CHDR
Centre for Human Drug Research

CLINICAL STUDY PROTOCOL

A randomized, double-blind, placebo controlled study to assess the pharmacodynamics, safety/tolerability and efficacy of omiganan in patients with mild to moderate atopic dermatitis

Short Title:	Pharmacodynamics of omiganan in patients with atopic dermatitis
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SUMMARY OF CHANGES

A randomized double-blind placebo controlled study to assess the pharmacodynamics, safety/tolerability and efficacy of omiganan in patients with mild to moderate atopic dermatitis

The following revisions were made to the protocol, which are also reflected in the synopsis, and other documents:

PROTOCOL VERSION 3, AMENDMENT 1&2, 17-JUN-2015 and 27-JUN-2016

Change	Rationale	Justification & Classification	Changed Document(s), Section
The frequency of emollients use for non-target lesion was changed to 'daily use' and predefined frequency of TCA use was deleted	To define the background treatment of non-target lesions less stringent	Minor change: Non-substantial	- Protocol, synopsis, sections 1.4.5; 3.3; 5.5.1 - SIS & ICF, Whole Document
Addition of 1 swab measurement (microbiome / SEB) of non-lesional skin at end-of-treatment visit.	Inclusion of non-lesional skin as benchmark	Correction minor scientific omission: non-substantial	- Protocol, Visit and assessment schedule - synopsis - SIS & ICF, Whole Document
Itch score will be monitored throughout the study. Therefore additional itch scores will be asked on day 35 and 42.	To be able to monitor the maximum duration of pharmacodynamic effects	Consistency change: Non-substantial	- Protocol, Visit and assessment schedule – synopsis and section 7.1.5
Duration of screening validity consistently changed to 35 days	Correct inconsistency in protocol	Consistency change: Non-substantial	-Protocol, section 3.1
Decrease diameter of skin punch biopsy from 4mm to 3 mm	Minimum diameter to decrease invasiveness	Reduction subject's burden: non-substantial	- Protocol synopsis, section 7.3.8 SIS & ICF, pag.4
Addition of one local biomarker measurement in biopsy sample	To correct minor scientific omission	Correct minor scientific omission: non-substantial	Protocol synopsis and sections 1.4.8, 6.1 and 7.3.9

PROTOCOL SYNOPSIS

Title

A randomized, double-blind, placebo controlled study to assess the pharmacodynamics, safety/tolerability and efficacy of omiganan in patients with mild to moderate atopic dermatitis.

Short Title

Pharmacodynamics of omiganan in patients with atopic dermatitis

Background & Rationale

Atopic dermatitis (AD) is a chronic, pruritic, inflammatory skin disease that occurs frequently in children, but also affects many adults. Clinical features of AD include skin dryness, erythema, oozing, crusting and lichenification. Pruritus is a major criterion for the diagnosis of AD and is the main driver of the high disease burden for patients and their families.

Two major models currently exist to explain the pathogenesis of AD. The predominant model describes AD as a result of impaired epidermal barrier function due to intrinsic structural and functional abnormalities in the skin. In this model, the disease evolves from the outside in, with an abnormal epidermal barrier as the primary defect. The second and more traditional model views AD primarily as an immune function disorder in which Langerhans cells, T-cells, and immune effector cells modulate an inflammatory response to environmental factors.

Colonization of S. aureus is found in 90% of chronic AD patients versus 5% in healthy individuals. Biofilm formation by AD-associated staphylococci almost certainly plays a major role in the occlusion of sweat ducts. This leads to inflammation and pruritus and may therefore play a role in exacerbation. Endogenous antimicrobial peptides are critical elements of the skin's innate immunity. In healthy skin, these peptides such as cathelicidins are induced upon colonization with certain bacteria or other external stimuli. However, in atopic skin the upregulation of cathelicidins is abrogated by the presence of Th2 cytokines. This results in lower levels of antimicrobial peptides, which could be a possible mechanism for staphylococcal colonization and superinfection.

LL-37 and indolicidin are antimicrobial peptides that are members of the cathelicidin family. Omiganan is a synthetic indolicidin analogue with antimicrobial and immunomodulatory activity. Recently it has been demonstrated that enhanced LL-37 expression improves barrier function in the skin. It disrupts the cytoplasmic wall of microorganisms, resulting in depolarization and cell death. Omiganan has shown to be effective against a wide variety of bacteria and fungi, including S. aureus. Immunomodulatory effects of omiganan were observed in a mouse model with TPA-induced ear edema. To date, omiganan was assessed in various clinical studies including patients with acne or rosacea where anti-inflammatory activity of this compound was demonstrated.

Due to its antimicrobial properties, the skin barrier enhancing properties of LL-37 and the immunomodulatory activity, we hypothesize that omiganan is a potential new treatment for AD.

This study is intended to investigate the pharmacodynamics of omiganan as a potential treatment for AD. Furthermore, exploratory efficacy by means of clinical outcomes (i.e. improvement in itch VAS and clearance of the lesion) and biomarkers will be assessed.

Primary objective

- To explore the pharmacodynamic effects on a target lesion of topically applied omiganan in AD patients

Secondary Objectives

- To assess safety and tolerability in AD patients
- To evaluate the efficacy of omiganan compared to placebo in AD patients

Design

A randomized, double-blind, vehicle controlled study to assess the pharmacodynamics, safety/tolerability, and efficacy of omiganan in patients with mild to moderate AD.

Principal Investigator & Trial Site

Prof. J. Burggraaf, MD, PhD, Centre for Human Drug Research, Zernikedreef 8, 2333 CL Leiden, The Netherlands

Subjects / Groups

In total 36 subjects with mild to moderate AD will be enrolled. The study will consist of one treatment arm with 2.5% omiganan*5HCL gel, one treatment arm with 1% omiganan*5HCL gel and one treatment arm with the vehicle gel. Subjects will administer a gel for four (4) weeks once daily on one antecubital fossa (the target lesion and treatment period). During treatment period and two weeks prior to first administration of the gel (run-in period), subjects administer emollients daily on the remaining affected skin and if necessary triamcinolone (TCA) topical 0.1%. During the run-in period the target lesion will be left untreated.

Study design:

Treatment	Subjects
CLS001 gel (1% omiganan *5HCL) x 4 weeks x QD on anticubital fossa + emollients daily on remaining skin*	12
CLS001 gel (2.5% omiganan*5HCL) x 4 weeks x QD on anticubital fossa + emollients daily on remaining skin*	12
Vehicle gel (placebo) x 4 weeks x QD + on anticubital fossa + emollients daily on remaining skin*	12

*= after a 2 week run-in period emollients treatment on a daily basis on the remaining affected skin;

TCA 0.1% as rescue medication

The study will last 11 weeks in total, with a screening period of 5 weeks, 2 weeks run-in period, 4 weeks of treatment and 2 weeks of follow-up.

Sample Size Justification

A group size of 12 subjects per treatment arm is justified by results from earlier trials investigating the role of LL-37 where significant changes in skin barrier transepidermal water loss upon application of topical formulations were observed [1]. However, no formal power calculation was performed given the exploratory character of the study.

Inclusion criteria

For enrollment of subjects the following criteria must be met:

1. Male and female subjects with mild to moderate AD 18 to 65 years of age, inclusive. The health status is verified by absence of evidence of any clinical significant active or

uncontrolled chronic disease other than AD following a detailed medical history, a complete physical examination including vital signs, 12-lead ECG, haematology, blood chemistry, and urinalysis. In the case of uncertain or questionable results, tests performed during screening may be repeated before randomization to confirm eligibility or judged to be clinically irrelevant for healthy subjects.

2. AD diagnosed by physician / medical specialist and that has been (intermittently) present for at least 1 year
3. At least one of the antecubital fossae must have an affected body surface area (BSA) of 0.5% with active dermatitis characterized by erythema and squamae at screening and end of the run-in period
4. Pruritus VAS score of target lesion of ≥30 at screening and end of the run-in period
5. oSCORAD-score of total body ≤40.
6. 2-15% body surface area (BSA) involved with AD lesions at screening.
7. Able to participate and willing to give written informed consent and to comply with the study restrictions.

Exclusion criteria

1. Have any current and / or recurrent clinically significant skin condition in the treatment area other than AD.
2. Use of topical medication (prescription or over-the-counter [OTC]) within 14 days of study drug administration, or less than 5 half-lives (whichever is longer) in local treatment area
3. Tanning due to sunbathing, excessive sun exposure, or a tanning booth within 3 weeks of enrollment.
4. Any confirmed, active significant allergic reactions (urticaria or anaphylaxis) including allergic reactions against any drug, multiple drug allergies or (ingredients of) emollients.
5. Participation in an investigational drug or device study within 3 months prior to screening or more than 4 times a year.
6. Loss or donation of blood over 500 mL within three months (males) or four months (females) prior to screening.
7. Unwillingness or inability to comply with the study protocol for any other reason.

Other qualifying criteria:

1. Subjects and their partners of childbearing potential must use two methods of contraception, one of which must be a barrier method for the duration of the study and for 3 months after the last dose (section 4.4.1).
2. Subjects *must not* have received treatments for AD within the intervals for the following medications:
    a. Cyclosporine/oral steroids/azathioprine/mycophenolate mofetil/other systemic immunosuppressants: 4 weeks
    b. Phototherapy: 3 weeks
    c. Topical calcineurin-inhibitors: 10 days

Concomitant medications

Prohibited are other treatments of AD, i.e. topical immunomodulators, oral steroids, cyclosporine, mycophenolate mofetil, methotrexate and azathioprine.

Allowed: Exceptions are paracetamol (up to 4 g/day), ibuprofen (up to 1 g/day), medication against asthma or allergic rhinitis including local corticosteroids; other medication against chronic diseases

as judged by the investigator. Other exceptions will only be made if the rationale is discussed and clearly documented between the investigator and the sponsor.

