CLINICAL STUDY PROTOCOL

Prot. no: E.HU.027-0080.05.004L_2018/2278

"A randomized, double blind, placebo controlled clinical study for evaluating the emollient, hydrating and soothing efficacy of a food supplement intended for subjects with atopic dermatitis".

Principal investigator:	Dr Enza Cestone, MD, Specialist in Dermatology and Venereology
Co-investigator:	Dr Marta Pisati, Efficacy research, Biologist
Sponsor:	Roelmi HPC Srl
In-site study Director and Quality Manager:	Dr Angela Michelotti, Biologist,
Study site:	COMPLIFE ITALIA Srl Via Mons. Angelini, 21 27028 San Martino Siccomario (PV)

FINAL VERSION NO. 1– 06th July 2018

GENERAL INFORMATION

TITLE:		
A Randomized, double blind, placebo controlled clinical study for evaluating the emollient, hydrating and soothing efficacy of a food supplement intended for subjects with atopic dermatitis.		
PROTOCOL NO. AND VERSION		
E.HU.027-0080.05.004L_2018/2278- VERSION NO. 1 – 06th July 2018		
SPONSOR:		
NAME AND ADDRESS:	**PERSONS AUTHORIZED TO SIGN THE PROTOCOL AND ITS AMENDMENTS:**	**MEDICAL STAFF:**
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MONITOR:		
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FACILITY:		
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OTHER LABORATORIES		
None		
OTHER DEPARTMENTS		
Not applicable		

PROTOCOL APPROVAL

INVESTIGATOR SIGNATURE

I have read the protocol E.HU.027-0080.05.004L_2018/2278- VERSION NO. 1 – 06th July 2018 "Randomized, double blind, placebo controlled clinical study for evaluating the emollient, hydrating and soothing efficacy of a food supplement intended for subjects with atopic dermatitis" and I agree. I am aware of my responsibilities as an Investigator under the declaration of Helsinki, local regulations and the study protocol. I agree to conduct the study according to these guidelines and to appropriately direct and assist the staff under my control, who will be involved in the study.

For and on behalf of the Study Sponsor:

Signature

Federica Carlomagno

R&D Manager

Date

10 / 07 / 2018

Study director and quality control:

Signature

Dr Angela Michelotti

General Manager

Date

10 / 07 / 2018

Principal Investigator:

Signature

Dr Enza Cestone, MD

Dermatologist

Date

10 / 07 / 2018

SUMMARY

1. PROTOCOL AMENDMENTS HISTORY

The table here below reports the list of the amendments to the protocol.

Amendments no.	Protocol vers.	Date	Author	Description
	1	06/07/2018	Enza Cestone Angela Michelotti	First drafting

2. BACKGROUND

Frequently encountered in clinical practice, in the last 30 years, the prevalence of AD (Atopic Dermatitis) has rapidly increased as a result of industrialization.

Thus, a new wave of quests for the treatment and prevention of AD has pushed forward. Hallmarks of atopic dermatitis include dry, itchy skin and red rashes that come and go. The rashes can occur on any part of the body, although the pattern tends to be different at different ages.

A large number of studies have explored the potential efficacy of probiotics in the prevention and treatment of AD (Pessi et al., 2000; Kalliomaki et al., 2001; Ouwehand et al., 2002; Hattori et al., 2003; Matsumoto et al., 2007; Park et al., 2008; Savilahti et al., 2008; Wickens et al., 2008; Adams, 2010; Batchelor et al., 2010; Chapman et al., 2011; Wickens et al., 2012; Morgan et al., 2014), yet the picture remains unclear and conflicting: several clinical studies show improvement in the severity of atopic dermatitis after taking probiotics but a strong evidence to support the effectiveness of the administration of probiotics at a clinical level remains elusive (Meninghin et al., 2012; Foolad and Armstrong, 2014).

Given that the gut bacterial flora is very different in patients with atopic dermatitis, the dysbiosis seems to be an essential step of dermatitis occurrence. In fact, recent studies have shown the importance of the gut-skin axis which link the gut dysbiosis to skin inflammation. Basically, gut dysbiosis can influence gut absorption allowing toxins or inflammatory agents going into the blood stream, so to the skin.

A paper in 2001 showed that the probiotic strain **Lactobacillus rhamnosus** GG reduced the incidence of atopic dermatitis in at-risk infants through the age of 7 years [Alliomaki 2001]. A randomized, double-blind, placebo-controlled study investigated the effects of the use of the **L. plantarum CJLP133** strain in the prevention of AD symptoms. The study was performed for a time period of 12 weeks among children between 1 to 12 years old. It was found that there was an improvement in AD scores (SCORAD), with a concomitant decrease in IFN-γ, eosinophil, and Interleukin-4 counts (Han et al., 2012).

Out thinking is that, the clinical administration of probiotics may become more widespread if the remaining questions are answered with strong evidence: what type of probiotic strain should be used? What dosage and time of administration should be used? This randomized, double-blinded and placebo-controlled study was designed to evaluate the efficacy and safety of a probiotic mix (L. plantarum, L. rhamnosus, L. reuteri) in the treatment of AD symptoms in an adult population.

