# CLINICAL STUDY PROTOCOL

**Prot. no: E.HU.111-0080.17.003L _2019/684**

# "Clinical assessment of the efficacy of a treatment (food supplement + cosmetic product) targeted to subjects with acne-prone skin. Double blind randomized placebo/reference product-controlled clinical study"


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|---|---|
| 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: | Dr Ileana De Ponti, Chemist and Pharmaceutical Tecnologist |
| Study site: | COMPLIFE ITALIA Srl<br>Via Mons. Angelini, 21<br>27028 San Martino Siccomario (PV) |


**VERSION NO. 2 – 19th March 2019**

# GENERAL INFORMATION

| | | |
|---|---|---|
| **TITLE:** | | |
| Clinical assessment of the efficacy of a treatment (food supplement + cosmetic product) targeted to subjects with acne-prone skin. Double blind randomized placebo/reference product-controlled clinical study. | | |
| **PROTOCOL NO. AND VERSION** | | |
| **E.HU.111-0080.17.003L _2019_2019/684- VERSION NO. 2 – 19th March 2019** | | |
| **SPONSOR:** | | |
| **NAME AND ADDRESS:** | **PERSONS AUTHORIZED TO SIGN THE PROTOCOL AND ITS AMENDMENTS:** | **MEDICAL STAFF:** |
| ROELMI HPC Srl,<br>Via Celeste Milani 24,<br>21040 Origgio (VA) | Dottoressa Federica Carlomagno<br>R&D Manager<br>ROELMI HPC Srl,<br>Via Celeste Milani 24, 21040 Origgio (VA) | Non applicabile |
| **MONITOR:** | | |
| Not applicable | | |
| **FACILITY:** | | |
| **NAME AND ADRESS:** | **INVESTIGATING SPECIALIST:** | **CO-INVESTIGATOR:** |
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| **MONITOR:** | | |
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| **STUDY MANAGEMENT** | | |
| **NAME AND ADDRESS:** | **PERSONS AUTHORIZED TO SIGN THE PROTOCOL AND ITS AMENDMENTS:** | |
| COMPLIFE ITALIA srl<br>Via Mons. Angelini, 21<br>27028 San Martino Siccomario (PV)<br>T. 0382 490286 - F. 0382 490886 | Dr. Ileana De Ponti<br>Via Mons. Angelini, 21<br>27028 San Martino Siccomario (PV)<br>T. 0382 490286 - F. 0382 490886 - Mail: ileana.deponti@complifegroup.com | |
| **OTHER LABORATORIES** | | |
| None | | |
| **OTHER DEPARTMENTS** | | |
| Not applicable | | |

# PROTOCOL APPROVAL

INVESTIGATOR SIGNATURE

I have read the protocol E.HU.111-0080.17.003L _2019/684- VERSION NO. 2 – 19th March 2019 "Clinical assessment of the efficacy of a treatment (food supplement + cosmetic product) targeted to subjects with acne-prone skin. Double blind randomized placebo/reference product-controlled clinical study" 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 _____ Date _____

Federica Carlomagno
20/03/2019
Federica Carlomagno
*R&D Manager*

**Principal Investigator**

Signature Date

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**Study Director**

Signature Date

Ileana De Ponti
20/03/2019
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*In Vivo Safety & Efficacy Technician and Sales Manager*

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 | 08/03/2019 | Enza Cestone<br>Ileana De Ponti | First drafting |
| | 2 | 19/03/2019 | Enza Cestone<br>Ileana De Ponti | Second drafting (change of the active and placebo food supplement composition) |

## 2. BACKGROUND

Skin represents the major organ of the body, with a main role of protection from the external environment. It has been shown that the skin is an active barrier, covered by a number of microorganisms, including bacteria, fungi and viruses. (Grice et al., 2011). The skin can be seen as an ecosystem, harbouring microbial communities that live in distinct niches.
Variations in the cutaneous microbiota of healthy individuals are associated with the sebaceous, dry, or moist environments of the skin. In addition, differences in skin microbiota have been associated with factors like age, diet, and gender, climate and geographic location (Perez et al., 2016).