Study periods
The total duration of the study will be 11 weeks: 5 weeks for screening including 2 weeks for run-in period, 4 weeks of treatment and 2 weeks of follow-up. During the treatment period subjects will apply omiganan at home. On day 0 subject's drug administration is in clinic and subjects will stay for further testing such as; clinical assessment (local o-SCORAD), microbiology (swab), routine safety lab, 12-lead ECG, temperature and vital signs. Also standardized high-resolution photos, a 3 mm punch biopsy and TEWL measurement are completed.

On all other study days the clinical assessment (oSCORAD) and skin swab are carried out. Additional activities such as TEWL measurements, and clinical photography are performed according to the visit and assessment schedule.

Investigational drug

CLS001 is a topical gel containing omiganan, a 12-amino-acid cationic peptide. Two previous phase 2 studies for other indications (i.e. rosacea and acne vulgaris) studied omiganan in dosages of 1%, 1.75% and 2.5%. Dosage in this phase I/II study will be 1% and 2.5% once daily (QD). The maximum treated body surface area will be approximately 1%. The maximum dose of topical omiganan is estimated at: 181 cm^2 (1% total average BSA of an adult with a total body surface area of 1.81 m^2) x 1.7 mg/cm^2 (average amount applied per cm^2) x 0.025(dose of 2.5% w/w) = 8 mg per day.

Comparative drug

- No comparator will be used.
- The vehicle gel will serve as placebo. This is comprised of hydroxyethyl cellulose, sodium benzoate, glycerine and purified water.

Pharmacodynamic endpoints

Pharmacodynamic effects of Omiganan will be assessed at the time points indicated in the Visit and Assessment Schedule (Table 1) by:

- Local (biopsy) biomarkers (IgE, IFN-α, IFN- γ IL-1b, IL4, IL-6, IL-8, IL-9, IL-10, IL-13, IL-18, IL-31, TARC, eotaxin, oncostatin, TLR-2, TSLP, filaggrin)
- Microbiome of skin lesion
- Bacterial colonization of skin lesions (S. aureus) including biomarkers (enterotoxins)
- Transepidermal water loss of lesional and non-lesional skin (volar forearm)
-

Efficacy endpoints
Efficacy will be assessed at the time points indicated in the Visit and Assessment Schedule (Table 1) by:

- Clinical assessment of lesion on-site with local objective SCORAD and pruritus VAS
- Lesion size and morphology assessment by standardized clinical photography and 3D photography

Tolerability / safety endpoints

Adverse events (AE) will be collected throughout the study, at every study visit. Laboratory safety testing, 12-Lead ECGs and vital signs will be performed and measured multiple times during the course the study according to the Visit and Assessment Schedule. Skin tolerance and cosmetic scores by patients will be collected on day 28.

Statistical methodology

Data listings and averages will be presented for safety and pharmacodynamic measures. Given the exploratory character of the study, efficacy / pharmacodynamic endpoints will be primarily analyzed using descriptive statistics.

All pharmacodynamic and efficacy endpoints will be summarized (mean and standard deviation of the mean, median, minimum and maximum values) by treatment and time, and will also be presented graphically as mean over time, with standard deviation as error bars. Both Nominal results, and log-transformed results and change from baseline results will be utilized in all data summaries.

All categorical PD endpoints will be summarised by frequencies.

To establish whether significant treatment effects can be detected on the repeatedly measured PD parameters, each parameter will be analysed with a mixed model analysis of covariance (ANCOVA) with treatment, time, and treatment by time as fixed factors, subject as random factor and the (average) baseline measurement as covariate.

Biopsy parameters will be analysed with a mixed model analysis of covariance (ANCOVA) with treatment as fixed factor and the baseline measurement as covariate.

The following contrasts will be calculated within the model:

- 2.5% CLS001 – vehicle
- 1% CLS001 – vehicle
- 2.5% CLS001 – 1% CLS001

Table 1 Visit and Assessment Schedule

Assessment	SCR	-2wks	Day 0					Day 1	Day 2	Day 3	Day 4-6	Day 7	Day 8-13	Day 14	Day 15-20	Day 21	Day 22-27	Day 28	Day 35	Day42
	Up to -28 d		Pre-dose	0 h	2 h	4 h	8 h											EOT	Follow-up	Follow-up EOS
Informed consent	X																			
Demography	X																			
Inclusion and exclusion criteria	X																			
Medical history	X																			
Genomic sampling (filaggrin mutations)					X															
Physical examination	X																			X
Concomitant medication	X	X	<----- continuous ----->																	
Meals / snack						X	X													
Virology	X																			
BsHaem, BsChem, Urinalysis	X																	X		X
UrDrug, pregnancy testing**	X		X																	X
Temperature	X		X															X		X
ECG	X																			X
General symptoms	X	X	X	X	X	X		X		X		X		X		X		X	X	X
Vital Signs (HR, BP, RR)	X		X				X	X		X		X		X		X		X	X	X
Study drug administration (verum and placebo) CLINIC				X																
Study drug administration (verum and placebo) HOME								X	X	X	X	X	X	X	X	X	X			
Emollients BID		X	X					X	X	X	X	X	X	X	X	X	X	X		
Microbiology / microbiome/SEB		X	X		X	X	X	X		X		X		X		X		X***	X	X
Clinical assessment (oSCORAD and 5D-pruritus)	X	X	X															X		
Clinical assessment (Local oSCORAD)	X	X	X					X		X		X		X		X		X	X	X
Clinical photography	X	X	X									X		X		X		X		X
Skin punch biopsy *			X															X		
Patient assessment treatment satisfaction																		X		
PD TEWL measurements		X	X		X	X	X	X				X		X		X		X		X
eDiary / local VAS itch		<----- continuous ----->																	X	X
(S)AE/Con-meds		<----- continuous ----->																		

BP = Blood Pressure, HR = Heart Rate, RR = Respiratory Rate, SCR = Screening, AE = Adverse Event, UrDrug = Urine Drug SCR = Screen, BsHaem = Blood Sample Haematology, BsChem = Blood Sample Chemistry

* on Day0 a skin punch biopsy will be taken of the lesional skin (pre-dose) and of non-lesional skin during the study day

** UrDrug only at screening and at the discretion of the investigator

*** Swab will be taken from target lesion and non-lesional skin during the study day 28 (EOT)

1 BACKGROUND AND RATIONALE

1.1 Context

Atopic dermatitis (AD) is a chronic, pruritic, inflammatory skin disease that occurs frequently in children, but also affects many adults. Clinical features of atopic dermatitis include skin dryness, erythema, oozing and crusting, and lichenification. Pruritus is a hallmark of the condition and is the main driver of the high disease burden for patients and their families.

Two major models currently exist to explain the pathogenesis of atopic dermatitis. The predominant model describes AD as a result of an impaired epidermal barrier function due to intrinsic structural and functional abnormalities in the skin. In this model, the disease evolves from the outside in, with an abnormal epidermal barrier as the primary defect. The second and traditional model views atopic dermatitis primarily as an immune function disorder in which Langerhans cells, T-cells, and immune effector cells modulate an inflammatory in response to environmental factors.

Colonization of S. aureus is found in 90% of chronic AD patients versus 5% in healthy individuals. Biofilm formation by AD-associated staphylococci almost certainly plays a major role in the occlusion of sweat ducts. This leads to inflammation, pruritus and may therefor play a role in exacerbation. Endogenous antimicrobial peptides are critical elements of the skin's innate immunity. In healthy skin, these peptides such as cathelicidins are induced upon colonization or other external stimuli. However, in atopic skin upregulation of cathelicidins is abrogated by the presence of Th2 cytokines. This results in lower levels of antimicrobial peptides, which could be a possible mechanism of staphylococcal superinfection.

LL-37 and indolicidin are antimicrobial peptides that are members of the cathelicidin family. Omiganan is a synthetic indolicidin analogue with antimicrobial and immuno-modulatory activity. Recently it has been demonstrated that enhanced LL-37 expression improves barrier function in the skin. Regarding the mechanism of action, omiganan disrupts the cytoplasmic wall of microorganisms, resulting in depolarization and cell death. Omiganan was effective against a wide variety of bacteria and fungi, including S. aureus. Immunomodulatory effects of omiganan were observed in a mouse model with TPA-induced ear edema, in a mouse model of LPS-induced cytokine expression, and in in-vitro studies of human monocytes expression of TLR-mediated inflammatory products. To date, omiganan was assessed in various clinical studies including patients with acne or rosacea where anti-inflammatory activity of this compound could be demonstrated.

1.2 Study rationale

1.2.1 Benefit and risk assessment

The risks associated with the topical administration of CLS001 to humans has been identified in over 2500 subjects in total in fourteen clinical trials completed with topical applications of omiganan in formulations ranging from 0.5% to 3% in an aqueous gel and from 1% to 5% in an alcoholic solution for the indications of various indications including treatment of the inflammatory lesions of rosacea, treatment of acne and treatment of S. aureus in the nasal carriage. Omiganan when applied topically to intact or abraded skin, intranasally or at peripheral and central venous catheter sites appears to be safe and well tolerated. In addition, omiganan was not detected in the plasma of subjects after topical application to intact or abraded skin, to the nasal mucosa or at peripheral catheter sites. The risk of topical application to a very restricted lesional area can be considered minimal. Potential beneficial effects on atopic dermatitis lesions are to be explored in this study.

Therefore, providing the protocol is adhered to, careful observation and medical management will minimize any associated risk in this study

For a structured risk assessment see Section **Error! Reference source not found.**.