3. OBJECTIVES

The study is aimed to assess the efficacy and the safety of a food supplement claimed to have an effect AD symptoms. In order to reach such goal a clinical study is carried out on 80 male and female subjects (n=40 subjects in the active group and n=40 in the placebo group according to a previously predisposed randomisation list) showing a mild – moderate score od AD diagnosed by SCORAD.

Study product efficacy is evaluated after 28 and 56 days of its daily uptake and 28 days after the end of treatment by means of SCORAD score calculation and by measuring skin moisturization and Trans Epidermal Water Loss using non-invasive bioengineering techniques. The study is then integrated with the dermatologist clinical analysis and the subjects self-assessment.

3.1. Primary objectives

The aim of this randomized double blinded active treatment vs placebo study is to evaluate the clinical efficacy of the intake of a combination of three probiotics for the treatment of adult AD patients.

3.2. Secondary objectives

Secondary objective of this study is the confirmation of the safety of use of the product.

4. STUDY DESIGN

This study will be carried as follows: single centre, randomized, double-blind, placebo-controlled, parallel group study.

4.1. Population characteristics

It is planned to enroll 80 male and female subjects showing a mild to moderate SCORAD (between 15 and 25 at the inclusion). Subjects are enrolled only if they satisfy all the inclusion/non-inclusion criteria reported in the sections 5.
Subjects will be randomly attributed to product or placebo treatment: 40 subjects for treatment.

4.2. Study structure

The study will be carried out by Complife Italia Srl, placed in Via Monsignor Angelini, 21 - 27028 San Martino Siccomario (PV) - Italy.

The principal investigator is Dr. Enza Cestone, Medical and Chirurgical Physician, Specialist in Dermatology and Venereology. The co-investigator is: Dr. Marta Pisati, Biologist, Efficacy Research, Nutritionist. The in site Study Director and Quality Manager is Dr. Angela Michelotti, Biologist.

5. STUDY POPULATION

A total of 80 male and female subjects will be enrolled. Withdrawn/lost to follow-up/drop-out subjects will not be replaced.

All inclusion and non-inclusion criteria will be checked by the principal investigator or delegate (co-investigator), through a questionnaire during the screening visit.

5.1. Inclusion criteria

- ✓ Good general health
- ✓ Female or male sex
- ✓ Photoype I to IV
- ✓ Age more than 18 years old
- ✓ Mild to moderate SCORAD (between 15 and 25)
- ✓ Subjects who have not been recently involved in any other similar study
- ✓ Willingness to follow the proposed alimentary supplement for all the study time
- ✓ Willingness to use for body care only the cream that will be consigned at the beginning of the study
- ✓ Willingness to submit to before and after pictures
- ✓ Willingness to use during all the study period only the product to be tested
- ✓ Willingness to not use products likely to interfere with the product to be tested
- ✓ Willingness to not vary the normal daily routine (i.e. lifestyle, physical activity, etc.)
- ✓ Subject is under effective contraception (oral/not oral); not expected to be changed during the trial
- ✓ Subject aware of the study procedures and having signed an informed consent form

5.2. Non-inclusion criteria

- ✓ Current antibiotic administration
- ✓ Known history of chronic medical condition such as congenital heart disease, liver or kidney disease, or immune deficiency
- ✓ Treatment with probiotics in the 6 months preceding enrollment
- ✓ Treatment with steroids and antihistamines systemically in the three months prior to enrollment
- ✓ Topical treatments with immunomodulators (tacrolimus or pimecrolimus) in the three months prior to enrollment
- ✓ Acute or chronic infectious diseases
- ✓ Pre-existing hypersensitivity to components contained in the probiotic
- ✓ Subject do not meet the inclusion criteria
- ✓ Pregnant woman or women intending to became pregnant during the study
- ✓ Breastfeeding women
- ✓ Subjects who has used sun-beds or self-tanning product for one month before the study or intend to use it during the present study
- ✓ Any condition that the principal investigator deems inappropriate for participation
- ✓ Adult protected by the law (under guardianship, or hospitalized in a public or private institution, for a reason other than the research, or incarcerated).
- ✓ Volunteer unable to communicate or cooperate with the Investigator due to language problems, poor mental development, or impaired cerebral function.

5.3. Subject withdrawal criteria

In compliance with the Helsinki Declaration (1964) and its successive, subjects have the right to exit from the study at any time and for any reason. In all cases, the Investigator should attempt to contact the subject as soon as possible for a final assessment in order to: i) have the subject's decision written on the consent form, ii) obtain the reason(s) for their withdrawal so they can be recorded, iii) evaluate the subject's clinical condition, iv) if necessary, take appropriate therapeutic measures (management of an AE or concomitant disease), v) recover the investigation product given to the subject.