As a complex ecosystem, composed by living and dead microorganisms, the skin can host transient species from the external environment or resident members of skin community. It seems essential that the communities remain in equilibrium for a good status of skin homeostasis. (Grice et al., 2011).
In fact, as skin microbiota activates and educates host immunities, a number of skin inflammatory diseases are associated with shifts in the resident microbiota from a "healthy" to a "disease" state. These diseases can be seen as dysbiotic host-microbial metaorganismal states. (Belkaid et al., 2014).
Differences within skin microbiota can be associated with conditions such as dandruff, acne, psoriasis, atopic dermatitis. (Perez et al., 2016), and also sunburn and vulvovaginitis (Mendling et al., 2016).
The effectiveness of antimicrobial agents for the management of skin disorders supports the link between microbes and pathophysiology (Grice et al., 2011). Therapies to maintain healthy skin require the promotion of the growth of symbiotic bacteria more than the inhibition of pathogens growth (Grice et al., 2009).
Other examples of a direct link between skin microbiota and inflammatory diseases are the infections caused by overgrowth of typically commensal bacteria like Staphylococcus epidermidis, or Propionibacterium acnes (Grice et al., 2011).

## 3. OBJECTIVES

The study is aimed to assess the efficacy and the safety of a complete acne treatment (active food supplement + active cosmetic product – "ProBeauty Shield") in improving skin appearance on adult subjects affected by active acne (inflammatory and noninflammatory acne), in normalizing the presence of sebum and its effect on skin moisturizing and skin pH.
In order to reach this goal a double-blind randomized placebo/reference product-controlled clinical study is carried out on 80 female subjects aged between 18 and 50 years old showing acne severity from 1 to 3 according to IGA (Investigator's Global Assessment) severity scale.
According to a predisposed randomisation list included subjects are divided in 4 groups:
- G1: 20 subjects use the complete active treatment (active cosmetic product + active food supplement)
- G2: 20 subjects use the active cosmetic product + the food supplement placebo
- G3: 20 subjects use the cosmetic reference product (benchmark present on the market) + the active food supplement
- G4: 20 subjects use the cosmetic reference product (benchmark present on the market) + the food supplement placebo.

The study foresees 56 days of products use. Instrumental evaluations of the skin parameters under study are carried out at baseline (T0) and after 28 days (T28) and 56 days (T56) of products use.
The study is moreover integrated with the Dermatologist clinical analysis and the subjects self-assessment.

### 3.1. Primary objectives

The aim of this study is to evaluate the efficacy of the tested treatment in promoting the improvement of the acne clinical signs. In particular, its efficacy in promoting the reduction of the number and appearance of acne lesions (inflammatory and noninflammatory acne) and in normalizing the presence of sebum on the skin are evaluated.

### 3.2. Secondary objectives

Secondary objective of this study is the confirmation of the safety of use of the products, the evaluation of their effects on skin moisturizing and skin pH.

## 4. STUDY DESIGN

A double blind randomized placebo/reference product-controlled clinical study is carried on 4 group of 20 subjects as follows: one group (G1) use the complete active treatment (active cosmetic product + active food supplement), one group (G2) use the active cosmetic product + the food supplement placebo, one group (G3) use the cosmetic reference product (benchmark present on the market) + the active food supplement and one group (G4) use the cosmetic reference product (benchmark present on the market) + the food supplement placebo.

**4.1. Population characteristics**

It is planned to enroll 80 female subjects showing acne severity from 1 to 3 according to IGA scale.
Subjects are enrolled only if they satisfy all the inclusion/non-inclusion criteria reported in the sections 5.
Subjects will be randomly attributed to each group of 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 is Dr. Ileana De Ponti, Chemist and Pharmaceutical Technologist.