1.2.2 Medical and regulatory background

Due to its antimicrobial properties, the skin barrier enhancing properties and the immunomodulatory activity of LL-37, we hypothesize that omiganan is a potential new treatment for AD.

This study is intended to assess the pharmacodynamics of omiganan as a potential treatment for AD. Furthermore, exploratory efficacy by means of clinical outcomes (i.e. clearance of the lesion) and sub-clinical parameters / biomarkers will be assessed.

1.2.3 Study population

This phase 2 trial, with administration of CLS001 as multiple topical administrations to patients with mild to moderate AD, will provide exploratory PD, efficacy and safety data on omiganan. Although no therapeutic benefit on the lesions of the study participants is expected a treatment effect on the target region is anticipated. Patients with mild to moderate AD (male, non-pregnant females), 18 to 65 years of age who have a minimum target lesion of 0.5% BSA on at least one of the antecubital fossae are planned for inclusion.

1.2.4 Study design

This is a placebo-controlled, double-blind, randomized, single center, exploratory study that allows a thorough profiling of the PD activity of CLS001 in patients with mild to moderate AD. An extensive test battery of various dermatological assessments will advance the understanding of the potential effect of CLS001 in the AD patient population.

1.2.5 Placebo

The target lesions in one treatment group will be treated with vehicle gel (placebo). The use of placebo can be justified since the treatment area is limited to the target lesion and the non-target lesions can be treated with bland emollients on a daily basis and the rescue medication TCA 0.1% ointment.

1.2.6 Dose selection

Two previous phase 2 studies for other indications (i.e. rosacea and acne vulgaris) studied omiganan in dosages of 1%, 1.75% and 2.5%. Dosage in this phase I/II study will be 1% and 2.5% once daily. The maximum treated body surface area will be approximately 1%. The maximum dose of topical omiganan is estimated at: 181 cm^2 (1% total average BSA of an adult with a total body surface area of 1.81 m^2) x 1.7 mg/cm^2 (average amount applied per cm^2) x 0.025(dose of 2.5% w/w) = 8 mg per day.

1.2.7 Treatment duration

Based on the experience of two previous phase 2 studies for previously investigated indications, i.e. rosacea and acne vulgaris, and common investigations in AD with topical formulations a treatment duration of 4 weeks is chosen. A 4-week treatment period is appropriate since study objectives such as assessment of pharmacodynamics and efficacy can be performed adequately. In aforementioned studies no pattern of safety issues or treatment-emergent AEs was observed. The maximum treated BSA in this study is approximately 1% of, preferably, the right antecubital fossa. The total duration of the study will be 11 weeks: 5 weeks for screening including 2 weeks run-in period, 4 weeks of active treatment and 2 weeks of follow-up. Since ample experience of systemic exposure with the investigational product is present, a treatment period of 4 weeks is considered adequate to evaluate safety.

1.2.8 Primary endpoints

Pharmacodynamic endpoints

Pharmacodynamic effects of Omiganan will be assessed at the time points indicated in the Visit and Assessment Schedule (Table 1) by:

- Local (biopsy) biomarkers (IgE, IFN-α, IFN- γ IL-1b, IL4, IL-6, IL-8, IL-9, IL-10, IL-13, IL-18, IL-31, TARC, eotaxin, oncostatin, TLR-2, TSLP, filaggrin)
- Microbiome of skin lesion
- Bacterial colonization of skin lesions (S. aureus) including biomarkers (enterotoxins)
- Transepidermal water loss of lesional and non-lesional skin (volar forearm)

Efficacy endpoints

Efficacy will be assessed at the time points indicated in the Visit and Assessment Schedule (Table 1) by:

- Clinical assessment of lesion on-site with local objective SCORAD and pruritus VAS
- Lesion size and morphology assessment by standardized clinical photography and 3D photography;

Tolerability / safety endpoints

Adverse events (AE) will be collected throughout the study, at every study visit. Laboratory safety testing, 12-Lead ECGs and vital signs will be performed and measured multiple times during the course the study according to the Visit and Assessment Schedule.

1.2.9 Statistical hypotheses and sample size

A group size of 12 subjects per treatment arm is justified by results from earlier trials investigating the role of LL-37 where significant changes in skin barrier transepidermal water loss upon application of topical formulations were observed [1]. However, no formal power calculation was performed given the exploratory character of the study.

2 STUDY OBJECTIVES

2.1 Primary objective

- To explore the pharmacodynamic effects on a target lesion of topically applied omiganan in AD patients

2.2 Secondary objectives

- To assess safety and tolerability in AD patients
- To evaluate treatment effect of omiganan compared to placebo in AD patients

3 STUDY DESIGN

3.1 Overall study design and plan

This study has a randomized, double-blind, placebo-controlled design to assess the pharmacodynamics, safety/tolerability, and efficacy of omiganan in patients with mild to moderate atopic dermatitis.

The total duration of the study for each subject will be up to 70 days divided as follows:

- Screening: Up to 35 days prior to first study day;
- Run-in period: 14 days
- Treatment and study assessments: Days 0 to 28
- In Clinic visits: Days 0, 1, 3, 7, 14, 21, 28
- Follow-up visit: Days 35 and 42

3.2 Screening

Within 5 weeks prior to study baseline visit (Day 0), patients will undergo a medical screening. Screening will be performed in a fasting state (≥4 hours), and consists of medical history, physical examination, 12-lead ECG, vital signs, weight, height, heart rate, blood sampling (haematology, biochemistry, virology) and urinalysis. An overview and detailed photograph, when appropriate will be taken of de atopic lesions during the screening visit for diagnosis.

During screening urine and blood samples will be collected from each patient for analysis as described in section 7.2.

In addition, skin types will be assessed according to the Fitzpatrick classification.

Fitzpatrick skin type classification [2]:
-I: Highly sensitive; always burn, never tan
-II: Highly sensitive; usually burn, tan less than average (with difficulty)
-III: Sensitive; sometimes mild burn, tan about average
-IV: Moderate sensitivity; rarely burn, tan more than average (with ease)
-V: Low sensitivity; rarely burn, tans profusely, dark skin color
-VI: Not sensitive: never burn, always tans, darkest skin color

3.3 Run-in period

Following screening, eligible patients will enter a 2 week run-in period at the end of which they must have demonstrated i) compliance with a protocol-specified regimen of TCS and emollients ii) continue to demonstrate mild to moderate AD and the target lesion according to the inclusion criteria to be eligible for entry to the treatment period. If applicable, the run-in period can start during the washout period of other treatments.

The protocol-specified topical therapy regimen requires patients to self-apply the following topical therapy regimen daily (unblended):

- Emollient to all xerotic skin surfaces other than target lesion, preferably twice daily
- If needed, topical corticosteroid (TCS) cream / ointment only to all active skin lesions other than the target lesion if needed, consisting of medium potency TCS (triamcinolone acetonide 0.1% cream or ointment) for use on the body. In consultation with the investigator also other medium potency topical glucocorticoids can be used if indicated.

3.4 Treatment and observation period

Subjects will visit the clinical unit on days 0, 1, 3, 7, 14, 21, 28 during the treatment period from day 0 through day 28. A fixed dose of CLS001 / placebo will be applied once daily on (approximately) 0.5-1% BSA of, preferably, the right antecubital fossa. Two follow up visits are scheduled on day 35 and 42.

During study execution, vital signs will be measured on days 0, 1, 3, 7, 14, 21, 28, 35, and 42. Pharmacodynamic assessments (TEWL and microbiology / microbiome) are performed pre-dose and on day 0, 1, 3, 7, 14, 21, 28, 35 and 42, respectively. Punch biopsies will be performed on day 0 (lesional and non-lesional skin) and on day 28 (lesional skin). If bleeding does not stop within 10 min a suture can be placed to stop the bleeding if deemed necessary by the physician.

Subjects are dosed 28 consecutive days with 24 hours between each treatment. Per calendar day 1 dose of CLS001 is applied, enabling a dose window of up to 16 hours. All study procedure performed pre-dose and during the observation period are outlined in table 1, Visit and Assessment Schedule.

Visit variances are allowed as described in table 2.

3.4.1 Follow-up

In total, two follow-up visits will be performed, 7 and 14 days after the last treatment. Each subject will complete the study with an End of Study (EOS) visit, which will take place on day 42, 14 days after the last dose application, which includes a final physical examination, safety laboratory tests (haematology, biochemistry and urinalysis) and the measurement of vital signs.

During this visit final PD assessments will be performed. The atopic lesions will be assessed on morphology and by standardized clinical photography.
A description of all procedures and analyses is included in section 7.

Guidelines for Administration of Non-Investigational Treatment After EOT Visit

After the EOT visit is complete, the following guidelines apply to the administration of non-investigational treatment for AD.
1) All patients are encouraged to remain off-treatment of the target lesion through the End-of-Study visit to permit assessment of the durability of response.
2) Patients who request treatment of symptomatic disease may resume non-investigational treatment for AD based on their decision with their physician. They should attend all scheduled visits for safety assessments through the EOS; all treatments should be recorded as concomitant medications.
After EOS visit is complete, all patients may resume non-investigational treatment for AD based on their decision with their physician.

4 STUDY POPULATION

4.1 Subject population

A total of 36 subjects (males, females) will be enrolled into the study following satisfactory completion of a screening visit where eligibility for the study will be checked. Subjects will be recruited via media advertisement or from the subjects database of the Centre for Human Drug Research, Leiden, The Netherlands.

Patients with mild to moderate AD, 18 to 65 years of age who have a minimum target lesion of 0.5% BSA on at least one of the antecubital fossa will be included.