The investigator can also interrupt the subject participation in the study prematurely in the case of a disease occurrence, a pregnancy or the occurrence of an adverse reaction or a serious adverse event, particularly if it is considered by the Investigator liable to threaten the health of the subject or if necessitates the prescription of a medication incompatible with the pursuit of the study. In this case, the Sponsor will be informed by phone or fax and a letter or report explaining the withdrawal will also be forwarded to him as soon as possible. Any premature discontinuation linked to an AE or a SAE will have to be followed-up (until final outcome). The Sponsor can demand that any subject be excluded from the study for major infringements to the protocol, for administrative reasons or any other motive. Nevertheless, premature removal of a high percentage of subjects from the study can make it difficult

or impossible to interpret. Consequently, any premature exit without valid reasons should be avoided as much as possible and is carefully documented in the case report form, the final report and, if necessary, in the AE form. Every premature exit must be classified as follows: i) presence of a non-inclusion criteria, ii) AE occurrence, iii) SAE occurrence, iv) withdrawal of consent, v) lost to follow-up, vi) appearance of non-inclusion criteria, vii) non-adherence to the protocol, viii) other reason (to be clearly specified).

5.4. Subject discontinuation

The subjects are entitled to discontinue the study for any reason at any time if they desire. Should this occur, the investigator or designee determines the reasons in order to know if it is linked to the study or not and the primary reason will be recorded in the Case Report Form (CRF). If the subject has withdrawn due to Serious Adverse Event (SAE), the subject will be followed until Serious Adverse Event (SAE) resolution.
In the case where subject does not present for a visit, the investigator or designee must attempt to contact the subject by telephone on two consecutive occasions. The subject will be considered as lost to follow-up if the investigator or designee fails to reach him/her. These attempts and the result must be recorded on source document.

5.5. Study completion

The study completion will be achieved by a subject when she will have performed the entire treatment and the check visits included the follow-up visit.

5.6. Subjects risk and benefit

Risks associated with the products intake are considered from low to very low, in absence of allergy/intolerances to product ingredients; other ingredients in the product formula are commonly used in dietary supplements.
All the instrumental measurements carried out are not invasive and no skin side effects are expected from the measurement process.
Benefits associated with product use are amelioration of skin imperfection due to AD.

6. STUDY FLOW CHART

The study duration is 56 days (8 weeks). Clinical visits are planned after 28 (T_{28d}) and 56 days (T_{56d}) of product/placebo use. A follow-up visit 28 days after the last intake of the product is planned.

6.1. Study schedule

Study schedule is as follows:

Study phases	Initial visit Start of the study (T0)	Intermediate Visit (T28)	Final visit (T56)	Follow-up (T84)
Sign Informed consent	X	---	---	---
Subject eligibility	X	X	X	X
Clinical assessment-safety of use	-	X	X	X
Clinical evaluation - SCORAD	X	X	X	X
Clinical evaluation of skin smoothness	X	X	X	X
Skin moisturization	X	X	X	X
Skin TEWL	X	X	X	X
Skin stripping	X	X	X	X
Self-assessment questionnaire	-	-	X	X
Alimentary diary distribution	X	X	X	-
Alimentary diary collection	-	X	X	X
Product distribution	X	-	-	-
Unused product collection	-	-	X	-

6.1.1. Screening – Initial visit

Subjects will be screened as follows:

- screening in the Complife Italia volunteers database*. The subjects identified by Complife Italia volunteers management database screened by appropriate personnel (authorized by the investigator, pursuant to and

for the effects of the legislation on protection of personal data). Screened subjects are then invited to participate to the study and it is make the date for the screening visit;

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* The database will be used only for screening purposes, without storing additional data that can allow the identification of the subject as a potential participant in the clinical study.

During the screening visit (T0)the principal investigator or her designee evaluates if the subject is eligible to participate in the study. The following procedures will be carried out:

- signature of the Informed Consent Form
- recording of the subject demographic data
- checking of the subject's medical history and previous and concomitant therapies
- checking of the inclusion/non-inclusion criteria
- Clinical evaluation – SCORAD
- Clinical evaluation – Skin smoothness
- Instrumental evaluations
- Skin stripping
- supplying the product / placebo in accordance with the randomization list
- supplying of an alimentary diary
- fixing the date of the 1st check visit after 28 days of treatment

6.1.2 1st check visit (T28)

The following procedures will be carried out:

- checking of subject eligibility
- Clinical assessment – safety of use
- Clinical evaluation – SCORAD
- Clinical evaluation – Skin smoothness
- Instrumental evaluations
- Skin stripping
- Collection of the Alimentary Diary and supplying a new one
- fixing the date of the final check visit after 56 days of treatment

6.1.3. Final check visit (T56)

The following procedures will be carried out:

- checking of subject eligibility
- Clinical assessment – safety of use
- Clinical evaluation – SCORAD
- Clinical evaluation – Skin smoothness
- Instrumental evaluations
- Skin stripping
- Collection of the Alimentary Diary
- filling of the self-assessment questionnaire
- Unused product collection
- fixing the date of the follow-up visit 28 days after the last product intake

6.1.4. Follow-up visit (T84)

The following procedures will be carried out:

- checking of subject eligibility
- Clinical assessment – safety of use
- Clinical evaluation – SCORAD
- Clinical evaluation – Skin smoothness
- Instrumental evaluations
- Skin stripping
- filling of the self-assessment questionnaire

7. TREATMENT

7.1. Products

7.1.1. Qualitative and Quantitative formula

Product to be tested:

Subjects will be administered with capsules of food supplement or placebo having the following composition:

product:

FORMA	INGREDIENTI	mg/CPS	COMPOSIZIONE %	Bill/CPS (dichiarati)
Capsula size 3* (100,00 mg)	*Lactobacillus plantarum* 500 Bill/g	14,00	14,00	1**
	Lactobacillus rhamnosus 300 Bill/g	24,00	24,00	1**
	Lactobacillus reuteri 200 Bill/g	35,00	35,00	1**
	Amido di mais	26,00	26,00	
	Magnesio stearato vegetale	1,00	1,000	
Totale		100,00	100,00	

* weight of the operculum 50.00 mg
** Bill=Bilions; probiotic mixtures have been overdosed with 5x

placebo:

FORMA	INGREDIENTI	mg / CPS	COMPOSIZIONE %
Capsula size 3* (100,00 mg)	Amido di mais	99,00	99,00
	Magnesio stearato vegetale	1,00	1,000
Totale		100,00	100,00

* weight of the operculum 50.00 mg

7.1.2. Products use

Product to be tested:

Subjects will take 1 capsule per day with a drink of water between meals

Moisturizing Cream,

A moisturizing cream will be supplied to all volunteers, who are instructed to use just it throughout the study period.

Volunteers will be instructed to use the cream as a substitute of their usual body cream, ie whenever they were used to apply it.

The supplied cream is a cosmetic formulation selected on the market whose composition is conform to Regulation (EC) 1223/2009.

7.1.3. Product supply, labeling, storage and accountability

7.1.3.1. Supply

Products will be supplied to COMPLIFE ITALIA srl by the Sponsor.

The shipment address is:

COMPLIFE ITALIA srl
Via Mons. Angelini, 21
27028 San Martino Siccomario (Pavia) - Italy

Contact person: dr. Angela Michelotti - T. +39 0382 25504

7.1.3.2. Labeling

Product and placebo will be packed in the same packaging. No obvious differences will be in the products pack. The products will be labeled as shown in figure 1.

Figure 1. Products label.

IT VERSION

INTEGRATORE ALIMENTARE
Prot. no: E.HU.027-0080.05.004L_2018/2278

SOGGETTO N° ------->
MODO D'USO: Assumere la capsula con un sorso d'acqua lontano dai pasti

AVVERTENZE: TENERE AL RIPARO DA FONTI DI LUCE E A TEMPERATURA <30°C, TENERE AL DI FUORI DALLA PORTATA DEI BAMBINI, NON ASSUMERE IL PRODOTTO OLTRE LA DOSE GIORNALIERA CONSIGLIATA (1 CAPSULA)

7.1.3.3 Storage

Product and placebo will be stored at room temperature at COMPLIFE ITALIA srl, protected from direct light, heat and source of water safe place with restricted access.

7.1.3.4 Accountability

The principal investigator and his collaborators will maintain a record of the products delivered to the subjects at the study starting and received by the subjects at the study ending.

7.1.3.5. Compliance to treatment

The compliance to treatment will be assessed by the principal investigator by asking the subject specific questions aimed to assess the actual use of the product.
The principal investigator may withdraw the subject in case of suspicion and/or if he has the evidence that the subject was not compliant to the treatment regimen.

7.1.4. Randomization

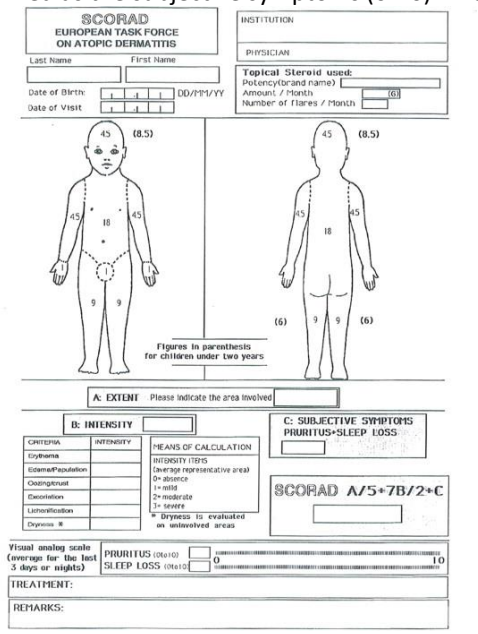
A restricted randomization list will be generated by the in site Study Director using an appropriate statistic algorithm ("Wey's urn"). For each subject participating in the study will be prepared an envelope containing the information on the product tested. Both the randomization list and the subjects envelopes will be stored by the in site Study Director under appropriate safety conditions in a place that is not accessible neither to volunteers nor to the experimenter.

8. EFFICAY ENDPOINTS AND EVALUATIONS

Parameters below reported are assessed under controlled ambient conditions (T = 22±2°C and RH = 40-60%).
Subjects are left to acclimatize to ambient condition for 15-20 minutes before the check visit.