**5. STUDY POPULATION**

A total of 80 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
- ✓ Caucasian ethnicity
- ✓ Female sex
- ✓ Photoype I to IV
- ✓ Age between 18 and 50 years old
- ✓ Acne severity from 1 to 3 according to IGA scale
- ✓ Subjects who have not been recently involved in any other similar study
- ✓ Willingness to use for face care only the creams that will be consigned at the beginning of the study
- ✓ Willingness to submit before and after pictures
- ✓ Willingness to use during all the study period only the products to be tested
- ✓ Willingness not to use similar products that could 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
- ✓ Subjects who accept not to expose in intensive way to UV rays during the whole study duration

**5.2. Non-inclusion criteria**

- ✓ Subjects who do not meet the inclusion criteria
- ✓ Pregnant/breastfeeding female or who have planned a pregnancy during the study period
- ✓ Positive history for atopy or hypersensitive skin
- ✓ Subjects under systemically pharmacological treatment
- ✓ Subjects under locally pharmacological treatment on the skin area monitored during the test
- ✓ Subjects with congenital or acquired immunodeficiency
- ✓ Subjects under treatment with food supplements which could interfere with the functionality of the product under study
- ✓ Subjects which show other skin alterations on the monitored area except for acne lesions
- ✓ Subjects considered as not adeguate to partecipate to the study by the investigator
- ✓ Subjects with known or suspected sensitization to one or more test formulation ingredients
- ✓ Adult protected by law (under control or hospitalized in public or private institutions for reasons other than research, or incarcerated)
- ✓ Subjects not able to communicate or cooperate with the investigator for problems related to language, mental retardation or impaired brain 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) of 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 adverse reactions 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 is achieved by a subject when she completes the entire treatment and she is undergone all the check visits.

### 5.6. Subjects risk and benefit

Risks associated with the products intake/application 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 carried out instrumental measurements are not invasive and no skin side effects are expected from the measurement process.

Benefits associated with product use are amelioration of skin imperfections due to acne.

## 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 products use. An intermediate visit after 28 days of products use 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) |
|---|---|---|---|
| Signed Informed consent | X | --- | --- |
| Subject eligibility | X | X | X |
| Clinical assessment-safety of use | - | X | X |
| Clinical evaluation of the numbers of acneic lesions (papules, pustoles, open and closed comedones) | X | X | X |
| Clinical evaluation of skin complexion evenness | X | X | X |
| Clinical evaluation of the improvement of the skin inflammatory status of the area interested by acne lesions | X | X | X |
| Instrumental evaluation of the skin sebum | X | X | X |
| Instrumental evaluation of the skin moisturization | X | X | X |
| Instrumental evaluation of the skin pH | X | X | X |
| Digital pictures of the treated areas | X | X | X |
| Self-assessment questionnaire | - | - | X |
| Products distribution | X | - | - |
| Unused product collection | - | - | X |

#### 6.1.1. Screening – Initial visit (T0)

Subjects are 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.

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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 are 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 evaluations
- instrumental evaluations
- digital pictures acquisition
- supplying the product / placebo in accordance with the randomization list
- fixing the date of the 1st check visit after 28 days of treatment

#### 6.1.2. 1st check visit (T28)

The following procedures are carried out:

- checking of subject eligibility
- clinical assessment-safety of use
- clinical evaluations
- instrumental evaluations
- digital pictures acquisition
- 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 are carried out:

- checking of subject eligibility
- clinical assessment-safety of use
- clinical evaluations
- instrumental evaluations
- digital pictures acquisition
- filling of the self-assessment questionnaire.