4.2 Inclusion criteria

For enrollment of subjects the following criteria must be met:

1. Male and female subjects with mild to moderate AD 18 to 65 years of age, inclusive. The health status is verified by absence of evidence of any clinical significant active or uncontrolled chronic disease other than AD following a detailed medical history, a complete physical examination including vital signs, 12-lead ECG, haematology, blood chemistry, and urinalysis. In the case of uncertain or questionable results, tests performed during screening may be repeated before randomization to confirm eligibility or judged to be clinically irrelevant for healthy subjects.
2. AD diagnosed by physician / medical specialist and that has been (intermittently) present for at least 1 year
3. At least one of the antecubital fossae must have an affected body surface area (BSA) of 0.5% with active dermatitis characterized by erythema and squamae at screening and end of the run-in period
4. Pruritus VAS score of target lesion of ≥30 at screening and end of the run-in period
5. oSCORAD-score of total body ≤40.
6. 2-15% body surface area (BSA) involved with AD lesions at screening.
7. Able to participate and willing to give written informed consent and to comply with the study restrictions.

Exclusion criteria

1. Have any current and / or recurrent clinically significant skin condition in the treatment area other than AD.
2. Use of topical medication (prescription or over-the-counter [OTC]) within 14 days of study drug administration, or less than 5 half-lives (whichever is longer) in local treatment area
3. Tanning due to sunbathing, excessive sun exposure, or a tanning booth within 3 weeks of enrollment.
4. Any confirmed, active significant allergic reactions (urticaria or anaphylaxis) including allergic reactions against any drug, multiple drug allergies or (ingredients of) emollients.
5. Participation in an investigational drug or device study within 3 months prior to screening or more than 4 times a year.
6. Loss or donation of blood over 500 mL within three months (males) or four months (females) prior to screening.
7. Unwillingness or inability to comply with the study protocol for any other reason

Other qualifying criteria:

1. Subjects and their partners of childbearing potential must use two methods of contraception, one of which must be a barrier method for the duration of the study and for 3 months after the last dose (section 4.4.1).
2. Subjects *must not* have received treatments for AD within the intervals for the following medications:
    a. Cyclosporine/oral steroids/azathioprine/mycophenolate mofetil/other systemic immunosuppressants: 4 weeks
    b. Phototherapy: 3 weeks
    c. Topical calcineurin-inhibitors: 10 days

4.3 Concomitant medications

All medications (prescription and over-the-counter [OTC]) taken within 30 days of study screening will be recorded.

4.3.1 Allowed concomitant medications

Paracetamol (up to 4g daily), Ibuprofen (up to 1g daily), birth control (oral or parenteral). Routine use of inhaled corticosteroids during the study is allowed.

Medication against asthma or allergic rhinitis including local corticosteroids; other medication against chronic diseases as judged by the investigator. Other exceptions will only be made if the rationale is discussed and clearly documented between the investigator and the sponsor.

The treatment of non-investigational lesional skin with bland emollients is allowed; TCA 0.1% will be used as rescue medication. Use of medications and approval thereof will be determined by the investigator individually.

4.3.2 Prohibited concomitant medications

Prohibited are other treatments of AD, i.e., topical immunomodulators, oral steroids, cyclosporine, mycophenolate mofetil, methotrexate and azathioprine.

Other exceptions will only be made if the rationale is discussed and clearly documented between the investigator and the sponsor.

4.3.3 Escape/rescue medications

All subjects will obtain 0.1% TCA or other medium-potent topical corticosteroids which can be used as rescue medication in the case an intolerable increase of the target lesion severity occurs. The subject and investigator / medical specialist will decide together if the use of rescue medication is necessary and if high-potent topical corticosteroids might be necessary.

4.4 Lifestyle restrictions

In the interest of their safety and to facilitate assessment of the treatment effect, the patients participating in this study will be requested to agree to the following restrictions during the study:

For the screening and on day 42 subjects will be required to fast for at least 4 hours. Water is allowed as required.
Bathing and washing of the target lesion is not allowed 24 hours prior to each study visit.

Subjects should avoid prolonged exposure of their involved skin to sunlight.

Alcohol consumption will not be allowed whilst in the study unit. At other times throughout the study, subjects should not consume more than 2 units of alcohol daily on average (one unit is 10 grams of alcohol). Subjects may undergo an alcohol breath test at the discretion of the investigator.

Smoking is not allowed during in- and outpatient visits at CHDR.

Approximate meal times will be according to the study schedule.

4.4.1 Contraception requirements

Nothing is known about the effect of omiganan on the human fetus. Therefore, all women of child bearing potential must practice effective contraception during the study and be willing and able to continue contraception for at least 90 days after their last dose of study treatment.

Women of child bearing potential are defined as all women physiologically capable of becoming pregnant, unless they meet one of the following conditions:

- Postmenopausal: 12 months of natural (spontaneous) amenorrhea or 6 weeks after surgical bilateral oophorectomy with or without hysterectomy;
- Posthysterectomy.

For the purposes of the study, effective contraception is defined as follows:

- Females: Using 1 or more of the following acceptable methods of contraception: surgical sterilization (e.g., bilateral tubal ligation), intrauterine contraception/device, hormonal contraception, or any 2 barrier methods (a combination of male or female condom with spermicide; diaphragm, sponge, cervical cap).
- Males: Effective male contraception includes a vasectomy with negative semen analysis at follow up, or the use of condoms with spermicide.

Abstinence can be considered an acceptable method of contraception at the discretion of the investigator. Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post ovulation methods) and withdrawal are not considered acceptable methods of contraception.

4.5 Study drug discontinuation and withdrawal

4.5.1 Study drug interruption or discontinuation

The investigator must temporally interrupt or permanently discontinue the study drug if continued administration of the study drug is believed to be contrary to the best interests of the subject. The interruption or premature discontinuation of study drug might be triggered by an Adverse Event (AE), a diagnostic or therapeutic procedure, an abnormal assessment (e.g., ECG or laboratory abnormalities), or for administrative reasons in particular withdrawal of the subject's consent. The reason for study drug interruption or premature discontinuation must be documented.

4.5.2 Subject withdrawal

Subjects have the right to withdraw from the study at any time for any reason. Should a subject decide to withdraw from the study, all efforts should be made to complete and report the observations, particularly the follow-up examinations, as thoroughly as possible.

4.5.3 Replacement policy

Subjects withdrawing for reasons other than adverse events or any other tolerability issues with the treatment may be replaced at the discretion of the investigator and sponsor.

4.5.4 Schedule of EOT and EOS Visits Based on Completion of Treatment

For subjects who terminate study treatment prematurely the following visits are performed:

- EOT visit 1 to 7 days after the last treatment administration.
- EOS visit 14 to 28 days after the last treatment administration

4.6 Study drug packaging and labelling

The study drug will be supplied in 20 g tubes, packaged and labelled in accordance with local regulations. Upon arrival at the pharmacy, the investigational products should check them for damage and verify proper identity, quantity, integrity of seals and temperature conditions, and report any deviations or product complaints upon discovery. The dispensing of the study drug will be performed by the pharmacy. The labelling will be according to GMP. Study drug will be dispensed for each subject according to the randomization list. Study drug packaging will be overseen by the Leiden University Medical Centre Pharmacy and bearing a label with the identification required by local law, the protocol number, drug identification, and dosage.

All drug supplies must be stored in a secure, temperature-controlled area with limited access. For batch-specific storage instructions, see the packaging.

4.7 Drug accountability

Drug accountability will be maintained by the Leiden University Medical Centre Pharmacy and assessed by maintaining adequate study drug dispensing records.
The investigator is responsible for ensuring that dosing is administered in compliance with the protocol. Delegation of this task must be clearly documented and approved by the investigator. The study drug administration will occur under medical supervision with instructions on day 0. Administrations from day 1 to day 28 will be performed by the subject at home or other location e.g. at the clinic. Subjects will keep a digital patient diary (e-diary) where all administrations can be recorded with a date and time stamp. Tubes are weighed prior to distribution to the subject, and upon return (Day 28).

4.8 Treatment assignment and blinding

4.8.1 Treatment assignment

Subjects in this study will be numbered sequentially from 1 to 36 in order of inclusion. Replacement subjects will be numbered starting from 101 and replacement subject number will be assigned by the unblinded statistician. Randomization is performed in blocks of three.

The randomization code will be generated by a study-independent, CHDR statistician. The randomization code will be unblinded/broken and made available for data analysis only after study closure, i.e., when the study has been completed, the protocol deviations determined, and the clinical database declared complete, accurate and locked. The randomization code will be kept strictly confidential. Sealed individual randomization codes, per subject and per treatment, will be placed in a sealed envelope, labelled 'emergency decoding envelopes' and will be kept in a safe cabinet at CHDR.

4.8.2 Blinding

This study will be performed in a double-blind fashion. The investigator, study staff, subjects, sponsor, and monitor will remain blinded to the treatment until study closure. The investigational drug and its matching placebo are indistinguishable and will be packaged in the same way.

The investigator will receive a set of sealed emergency codes to be broken in case of emergency situations. If the identity of the study drug administered needs to be known in order to manage the subject's condition i.e., in case of a medical emergency or in the case a SUSAR occurs, the

treatment emergency code for that subject may be broken and the study drug identified. All such occurrences should be documented in the study file. Treatment emergency codes should not be broken except in emergency situations and, if possible, the investigator should be contacted before the emergency code is opened. Prior to database lock the unused emergency code labels will be checked and a statement to the effect that all are intact (or not as the case may be) will be made on the database lock form.

4.9 Non-investigational medicinal products

4.9.1 Unguentum leniens

All subjects will receive emollients (Unguentum leniens) to be applied on a daily basis, preferably twice daily from the start of the run-in period until EOT visit. Tubes will be weighed pre-dose and at EOT. Treatment compliance will be monitored with the ediary app.