8.1. SCORAD

The European Task Force on Atopic Dermatitis (ETFAD) has developed the SCORAD (SCORing AD) index to create a consensus on assessment methods for AD, so that study results of different trials can be compared. To measure the extent of AD, the rule of nines is applied on a front/back drawing of the patient's inflammatory lesions. The extent can be graded 0-100. The intensity part of the SCORAD index consists of six items: erythema, oedema/papulation, excoriations, lichenification, oozing/crusts and dryness. Each item can be graded on a scale 0-3. The subjective items include daily pruritus and sleeplessness. Both subjective items can be graded on a 10-cm visual analogue scale. The maximum subjective score is 20. All items should be filled out in the SCORAD evaluation form. The SCORAD index formula is: A/5 + 7B/2 + C. In this formula A is defined as the extent (0-100), B is defined as the intensity (0-18) and C is defined as the subjective symptoms (0-20). The maximum SCORAD score is 103.



8.2. Skin smoothness

Skin smoothness is clinically evaluated by the dermatologist or her collaborators in accordance with the clinical scores reported in table A

Table A - Clinical classification of skin smoothness	Score
Not smooth skin	**1**
Little smooth skin	**2**
Smooth skin	**3**
Well smooth skin	**4**

8.3. Evaluation of the skin moisturization

The measurement of skin moisturization is based on the Corneometer® method. Corneometer® method is based on the dielectric constant of water. The probe shows changes of capacitance according to the moisture content of the skin. An electric scatter field penetrates the very first layers of the skin (10-20 µm) and determines the dielectricity. The used device is the Corneometer® CM 825 (Courage+Khazaka, electronic GmbH)..

8.4. Evaluation of the skin Trans Epidermal Water Loss

Transepidermal water loss is measured indirectly using a Tewameter® TM 300 (Courage+Khazaka, electronic GmbH). The measurement is based on the diffusion law, as described by the equation here below:

$$\frac{dm}{dt} = -D \cdot A \cdot \frac{dp}{dx}$$

where: A is the surface in m² | m is the water transported (in g) | t is the time (h) | D is the diffusion constant (0.0877 g/m(h(mm Hg)) | p is the vapor pressure of the atmosphere (mm Hg) | s is distance from skin surface to point of measurement (m)

The diffusion flow dm/dt indicates the mass of water, which is transported per cm² in a specific period. It is proportional to the area A and the change of concentration per distance (dc/dx). D is the diffusion coefficient of water vapor in the air. The resulting density gradient is measured indirectly by two pairs of sensors (temperature and relative humidity) and is analyzed by a microprocessor. The measuring head of the probe is a narrow hollow cylinder (10 mm diameter and 20 mm height), in order to minimize influences of air turbulence inside the probe.

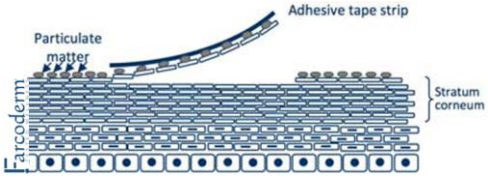
8.5. Self-assessment questionnaire

At the end of the treatment period and at the end of the follow-up period the subjects will be asked to express their personal opinion on the treatment by answering to a questionnaire about product acceptability and effects.

8.6. Cytokine expression

Skin stripping is performed using Corneofix® (Courage+Khazaka). This technique allows to take serial layers of the stratum corneum. In accordance with the standard operative procedure, skin stripping is performed using a device that allows to standardize the pressure applied on the stripping. Ten strips are collected for each study time for each volunteer on a selected skin area.

Skin stripping technique. Different layers of the stratum corneum are collected using an adhesive tape strip (Corneofix).



The following Cytokines could be evalued (to be decided after clinical and instrumental parameters analysis) on the skin strippings extracts:

- scTARC (Thymus and activation-regulated chemokine in the stratum corneum) or IL-8 as biochemical indicator of the severity of skin lesions
- scTSLP (Cytokine thymic stromal lymphopoietin in the stratum corneum) as trigger factor in the initiation, development and progression of atopy and atopic diseases
- scTNFalpha (Tumor Necrosis Factor alpha in stratum corneum) as master cytokine regulator of inflammatory status

9. SAFETY ENDPOINTS AND EVALUATIONS

Tolerability of the treatment will be closely followed by the study principal investigator during the course of the study. Subjects will have access to the investigators in case of intolerance reactions via a contact phone number provided with the informed consent form. If a subject reports an event, the principal investigator has to decide if it is pertinent or not. If yes, she will report it as a cosmetic or an adverse event.

Any unexpected related side effect judged as severe by the principal investigator will be reported to the Sponsor. Upon investigator judgment, the subject may be withdrawn from the study and the side effect will be followed until resolution (maximum until the end of the study).

9.1. Adverse Events (AE) and Serious Adverse Events (SAE)

9.1.1. Definition of Adverse Event (AE)

An Adverse Event is any untoward medical occurrence in a clinical investigation subject administered a test product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a test product, whether or not related to the test product.

9.1.2. Definition of Serious Adverse Event (SAE)

A Serious Adverse Event is any untoward medical occurrence that: i) results in death, ii) is life-threatening, iii) requires inpatient hospitalization or prolongation of existing hospitalization, iv) results in persistent or significant disability/incapacity, or v) is a congenital anomaly/birth defect

9.1.3. Documentation of AE and SAE

All concomitant treatments are reported in the data collecting sheet and the study report. All Adverse Events likely to be related to the studied product (adverse reactions) are reported in the data collecting sheet and the study report. All Serious Adverse Events are reported in the data collecting sheet and the study report.