4.9.2 Triamcinolone 0.1%

As rescue (‘escape’) medication all subjects will receive TCA 0.1%. The investigator will decide if an ointment or cream is appropriate if necessary in consultation with the dermatologist. In case TCA 0.1% needs to be applied, the subjects will record application in the ediary app.

5 STUDY ENDPOINTS

5.1 Pharmacodynamic endpoints

Pharmacodynamic effects of CLS001 on the target lesion will be assessed at the time points indicated in the Visit and Assessment Schedule (Table 1).

- Local (biopsy) biomarkers (IgE, IFN-α, IFN- γ IL-1b, IL4, IL-6, IL-8, IL-9, IL-10, IL-13, IL-18, IL-31, TARC, eotaxin, oncostatin, TLR-2, TSLP, filaggrin)
- Microbiome of skin lesion
- Bacterial colonization of skin lesions (S. aureus) including biomarkers (enterotoxins)
- Transepidermal water loss of lesional and non-lesional skin

5.2 Safety and tolerability endpoints

Adverse events (AE) will be collected throughout the study, at every study visit. Laboratory safety tests will be performed at time of screening, End-of-Treatment and at End-of-Study. Vital signs will be measured at baseline and days 1, 3, 7, 14, 21, 28, 35, and 42. 12-Lead ECGs will be performed at screening and End-of-Study.

Safety endpoints include:

- Treatment-emergent (serious) adverse events ((S)AEs).
- Skin tolerance and treatment satisfaction
- Concomitant medication
- Clinical laboratory tests
    - Haematology
    - Chemistry
    - Urinalysis
- Vital signs
    - Pulse Rate (bpm)
    - Systolic blood pressure (mmHg)
    - Diastolic blood pressure (mmHg)
- Electrocardiogram (ECG)
    - Heart Rate (HR) (bpm), PR, QRS, QT, QTcB,

5.3 Efficacy endpoints

Change from baseline to each time point of measurement during each treatment period for the following assessments:

- Clinical assessment of lesion on-site with local objective SCORAD and pruritus VAS
- Lesion size and morphology assessment by standardized clinical photography and 3D photography;

6 STUDY ASSESSMENTS

See Visit and Assessment Schedule (Table 1) for the time points of the assessments.

6.1 Efficacy assessments

6.1.1 Target Lesion Size

Target lesions will be recorded by measuring the widest diameter and shortest diameter perpendicular to first measurement, all dimensions measured in millimetres by photography, see 7.1.6. The size of the target lesion will be calculated.

6.1.2 SCORAD

The SCORAD is a validated tool used in clinical research and clinical practice that was developed to standardize the evaluation of the extent and severity of AD (Dermatology 1993).

The extent of AD is assessed as a percentage of each defined body area and reported as the sum of all areas, with a maximum score of 100% (assigned as “A” in the overall SCORAD calculation). The severity of 6 specific symptoms of AD is assessed using the following scale: none (0), mild (1), moderate (2), or severe (3) (for a maximum of 18 total points, assigned as “B” in the overall SCORAD calculation). Subjective assessment of itch and sleeplessness is recorded for each symptom by the patient or relative on a visual analogue scale (VAS), where 0 is no itch (or sleeplessness) and 10 is the worst imaginable itch (or sleeplessness), with a maximum possible score of 20. This parameter is assigned as “C” in the overall SCORAD calculation. The SCORAD is calculated as: A/5 + 7B/2 + C. Patients will undergo this assessment according to the Assessment Schedule (Table 1) or early termination.

The objective SCORAD (oSCORAD) leaves out the subjective, i.e. patient reported, parameters sleeplessness and itch. In the assessment of the local oSCORAD, the severity is scored as described above, the extent is relative to the surface of the anterior upper limb.

6.1.3 Body Surface Area Involvement of Atopic Dermatitis

Body surface area affected by AD will be assessed for each major section of the body (head, trunk, arms, and legs) and will be reported as a percentage of all major body sections combined. Patients will undergo this assessment at the following visits: screening, day0/baseline (pre-dose), and day 48 (EOT) or early termination.

6.1.4 5-D Pruritus Scale

The 5-D Pruritus Scale is a 1-page, 5-question, validated questionnaire developed by Elman et al. 2010 [3] used in clinical trials to assess 5 dimensions of background itch: degree, duration, direction, disability, and distribution. Each question corresponds to 1 of the 5 dimensions of itch; patients will rate their symptoms over the preceding 2-week period as “present” or on a 1 to 5 scale, with 5 being the most affected. Patients will undergo this assessment according to the Assessment Schedule (Table 1) or at early termination.

6.1.5 Pruritus Visual Analogue Scale (VAS)

The pruritus VAS is a single-question assessment tool that will be used to assess the patient’s worst itch as a result of AD in the previous 12 hours. Patients will fill-in the e-diary daily from the evening of the run-in visit and be asked the following question; “on a scale of 0 – 100, with 0 being „no itch” and 100 being the „worst itch imaginable“, how would you rate your worst degree of itch experienced during the previous 12 hours?” Patients will be instructed on daily reporting at the visit of the run-in period and will be queried for compliance at every clinic visit. Patients will complete the rating scale twice daily through the last study visit (EOT). At the post-treatment visits the average itch of subjects during the last 7 days will be asked.

6.1.6 Clinical Photography

On Days 0, 7, 14, 21, 28, and 42 a standardized set of photographs will be taken of the lesion that is included in the study; photographs will include a ruler labelled with subject initials, subject study number, and calendar date and nominal study date/ occasion number. Pictures will be taken by a 3D stereo-camera system (LifeViz™ QuantifiCare, Valbonne, France). A quantitative analysis of the photographs will be performed by one or more blinded, experienced (sub-)investigators.

6.2 Safety and tolerability assessments

The definitions, reporting and follow-up of AEs, SAEs and potential pregnancies are described in section 7.

6.2.1 Vital signs

Evaluations of systolic and diastolic blood pressure, pulse rate, respiratory rate, and temperature will be performed throughout the study. Pulse and blood pressure will be taken after 5 minutes in the supine position. Automated oscillometric blood pressures will be measured using a Dash 3000, Dash 4000, Dynamap 400 or Dynamap ProCare 400.

6.2.2 Weight and height

Weight (kg) will be recorded at screening and the follow-up visit or upon early termination. Height (cm) will be recorded and body mass index (BMI) calculated at screening.

6.2.3 Physical examination

Physical examination (i.e., inspection, percussion, palpation and auscultation) is performed during the course of the study. Clinically relevant findings that are present prior to study drug initiation must be recorded with the subject's Medical History. Clinically relevant findings found after study drug initiation and meeting the definition of an AE (new AE or worsening of previously existing condition) must be recorded.

6.2.4 Electrocardiography

12-lead electrocardiographs (ECGs) will be obtained during the course of the study using Marquette 800/5500 or Dash3000 and stored using the MUSE Cardiology Information System. The investigator will assess the ECG recording as 'normal', 'abnormal - not clinically significant', or 'abnormal - clinically significant' and include a description of the abnormality as required. The ECG parameters assessed will include heart rate, PR, QRS, QT and QTc (calculated using Bazett's method).

6.2.5 Laboratory assessments

Laboratory parameters

Blood and other biological samples will be collected for the following clinical laboratory tests:

Lab	Tests	Collection & Analysis
Haematology	Haemoglobin [including Mean Corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC)], haematocrit, red cell count (RBC), total white cell count (WBC), leukocyte differential count and Platelet count. Differential blood count, including: basophils, eosinophils, neutrophils, lymphocytes, and monocytes.	4 mL of venous blood in a BD Vacutainer® K_2EDTA tube. Samples will be analysed by the Central Clinical Hematology Laboratory (CKHL) of Leiden University Medical Center.
Chemistry and electrolytes	Sodium, potassium, calcium, chloride, inorganic phosphate, total protein, albumin, glucose[1], total cholesterol, triglycerides, blood urea nitrogen (BUN), creatinine, uric acid, total	8.5 mL of venous blood in a BD Vacutainer® SST Gel and Clot Activator tube. Samples will be analysed by the Central

	bilirubin[2], CPK, , alkaline phosphatase, AST, ALT, gamma-GT and LDH.	Clinical Chemistry Laboratory (CKCL) of Leiden University Medical Center.
Serology	HIV1 and HIV2 antibodies, Hepatitis B antigen and Hepatitis C antibodies	5 mL of venous blood in a BD Vacutainer® SST Gel and Clot Activator tube. Samples will be analysed by the Central Clinical Microbiology Laboratory (CKML) of the Leiden University Medical Center.
Urinalysis	Leucocytes, blood, nitrite, protein, urobilinogen, bilirubin, pH, specific gravity, ketones, glucose. If there is a clinically significant positive result, urine will be sent to the CKCL for microscopy and/or culture.	A midstream, clean-catch urine specimen will be analysed by dipstick (Multistix® 10 SG, Siemens Healthcare Diagnostics, Frimley, UK).
Pregnancy[3]	hCG. If there is a clinically significant, positive result, urine will be sent to the CKCL for confirmation.	A urine specimen will be analysed at CHDR by test kit (InstAlert, Innovacon, San Diego, USA).
Urine drug screen	Cocaine, amphetamines, opiates (morphine), benzodiazepines and cannabinoids.	A urine specimen will be analysed at CHDR by test kit (InstAlert, Innovacon, San Diego, USA).
Genotyping sampling	Screening for the 4 most prevalent filaggrin mutations	3 mL of venous blood in a BD Vacutainer® K_2EDTA tube. Samples will be analysed by DDL.
[1]After 4-hours fasting, not at EOT visit. [2]Conjugated bilirubin will be reported only when total bilirubin is outside the reference range. [3]Pregnancy test for women of childbearing potential will be performed at screening and Day0 and if pregnancy is suspected during the study.		