9.1.4. Notification to the Sponsor

9.1.4.1. Notification of reaction to the Sponsor

AEs occurring during the study or after the study must be reported to the Sponsor's vigilance officer by email with a copy to the project manager. SAE must be send within 24 hours after the observation. Reactions related to the product must be reported as soon as possible. If picture of the reactions are available, they should be enclosed with the notification.

9.1.5. Follow-up

SAE and reactions related to the product must be followed up until resolution or stabilization. To inform Sponsor's vigilance officer of any new information the investigator must use the appropriate forms filled in with results collected from the examination carried out. Reports of hospitalization must be enclosed with the notification form.

9.2. Local tolerance

The Dermatologist Investigator must be the same throughout the study for each subject. The skin tolerability of the product will be closely followed by the study principal investigator and her collaborator during the study period. Subjects will have access to the investigator in case of intolerance reactions via a contact phone number provided with the study information sheet. If a subject reports an event, the principal investigator has to decide if it is related to the product or not. If yes, he will report it as an intolerance. Any unexpected related side effect judged as severe by the principal investigator will be reported to the Sponsor. Upon investigator judgment, the subject may be withdrawn from the study and the side effect will be followed until resolution.

9.2.1. Local tolerance assessment

A table summarizes the signs reported/observed for each subject. Local tolerability reactions, both subjective and objective, are scored according to a clinical score scale (Fig. 3). Each time a sign (physical or functional) appears (new sign or worsened compared to the previous evaluation), the investigator has to judge its causality with the use of the tested product.

Figure 3 Clinical scoring system of local tolerance reaction.

Physical	0: none	1: very mild	2: mild	3: moderate	4: severe
Erythema	☐	☐	☐	☐	☐
Oedema	☐	☐	☐	☐	☐
Dryness	☐	☐	☐	☐	☐
Desquamation	☐	☐	☐	☐	☐
Roughness	☐	☐	☐	☐	☐
Crackings	☐	☐	☐	☐	☐
Vesicule	☐	☐	☐	☐	☐
Papule	☐	☐	☐	☐	☐
Others:	☐	☐	☐	☐	☐
Please specify:.....					
Functional	0: none	1: very mild	2: mild	3: moderate	4: severe
Tightness	☐	☐	☐	☐	☐
Stinging/prickling	☐	☐	☐	☐	☐

Itching/pruritus	☐	☐	☐	☐	☐
Warm sensation	☐	☐	☐	☐	☐
Burning sensation	☐	☐	☐	☐	☐
Pain	☐	☐	☐	☐	☐
Others:	☐	☐	☐	☐	☐

Please specify:.....

For each sign, intensity, location, duration (hours, minutes), frequency are recorded. Moreover, the investigator collects all discomfort or reactions reported by the subjects. Each time a sign (physical or functional) appears (new sign or worsened compared to the baseline evaluation i.e evaluation at day 1), a reaction has to be recorded. For moderate or severe physical signs and/or for each relevant reaction (>2), pictures have to be taken. All the reactions observed by the dermatologist and reported by the subject are recorded.

The following information is recorded: i) subject characteristics, ii) details about study product (product code or name, date of first application, application modalities), iii) description of the reaction (functional and physical signs, intensity of the signs, location, date/time of onset, timeframe between product application and onset of the reaction, date/time of end or duration (hours, minutes), frequency, diagnosis/nature of the reaction), iv) significant medical history , v) concomitant events: cutaneous diseases (atopic dermatitis flare), medical treatments, sunscreen product application, food, external factors (weather conditions), other diseases, vi) outcome and actions taken (application modalities modification, temporary interruption, definitively discontinuation, medical treatment, care), and viii) relationship to the product (study product and/or associated product) (causality assessment): analysis of the probability that the reaction is attributable to the product(s) used in the study. This assessment has to be done in conjunction with clinical expertise, knowledge of the product (type of product, conditions of use…), identification of concomitant events.

9.2.2. Causality assessment of local tolerance

Three levels of causality can be described.

9.2.2.1. Very likely/likely

Very likely

Clinical signs suggest a link with the product; the reaction follows a definite reasonable temporal sequence from the time of the product application and rechallenge is positive

Likely

Clinical signs suggest a link with the product; the reaction follows a definite reasonable temporal sequence from the time of the product application and there hasn't been a rechallenge or results of rechallenge are ambiguous. Or Clinical signs suggest a link with the product; the reaction follows a partially reasonable or unknown temporal sequence from the time of the product application and rechallenge is positive. Or Clinical signs only partially suggest or do not suggest a link with the product, the reaction follows a definite reasonable temporal sequence from the time of the product application and rechallenge is positive.