6.2.6 Labelling

Pre-printed, waterproof labels will be used to identify the tubes used during sample collection and for storage of separated plasma. Each label will contain the following information:

- CHDR Protocol number
- Subject Number
- Occasion number (date)
- Protocol (delta) time
- Activity: Sample type (blood) & purpose

6.2.7 Shipping Procedures

CHDR will arrange shipment of the samples. The samples must be packed securely together with completed shipment forms in polystyrene insulated shipping containers Samples must be shipped to the DDL laboratory at time intervals agreed with the sponsor.

6.3 Pharmacodynamic assessments and questionnaires

6.3.1 Concomitant medications

Concomitant medications initiated, stopped, up-titrated or down-titrated for an AE will be recorded.

6.3.2 Transepidermal water-loss (TEWL)

To assess the barrier status of the skin the TEWL will be measured at multiple time points during the study (Table 1). The water loss will be measured non-invasively using an AquaFlux AF200 system (Biox, London, UK) according to the standard operating procedure of CHDR. A circular area of 7 mm diameter of skin will be enclosed by the measuring probe. The flux of water that enters the chamber will be measured until a steady-state flux is reached. Minimal measurement time is 90 seconds and maximum measurement time is 200 seconds. All measurements will be performed under standard environmental conditions (temperature 22°C±2°C; relative humidity <60%), and subjects will be acclimatized under relaxed conditions for at least 15 minutes prior to testing.
Three sites will be measured: lesional skin (target region); lesional skin (off-target region, preferably contralateral); non-lesional skin (preferably volar forearm).

6.3.3 Swab sampling for microbiology – S.aureus quantification

Collection of skin culture samples is a non-invasive procedure where a sterile cotton swab (Puritan Sterile Polyester Tipped Applicators REF 25-806-1PD) is passed along the lesional surface of the target area. The skin swab will be placed in a Micro tube (REF 72.694.105, Sarstedt, Numbrecht, Germany) containing 0.9% NaCl and 0.1% Tween 20 and analyzed for the presence of bacteria. Samples will be collected according to the Assessment Schedule (Table 1). The tubes will be stored in the freezer at -80°C, to be shipped to DDL at the end of the study. The microbiology samples will be analysed at DDL Laboratory, Rijswijk, The Netherlands for the presence and quantity of S. aureus DNA.

6.3.4 Swab sampling for microbiome and SEB

Same material and procedure as described in 7.3.3 will be used.

6.3.5 Collecting DNA from swabbed microbiome

The DNA extraction will be performed automatically with the NucliSENS easyMag (bioMérieux) using 500µl of input and an elution volume of 50µl.

6.3.6 Microbiology analysis – *S.aureus* quantification

A single-plex quantitative PCR (qPCR) adapted from literature (Pichon et al., 2012 (J Antimicrob Chemother. 2012 Oct;67(10):2338-41)) targeting the *nuc* gene will be applied to quantify the *S.aureus* bacterial load. The input for this qPCR will be 5µl of DNA in total volume of 25µl. Quantification of bacterial load will be done by comparing the results of the samples with the results of a standard curve with known concentrations. This standard curve is tested in parallel to the samples in each experiment.

6.3.7 Microbiome analysis

After DNA extraction, the variable regions 3 and 4 of the 16S rRNA gene are amplified giving an amplicon of around 460 base pairs. This amplicon is analyzed on agarose gels or capillary systems using standard protocols, to confirm successful amplification of a PCR fragment of the expected size, and to determine whether the quantity of product is sufficient for successful next-generation sequencing. As a next step, PCR products are cleaned up by Ampure XP beads (Beckman Coulter) to remove primer-dimers and small a-specific PCR products and the purified PCR products are quantified using the Quant-iT PicoGreen dsDNA kit (Life Technologies), followed by serial dilution steps to reach the correct amount of input DNA. Index primers (Nextera XT Index kit) will be added

by limited cycle PCR to the diluted PCR products. Prior to pooling, samples are normalized by using beads with maximum binding capacity (Nextera XT sample preparation kit).

The sequencing is performed on the Illumina MiSeq platform by using the MiSeq v2 sequencing kit with 500 cycles (Illumina). De-multiplexed FASTQ files are generated as output and the sequences of the FASTQ files are clustered together on similarity. The clustered reads are then classified to the taxonomy database, resulting in a taxonomy percentage summary of the sequenced bacterial sample.

6.3.8 Biopsy sample collection

Three-millimeter punch biopsies are taken from skin with active eczema (lesional skin), pre-dose and at Day 28, and from clinically normal skin (non-lesional) pre-dose preferably antecubital fossa or lower forearm. The biopsy procedure will be performed according to SOP CGESPBIO section 3.2 “performance biopsy” and section 3.3 “wound care and advice” (CHDR) for skin punch biopsies with local anaesthetics. The biopsies will be placed in RNAlater medium directly after harvest of the biopsy and stored at -80°C. The biopsy sample will be analysed at DDL Laboratory, Rijswijk, The Netherlands for local biomarkers.

Sample collection materials will be provided by DDL Diagnostic Laboratory.

6.3.9 Local biomarker sequencing

RNA extraction and quantitative PCR will be performed for the following biomarkers: IgE, IFN-α, IFN-γ IL-1b, IL4, IL-6, IL-8, IL-9, IL-10, IL-13, IL-18, IL-31, TARC, eotaxin, oncostatin, TLR-2, TSLP and filaggrin. Sample analysis will be performed by DDL Diagnostic Laboratory. The biomarker battery may be extended if further analysis is deemed necessary.

6.3.10 Filaggrin mutation screening

All subjects will be screened on the four most prevalent mutations found in European Caucasians (2282del4, R501×, S3247×, and R2447×), covering around 93% of all FLG mutations known to date [4]. Plasma samples will be collected using 3.0ml K2EDTA-tubes (Vacutainer, BD, The Netherlands) according to standard operating procedures of the Centre of Human Drug Research, Leiden, The Netherlands. The samples will be stored at -20°C and processed for batch analysis . Mutations will be determined by genotyping after DNA extraction according to a modified protocol of [5] by DDL Diagnostic Laboratory.

6.3.11 Staphylococcal Enterotoxin Type B (SEB) analysis

SEB analysis will be performed from the obtained swab samples. After extraction, the samples will be analysed using a RUO commercial kit (Luminex Corporation, Austin, Texas) performed by DDL Diagnostic Laboratory.

6.3.12 Skin tolerance / treatment satisfaction score

The tolerance and treatment satisfaction evaluation will be performed using a questionnaire that will be handed out at the EOT visit (Day28), adapted from [6;7]. The Dutch questionnaire is provided in the clinical trial application dossier.

Following questions regarding gel properties will be graded by subjects on a 5-point scale:application on the skin, visibility on the skin, the skin being able to breathe, stickiness on the skin, greasiness on the skin absorption on the skin.

Investigator assessments, including any treatment-related adverse events (at the administration site) or events will be performed at each study visit.

6.3.13 E-diary

All subjects will be asked to fill in an ediary from start of the run-in period up to EOT. For this purpose subjects will make use of an app. The app is intended to be used as e-diary in order to monitor and promote treatment compliance in the clinical trial. The e-diary app captures data of the treatment application by means of a photo, records the time, the date and the geographical location of the photo. Furthermore, the VAS itch can be recorded as well as the use of emollients and rescues medication. When the photos are taken and data is entered into the app, the trial subject can immediately send the electronic data to the CHDR SQL server via a secured connection.

6.4 Sequence of assessments and time windows

On study day 0 study assessments will be performed prior to dose administration in the following order, where possible: urine pregnancy test (females only), vital signs (including temperature), PD assessments.

The deviations of actual time points from the expected time points will be within ten percent, calculated from the zero point (time of drug administration) or the last relevant activity. Deviations of more than 10% will be explained in a note (this does not apply dose administration as described in section 3.1.2). Pre-dose assessments are given in indicative expected times. Pre-dose assessments are given in indicative expected times.

Visit variances are allowed as described in Table 2:

Table2 . Visit Variances.

Protocol Procedure	**Approved Time Window**
Day 0	Per protocol.
Day 1	Per protocol;
Day 3	Per protocol
Day 7, 14, 21, 28 (EOT)	May be done plus or minus two (2) calendar days from scheduled.
Day 35, 42	May be done plus or minus four (4) calendar days from scheduled.

6.5 Total blood volume

During execution of the study a total of 48mL blood will be sampled:

Sample	**Samples taken**		**Sample Volume***			**Volume**	
Haematology	3	x	4	mL	=	12	mL
Chemistry	3	x	8.5	mL	=	25.5	mL
Serology	1	x	5	mL	=	5	mL
Filaggrin	1	x	5	mL	=	5	mL
* inclusive discarded volume			**Total blood volume/subject**			**47.5**	**mL**

7 SAFETY REPORTING

7.1 Definitions of adverse events

An Adverse Event (AE) is any untoward medical occurrence in a subject who is participating in a clinical study performed. The adverse event does not necessarily have to follow the administration of a study drug, or to have a causal relationship with the study drug. An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory or vital sign finding), symptom, or disease temporally associated with the study participation, whether or not it is related to the study drug.

7.1.1 Intensity of adverse events

The intensity of clinical AEs is graded three-point scale as defined below:

- Mild: discomfort noticed but no disruption of normal daily activity;
- Moderate: discomfort sufficient to reduce or affect normal daily activity;
- Severe: inability to work or perform daily activity.

7.1.2 Relationship to study drug

For each adverse event the relationship to drug as judged by the investigator:

- Probable;
- Possible;
- Unlikely;
- Unrelated.