9.2.2.2. Not clearly attributable / Unlikely

Not clearly attributable

Clinical signs suggest a link with the product; the reaction follows a definite reasonable temporal sequence from the time of the product application and rechallenge is negative. Or clinical signs suggest a link with the product; the reaction follows a partially reasonable or unknown temporal sequence from the time of the product application and there hasn't been a rechallenge or results of rechallenge are ambiguous. Or clinical signs only partially suggest or do not suggest a link with the product; the reaction follows a definite reasonable temporal sequence from the time of the product application and there hasn't been a rechallenge or results of rechallenge are ambiguous. Or clinical signs only partially suggest or do not suggest a link with the product; the reaction follows a partially reasonable or unknown temporal sequence from the time of the product application and rechallenge is positive.

Unlikely

Clinical signs suggest a link with the product; the reaction follows a partially reasonable or unknown temporal sequence from the time of the product application and rechallenge is negative. Or: clinical signs only partially suggest or do not suggest a link with the product; the time sequence between use of the product and occurrence of the symptoms is compatible; and rechallenge is negative. Or clinical signs only partially suggest or do not suggest a link with the product; the reaction follows a partially reasonable or unknown temporal sequence from the time of the product application and there hasn't been a rechallenge or results of rechallenge are ambiguous. Or clinical signs only partially suggest or do not suggest a link with

the product; the reaction follows a partially reasonable or unknown temporal sequence from the time of the product application and rechallenge is negative.

9.2.2.3. Excluded

Excluded

Causality can only be excluded if another aetiology has been medically validated or when time sequence between exposure and signs occurrence is incompatible.

If necessary, in case of adverse events, subjects can also contact the Investigation Centre. If required, they would be assessed by the Dermatologist who would perform the clinical assessment and decide the appropriate measures to take (i.e. medical treatment, withdrawal …).

For each reaction with a physical sign with an intensity of 3 (moderate) and higher and/or for each relevant reaction, photographs will be taken, and joined to results at the end of the study.

10. STATISTIC

10.1. Study population for Analysis

A total of 80 subjects will be enrolled in the study, and randomly assigned to product or placebo treatment.

Efficacy data analysis is based on the Per Protocol Population. In addition, an Intent-To-Treat (ITT) analysis may be carried out. ITT population is defined as any subject have been assigned a subject number, received the study treatment and has at least one efficacy evaluation.

The per-protocol (PP) population is defined as all subjects who will complete the study without any major protocol violations. Subjects will be excluded from the per-protocol population if:

- they miss the evaluation visit after 28 or 56 days of product use;

or

- they do not used the product properly during the study period.

Analysis of safety will be based on the Safety Population that is defined as all subjects that have been assigned a subject number and received the study treatment.

Analysis of questionnaire is reported as percentage of subjects giving a certain answer to each question.

10.2. Descriptive analysis

Demographic variables (age, sex, etc.) will be reported. Data will be summarized using frequency distributions (number and percentage) for categorical/ordinal variables. For continuous variables the following value will be calculated:

- the mean value
- the minimum value
- the maximum value
- the standard error of the mean (SEM)
- the individual variation/the individual percentage variation
- the mean variation/ the mean percentage variation

10.3. Statistical methods

The instrumental data are submitted to ANOVA test followed by Tukey-Kramer post test (intra-group analysis); the inter-group statistical analysis is made on the data variations versus T0 by means of Bilateral Student's Test t for unpaired data.

The following comparison is carried out for each parameter:

-Intra-group comparison: comparison of all experimental times vs T0

-Inter-group comparison: comparison of the two study groups at all experimental times.

Results of the safety evaluation will be based on AE/SAE listing, and will be presented descriptively as absolute and relative frequencies.

The statistical software used for statistical analysis is: NCSS 10 statistical software (NCSS, LLC. Kaysville, Utah, USA) running on Windows Server 2008 R2 Standard (Microsoft, USA).

11. STUDY MANAGEMENT

11.1. Data recording of Study Data

The medical records/medical notes, etc., will be clearly marked and will permit easy identification of a subject's participation in the specified clinical trial. The principal investigator will record manually all data with respect to protocol procedures, safety data and efficacy ratings related to the treatment on the data collecting sheet.

The investigator may delegate the authority to fill the data collecting sheet to appropriately qualified staff to complete data collecting sheet, by authorizing and completing the signature log.

11.2. Source Data Verification

The Investigator must, as a minimum, review and sign all SAE forms, and the data collecting sheet to attest the accuracy and completeness of all the data. All corrections on data collecting sheet and on source documents must be made by the originator (or authorized delegate) in a way that does not obscure the original entry. The correct data must be inserted, dated and initialed/authorized by study site personnel. If it is not obvious why a change has been made, a reason must be provided.

11.3. Data Quality

The entire file (protocol, results, final reports and study-related documents) is subject to quality assurance procedures in compliance with regulatory requirements. The investigating laboratory authorizes the inspections by the Regulatory Body and the audit or the control by the Sponsor and allows them to access to raw data.

11.4. Data Management

The investigator will allow direct access to all relevant files (for all subjects) for the purpose of verifying entries made in the data collecting sheet, and assist with the monitor's activities, if requested.

The subject must have consent to their records being viewed by sponsor-authorized personnel, and by local and possibly foreign Competent Authorities. This information should be included in the informed consent documents.