7.1.3 Chronicity of adverse events

The chronicity of the event will be classified by the investigator on a three-item scale as defined below:

- Single occasion: single event with limited duration;
- Intermittent: several episodes of an event, each of limited duration;
- Persistent: event which remained indefinitely.

7.1.4 Action

Eventual actions taken will be recorded.

7.1.5 Serious adverse events

A Serious Adverse Event (SAE) is defined by the International Conference on Harmonization (ICH) guidelines as any AE fulfilling at least one of the following criteria:

- Is fatal
- Is life-threatening
- Is disabling
- Requires or prolongs in-patient hospitalisation
- Causes congenital anomaly

will be described as a SAE. Important medical events that may not be immediately life threatening or result in death or hospitalisation may be considered a serious adverse event when, based on appropriate medical judgement, they may jeopardise the subject or may require medical or surgical intervention to prevent one of the outcomes listed above.

7.1.6 Suspected unexpected serious adverse reactions

A SUSAR (Suspected Unexpected Serious Adverse Reaction) is a serious adverse event that is unexpected, (nature or severity of which is not consistent with the applicable product information (e.g., investigator's brochure for an unauthorised investigational product or summary of product characteristics for an authorised product)) and suspected (a reasonable possibility of causal relationship with investigational drug).

7.1.7 Reporting of serious adverse events

SAEs and SUSAR's will be reported according to the following procedure.

All SUSARs and SAEs must be reported to the sponsor by telephone and in writing as soon as practical, but at least within 24 hours of awareness, except for those SAE's that the protocol or investigator's brochure identifies as not requiring immediate reporting
The investigator must report all SAEs and SUSAR's to the EC that approved the study, in writing as soon as practical, but at least within 15 days. Fatal and life-threatening suspected SUSAR's should be reported within 7 calendar days, with another 8 days for completion of the report.

The sponsor must report all SUSAR's to the CA, in writing as soon as practical, but at least within 15 days. Fatal and life-threatening suspected SUSAR's should be reported within 7 calendar days, with another 8 days for completion of the report. SAE's do not have to be reported to the CA.

The sponsor must furthermore report all SUSAR's to EMA's EudraVigilance database within 15 days. Fatal and life-threatening suspected SUSAR's should be reported within 7 calendar days, with another 8 days for completion of the report.

The sponsor can prepare additional reports for other authorities (e.g. FDA).

7.1.8 Follow-up of adverse events

All adverse events will be followed until they have abated, returned to baseline status or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

7.2 Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the EC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the EC, except insofar as suspension would jeopardise the subjects' health. The investigator will ensure that all subjects are kept informed.

7.3 Annual safety report or development safety update report

In addition to the expedited reporting of SUSARs, the investigator will submit, once a year throughout the clinical trial, a safety report to the EC, CA, MEB and CAs of the concerned Member States.
This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

7.4 Pregnancy

7.4.1 Teratogenicity

If a woman becomes pregnant when on study drug, permanent discontinuation of study drug should be considered as appropriate. The investigator must counsel the subject and discuss the risks of

continuing with the pregnancy and the possible effects on the foetus. Monitoring of the subject should continue until the outcome of the pregnancy is known.

7.4.2 Reporting of pregnancy

Irrespective of the treatment received by the subject, any pregnancy occurring during study drug administration until follow-up, must be reported within 24 hours of the investigator's knowledge of the event to the sponsor.

8 STATISTICAL METHODOLOGY AND ANALYSES

8.1 Statistical analysis plan

All safety and statistical programming is conducted with SAS 9.1.3 for Windows (SAS Institute Inc., Cary, NC, USA).

Data listings and averages will be presented for safety measures. Given the exploratory character of the study, efficacy / pharmacodynamic endpoints will be analysed using primarily descriptive statistics.

Major and minor protocol deviations will be identified before the study closure.

A Statistical Analysis Plan (SAP) will be written and finalized before the study closure, i.e., database closure and unblinding of the randomization code. The SAP will provide full details of the analyses, the data displays and the algorithms to be used for data derivations.
The SAP will include the definition of major and minor protocol deviations and the link of major protocol deviations to the analysis sets.

8.2 Protocol violations/deviations

Protocol deviations will be identified based on conditions related to the categories below:

- Protocol entry criteria
- Forbidden concomitant medications
- Missing evaluations for relevant endpoints
- Other protocol deviations occurring during study conduct.

Major protocol deviations will be identified before the study closure, and listed where appropriate.

8.3 Missing, unused and spurious data

The handling of missing, unused and spurious data will be documented in the study report.

All missing or incomplete safety and PD data, including dates and times, are treated as such. Missing test results or assessments will not be imputed. Missing PD data, indicated as ‘M’ in the data listing, will be estimated within the statistical mixed model using SAS PROC MIXED.

For graphical and summary purposes PD and safety values below the limit of quantification will be set to half (½) of the limit of quantification. For analysis no undetermined values will be replaced.

8.4 Analysis sets

Data of all subjects participating in the study will be included in the analyses if the data can meaningfully contribute to the objectives of the study.

- Safety population - all subjects who were validated (randomised) and received at least one topical administration of study medication.
- Intent-to-treat Population – All subjects who received at least twenty-one (21) topical administrations of study medication.
- Clinical Evaluable Population – All subjects who completed four weeks of treatment and the EOT visit and have no major protocol deviations as determined by review by the investigator prior to unblinding.

8.5 Subject disposition

The following subject data will be summarized by treatment and overall:

- Number and percentage of subjects enrolled in each analysis set for all randomized subjects;

Subject disposition will be listed.

8.6 Baseline parameters and concomitant medications

8.6.1 Demographics and baseline variables

Continuous demographic variables (e.g., age, height, weight, BMI) will be summarized by descriptive statistics (n, mean, SD, median, Min, Max).

Qualitative demographic characteristics (sex, race/ethnicity) will be summarized by counts and percentages.

8.6.2 Medical history

Medical history will only be listed.

8.6.3 Concomitant Medications

All concomitant medications will be displayed in a listing.

8.6.4 Treatment compliance/exposure

Exposure to study treatment is described in terms of duration of treatment and total amount of CLS001 applied. The average applied dose per cm^2 will be calculated. The average applied dose (mg/d) is summarized by mean, SD, median, Q1, Q3, Min, Max.

The compliance with protocol-defined investigational product will be calculated as follows: Treatment Compliance = (Number of investigational product administrations during exposure period) / (Number of planned investigational product administrations during exposure period) x 100%. The treatment compliance will be presented by specific ranges for each treatment group. The ranges of interest will be specified in the SAP.

8.7 Safety and tolerability endpoints

The safety set is used to perform all safety analyses. Baseline is defined as the last value prior to dosing. Change from baseline will be calculated for all continuous safety parameters.

8.7.1 Adverse events

The AE coding dictionary for this study will be Medical Dictionary for Regulatory Activities (MedDRA). It will be used to summarize AEs by primary system organ class (SOC) and preferred term (PT). All adverse events will be displayed in listings.

A treatment-emergent adverse event (TEAE) is defined as an adverse event observed after starting administration of the specific treatment, OR up to 5 days (96 hours) after study drug administration. If a subject experiences an event both prior to and after starting administration of a treatment, the event will be considered a TEAE (of the treatment) only if it has worsened in severity (i.e., it is reported with a new start date) after starting administration of the specific treatment, and prior to the start of another treatment, if any. All TEAEs collected during the investigational period will be summarized.

The number of subjects with treatment emergent AEs will be summarized by treatment, MedDRA SOC, PT and drug relatedness.

8.7.2 Vital signs

At each time point, absolute values and change from baseline of supine BP and HR will be summarized with n, mean, SD, SEM, median, Min, and Max values.

8.7.3 ECG

At each time point, absolute values and change from baseline of ECG numeric variables will be summarized with n, mean, SD, SEM, median, Min, and Max values.

8.7.4 Clinical laboratory tests

All laboratory data (including re-check values if present) will be listed chronologically. 'H' and 'L', denoting values above or below the investigator reference range (when present), will flag out-of-range results.

9.7.4.1 Haematology and chemistry

At each time point absolute values of the haematology and chemistry variables will be summarized by treatment and time with n, mean, SD, SEM, median, Min, and Max values.

9.7.4.2 Urinalysis

The categorical data of the urinalysis will be summarized by treatment and time in frequency tables by variable.

8.8 Pharmacodynamic endpoints

8.8.1 Pharmacodynamics

The final analysis will be preceded by an administrative blind data review which consists of individual graphs per visit by time of all pharmacodynamic measurements by time. The graphs will be used to detect outliers and measurements unsuitable for analysis.

The PD parameters will be listed by treatment, subject, visit and time. Individual graphs by time will be generated.

All PD endpoints will be summarised (n, mean, SD, SEM, median, Min and Max values) by treatment and time, and will also be presented graphically as mean over time, with standard deviation as error bars. Both Nominal results, and log-transformed results and change from baseline results will be utilized in all data summaries.

All categorical PD endpoints will be summarised by frequencies.

Parameters will initially be analysed without transformation, but if the data suggest otherwise, log-transformation may be applied. Log-transformed parameters will be back-transformed after analysis where the results may be interpreted as percentage change.

To establish whether significant treatment effects can be detected on the repeatedly measured PD parameters, each parameter will be analysed with a mixed model analysis of covariance (ANCOVA) with treatment, time, and treatment by time as fixed factors, subject as random factor and the (average) baseline measurement as covariate.

Biopsy parameters will be analysed with a mixed model analysis of covariance (ANCOVA) with treatment as fixed factor and the baseline measurement as covariate.

The Kenward-Roger approximation will be used to estimate denominator degrees of freedom and model parameters will be estimated using the restricted maximum likelihood method.