Data must be entered onto collecting data sheet. All forms must be completed in blue ballpoint pen. All study documents must provide adequate verification of the content of the collecting data sheet.

Definition of source data and source documents are given below:

Source Data:	All original records and certified copies of original records of clinical findings, observations, or other activities necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies)
Source Documents:	Original documents, data and records (subject file, collecting data sheet notes, evaluation check list)

All information, data and results of the study are confidential. All people having access to such data are informed of its confidentiality. In all cases, nominative information shall not be transmitted to the study sponsor. Whenever a subject name is revealed on a document required by the Sponsor (e.g., photographs) the name must be blacked out permanently by the site personnel, leaving the initials visible, and annotated with the subject number as identification.

Data capture is performed by Complife Italia under Microsoft® Excel 2010 (vers. 14.0.4760.1000; Microsoft, USA) worksheet running on Microsoft® Windows 8.1 Professional (Microsoft, USA). Data entry and quality control are performed by two different persons. Calculated cells and formulas in Excel are also checked by the quality assurance. Statistical analysis was carried out using NCSS 10 statistical software (NCSS, LLC. Kaysville, Utah, USA) running on Windows Server 2008 R2 Standard (Microsoft, USA).

11.5. Record Archiving and Retention

An original copy of all the data of the study (signed protocol, safety assessment letter of the Sponsor, case study report form, extracted raw data, administrative file including all the correspondence) is kept in the records of the Complife Italia for 10 years. The archives are destructed only after reception of a written and signed permission from the Sponsor. However, these documents should be retained for a longer period if required by the applicable regulatory requirements or by an agreement with the Sponsor. The investigator should take measures to prevent accidental or premature destruction of these documents. The archiving arrangements will be addressed by the monitor when closing-out the site. The Sponsor will inform Complife srl, in writing, as to when these documents no longer need to be retained.

12. COMPLIANCE WITH DECLARATION OF HELSINKI

12.1. Compliance with declaration of Helsinki

This study will be carried out in the spirit of informed consent regulations, and the Declaration of Helsinki.

12.2. Informed Consent

Prior to study entry, the investigator, or a person designated by the investigator, will explain the nature, purpose, benefits and risks of participation in the study to each subject. Informed consent must be obtained prior to the subject entering the study (before initiation of any study-related procedure). Sufficient time will be allowed to discuss any questions raised by the subject.

The final informed consent form must be agreed by the Sponsor and must contain all elements in the sample form, in language readily understood by the subject. Each subject's original consent form, personally signed and dated by the

subject and by the person who conducted the informed consent discussion, will be retained by the investigator. The investigator will supply all enrolled subjects with a copy of their signed informed consent.
The consent form may need to be revised during the study should important new information become available that may be relevant to the safety of the subject or as a result of protocol amendments.
It is the investigator's responsibility to ensure that the amended form is signed by all subjects subsequently entered into the study and those currently in the study. This is documented in the same way as previously described.

12.3. Subjects Confidentiality

In accordance with applicable law on data protection (EU Regulation 679/2016), the personal data, which may be sensitive, including date of birth, sex, race, etc.., the information resulting from clinical studies and on your health status (that you freely supply to us) will be processed by Complife Italia Srl in confidence, only for research purposes in relation with this study. If the results arising from the clinical study should be published or disseminated in scientific journals or conferences, this will be done in confidence. For this purpose, the subject medical information, cosmetic information and information related to subject lifestyle as well as, if necessary for this research, the data about ethnic origins will be forwarded to the Sponsor of the study or to Sponsor partners in France or abroad. In each case, data will be anonymized and will be identified by a code number and initials. The investigator has the responsibility to keep the list of codes to enable the link between the subject assigned number and the subject name. The data will remain strictly confidential and will not be made public. At any time during or after the study, health authorities may have direct access to the records to check the accuracy of the information collected. In such circumstances, it is possible that the subject identity will be known. All of the person mentioned here above are bound by professional secrecy.

13. ADMINISTRATION PROCEDURES

13.1. Publication Policy

The results of the study as well as any other data disclosed or generated in the context of the study are confidential. Any publication in relation to the study shall be subject to Sponsor's prior written approval.

13.2. Clinical Study Report

Clinical study report will contain Safety results based on the Safety Population and Efficacy results based on the Intent to Treat and Per Protocol Population.

13.3. Contractual and Financial Details

The principal investigator and the Sponsor will sign a clinical study agreement prior to the start of the study, outlining overall sponsor and investigator responsibilities in relation to the study. Financial remuneration will cover the cost per included subject, based on the calculated costs of performing the study assessments in accordance with the protocol, and the terms of payment will be described in the contract.

13.4. Insurance

A product liability insurance is provided by the Sponsor

13.5. Protocol Amendments (If applicable)

All amendments to the protocol shall be agreed upon by the sponsor and the investigator. Deviations should be reviewed to determine the need to amend the protocol or to terminate the investigation.
However, when there are changes to the initial list of investigators and Centre this list will not be formally updated by amendments at each change; the sponsor will maintain an updated list which will be available on request. The definitive list of all Centre and investigators will be provided with the final report.