The general treatment effect and specific contrasts will be reported with the estimated difference and the 95% confidence interval, the least square mean estimates and the p-value. Graphs of the Least Squares Means(LSM) estimates over time by treatment will be presented with 95% confidence intervals as error bars, as well as change from baseline LSM estimates.

The following contrasts will be calculated within the model:

- 2.5% CLS001 – vehicle
- 1% CLS001 – vehicle
- 2.5% CLS001 – 1% CLS001

- The following additional metrics will be analysed for each treatment. Proportion of patients who achieve a local SCORAD 0 ; 5 or 10 of target lesion at day 28 and EOS
- Percent improvement in BSA, local oSCORAD of target lesion, and 5-D pruritus scale from baseline to each visit

8.8.2 Inferential methods

The study is exploratory and no formal null hypothesis is set. No adjustments for multiple comparisons will be applied.

8.9 Exploratory analyses and deviations

Exploratory data-driven analyses can be performed with the caveat that any statistical inference will not have any confirmatory value.
Deviations from the original statistical plan will be documented in the clinical study report.

8.10 Interim analyses

No interim analysis is planned.

9 GOOD CLINICAL PRACTICE, ETHICS AND ADMINISTRATIVE PROCEDURES

9.1 Good clinical practice

9.1.1 Ethics and good clinical practice

The investigator will ensure that this study is conducted in full compliance with the protocol, the principles of the Declaration of Helsinki, ICH GCP guidelines, and with the laws and regulations of the country in which the clinical research is conducted.

9.1.2 Ethics committee / institutional review board

The investigator will submit this protocol and any related documents to an Ethics Committee (EC) and the Competent Authority (CA). Approval from the EC and the statement of no objection from the CA must be obtained before starting the study, and should be documented in a dated letter/email to the investigator, clearly identifying the trial, the documents reviewed and the date of approval. A list of EC members must be provided, including the functions of these members. If study staff were present, it must be clear that none of these persons voted.

Modifications made to the protocol after receipt of the EC approval must also be submitted as amendments by the investigator to the EC in accordance with local procedures and regulations.

9.1.3 Informed consent

It is the responsibility of the investigator to obtain written informed consent from each individual participating in this study after adequate explanation of the aims, methods, objectives and potential hazards of the study. The investigator 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 for any reason.

The Informed Consent and Subject Information will be provided in Dutch.

9.1.4 Insurance

The investigator has a liability insurance which is in accordance with article 7, subsection 6 of the WMO.
The investigator has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23rd June 2003). This insurance provides cover for damage to research subjects through injury or death caused by the study.

- € 450,000.- (i.e., four hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
- € 3,500,000.- (i.e., three million five hundred thousand Euro) for death or injury for all subjects who participate in the Research;
- € 5,000,000.- (i.e., five million Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the Sponsor as ‘verrichter’ in the meaning of said Act in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

9.2 Study funding

Cutanea Life Sciences is the sponsor of the study and is funding the study. All financial details are provided in the separate contract(s) between the CHDR, and the sponsor.

9.3 Data handling and record keeping

9.3.1 Data collection

Data will be recorded electronically on case report forms and will be entered after quality control in a Promasys database for subsequent tabulation and statistical analysis. The data will be handled confidentially and if possible anonymously.
A Subject Screening and Enrolment Log will be completed for all eligible or non-eligible subjects with the reasons for exclusion.

9.3.2 Database management and quality control

All data from the case report form will be entered into the database twice, by two different individuals. A quality control check will be done by CHDR staff using data entry progress checks and database listings (blind data review). Errors with obvious corrections will be corrected before database lock.
Results of clinical laboratory analyses will be sent electronically to CHDR and loaded into the database.
After the database has been declared complete and accurate, the database will be locked. Any changes to the database after that time can only be made by joint written agreement between the investigator, sponsor and the statistician.

9.4 Access to source data and documents

All study data will be handled confidentially. The investigator will retain the originals of all source documents generated at CHDR for a period of 2 years after the report of the study has been finalised, after which all study-related documents will be archived (at a minimum) on micro-film which will be kept according to GCP regulations. After 2 years the sponsor will be notified that the source documents can be retained with the sponsor or destroyed.
The investigator will permit trial-related monitoring, audits, EC review and regulatory inspections, providing direct access to source data and documents.

9.5 Quality control and quality assurance

This study will be conducted according to applicable Standard Operating Procedures (SOPs). Quality assurance will be performed under the responsibility of CHDR's Quality Assurance manager.

9.5.1 Monitoring

An initiation visit will be performed before the first subject is included. Monitoring visits and contacts will occur at regular intervals thereafter, according to a frequency defined in the study-specific monitoring plan. A close-out visit will be performed after study closure.

9.6 Protocol amendments

Any change to a protocol has to be considered as an amendment.

9.6.1 Non-substantial amendment

Administrative or logistical minor changes require a non-substantial amendment. Such changes include but are not limited to changes in study staff or contact details or minor changes in the packaging or labelling of study drug. Non-substantial amendments will be approved (signed) by the investigator(s) and will be recorded and filed by the investigator/sponsor but will not be notified to the EC and the CA.

The implementation of a non-substantial amendment can be done without notification to the appropriate EC or CA. It does not require their approval.

The following amendments will be regarded as non-substantial:

- change in timing of the samples;
- changes in assay-type and / or institution where an assay will be performed, provided that validated assays will be used;

- editorial changes to the volunteer information sheets;
- determination of additional parameters in already collected materials, which are in agreement with the study objectives and do not provide prognostic or genetic information;
- other statistical analyses than described in the protocol.

9.6.2 Substantial amendment

Significant changes require a substantial amendment. Significant changes include but are not limited to: new data affecting the safety of subjects, change of the objectives/endpoints of the study, eligibility criteria, dose regimen, study assessments/procedures, treatment or study duration, with or without the need to modify the core Subject Information and Informed Consent Form.

Substantial amendments are to be approved by the appropriate EC and the CA will need to provide a 'no grounds for non-acceptance' notification prior to the implementation of the substantial amendment.

Urgent amendment

An urgent amendment might become necessary to preserve the safety of the subjects included in the study. The requirements for approval should in no way prevent any immediate action being taken by the investigators in the best interests of the subjects. Therefore, if deemed necessary, an investigator can implement an immediate change to the protocol for safety reasons. This means that, exceptionally, the implementation of urgent amendments will occur before submission to and approval by the EC(s) and CA.

9.7 End of study report

The sponsor will notify the EC and the CA of the end of the study within a period of 90 days. The end of the study is defined as the last subject's last visit.
In case the study is ended prematurely, the investigator will notify the EC and the CA within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator will submit a final study report with the results of the study, including any publications/abstracts of the study, to the EC and the CA. The principal investigator and the sponsor's representative will be the signatories for the study report.

9.8 Public disclosure and publication policy

In accordance with standard editorial and ethical practice, the results of the study will be published, Conditions are subject to a separate contract between CHDR and the sponsor. The authorship guidelines of the Vancouver Protocol[1] will be followed regarding co-authorship.
In accordance with standard editorial and ethical practice, the results of the study will be published. The authorship guidelines of the Vancouver Protocol[2] will be followed regarding co-authorship.

The principal investigator will have the opportunity to review the analysis of the data and to discuss with the sponsor the interpretation of the study results prior to publication.

Any study-related article or abstract written independently by investigators should be submitted to the sponsor for review at least 60 days prior to submission for publication or presentation.

The list of authors of any formal publication or presentation of study results may include, as appropriate, representatives of the sponsor and will be determined by mutual agreement.

[1] http://www.icmje.org/

[2] http://www.icmje.org/

References

1. Grether-Beck S, Felsner I, Brenden H, Kohne Z, Majora M, Marini A, Jaenicke T, Rodriguez-Martin M, Trullas C, Hupe M, Elias PM, Krutmann J. Urea uptake enhances barrier function and antimicrobial defense in humans by regulating epidermal gene expression. *J Invest Dermatol.* 2012; 132: 1561-1572.

2. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. *Arch.Dermatol.* 1988; 124: 869-871.

3. Elman S, Hynan LS, Gabriel V, Mayo MJ. The 5-D itch scale: a new measure of pruritus. *Br J Dermatol.* 2010; 162: 587-593.

4. Chen H, Common JE, Haines RL, Balakrishnan A, Brown SJ, Goh CS, Cordell HJ, Sandilands A, Campbell LE, Kroboth K, Irvine AD, Goh DL, Tang MB, van Bever HP, Giam YC, McLean WH, Lane EB. Wide spectrum of filaggrin-null mutations in atopic dermatitis highlights differences between Singaporean Chinese and European populations. *Br J Dermatol.* 2011; 165: 106-114.

5. Sandilands A, Terron-Kwiatkowski A, Hull PR, O'Regan GM, Clayton TH, Watson RM, Carrick T, Evans AT, Liao H, Zhao Y, Campbell LE, Schmuth M, Gruber R, Janecke AR, Elias PM, van Steensel MA, Nagtzaam I, van GM, Steijlen PM, Munro CS, Bradley DG, Palmer CN, Smith FJ, McLean WH, Irvine AD. Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis vulgaris and atopic eczema. *Nat Genet.* 2007; 39: 650-654.

6. Atkinson MJ, Sinha A, Hass SL, Colman SS, Kumar RN, Brod M, Rowland CR. Validation of a general measure of treatment satisfaction, the Treatment Satisfaction Questionnaire for Medication (TSQM), using a national panel study of chronic disease. *Health Qual.Life Outcomes* 2004; 2: 12.

7. Atkinson MJ, Kumar R, Cappelleri JC, Hass SL. Hierarchical construct validity of the treatment satisfaction questionnaire for medication (TSQM version II) among outpatient pharmacy consumers. *Value Health* 2005; 8 Suppl 1: S9-S24.