## 7. TREATMENT

### 7.1. Products

#### 7.1.1. Qualitative and Quantitative formula

**Active cosmetic product - Aective**

| Ingredient list | Composition % |
|---|---|
| Aqua | 74,30% |
| Disodium EDTA | 0,10% |
| Glycerin | 1,00% |
| Polyglyceryl-3 Methyl Glucose Distearate | 4,00% |
| Glyceryl Stearate | 2,50% |
| Cetearyl Alcohol | 2,50% |
| Prunus Amygdalus Dulcis Oil | 8,30% |
| Coco Caprylate/Caprate | 5,00% |
| Ectoin | 1,00% |
| Aqua, Sodium Benzoate, Potassium Sorbate | 1,00% |
| Parfum | 0,30% |

**Benchmark cosmetic product present on the market - Sebium Global (Bioderma)**

AQUA/WATER/EAU, C12-13 ALKYL LACTATE, DIPROPYLENE GLYCOL, CITRIC ACID, CYCLOPENTASILOXANE, SODIUM HYDROXIDE, GLYCERIN, ZINC GLUCONATE, METHYL METHACRYLATE CROSSPOLYMER, SALICYLIC ACID, ARACHIDYL ALCOHOL, DIMETHICONE, MANNITOL, XYLITOL, RHAMNOSE, FRUCTOOLIGOSACCHARIDES, LAMINARIA OCHROLEUCA EXTRACT, GINKGO BILOBA LEAF EXTRACT, BEHENYL ALCOHOL, GLYCERYL STEARATE, PEG-100 STEARATE, SILICA, HYDROXYETHYL ACRYLATE/SODIUM ACRYLOYLDIMETHYL TAURATE COPOLYMER, XANTHAN GUM, ARACHIDYL GLUCOSIDE, C30-45 ALKYL CETEARYL DIMETHICONE CROSSPOLYMER, GLYCYRRHETINIC ACID, PROPYLENE GLYCOL, POLYSORBATE 60, CAPRYLIC/CAPRIC TRIGLYCERIDE, BAKUCHIOL, SQUALANE, FRAGRANCE (PARFUM). [BI 673]

**Active food supplement - ProBeauty Shield 2.0**

| Galenic formula | Ingredient list | mg/CPS |
|---|---|---|
| Capsule size 3 | *Lactobacillus plantarum* LA001 500 B/g | 7 B |
| | *Lactobacillus rhamnosus* LRH020 300B/g | 7 B |
| | *Lactobacillus reuteri* PBS072 200B/g | 7 B |
| | Amido di mais essiccato | QB |
| | Magnesio stearato | QB |
| | Biossido di silicio | QB |
| | Capsula vegetale DR | 47 |
| Total | | 200 mg |

**Food supplement placebo**

| Galenic formula | Ingredient list | mg/CPS |
|---|---|---|
| Capsule size 3 | Amido di mais essiccato | QB |
| | Magnesio stearato | QB |
| | Biossido di silicio | QB |
| | Capsula vegetale DR | 47 |
| Total | | 135 mg |

### 7.1.2. Products use

**Active cosmetic product**

Apply once a day (in the morning) on the face.

**Benchmark cosmetic product present on the market**

Apply once a day (in the morning) on the face.

**Active food supplement**

Take 1 cachet a day with a glass water (not sparkling water) away from meals.

**Food supplement placebo**

Take 1 cachet a day with a glass water (not sparkling water) away from meals.

### 7.1.3. Product supply, labeling, storage and accountability

#### 7.1.3.1. Product supply

Products are 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. Ileana De Ponti - T. +39 0382 25504

#### 7.1.3.2. Labeling

**Figure 1**. Products label.

*IT VERSION*

**INTEGRATORE ALIMENTARE**

**Prot. no:**

**SOGGETTO N°** ------->
**MODO D'USO: assumere 1 capsula al giorno accompagnata da un bicchiere di acqua non gasata, 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**

**Figure 2**. Products label.

*IT VERSION*

**CREMA VISO**

**Prot. no:**

**SOGGETTO N°** ------->
**MODO D'USO: applicare una volta al giorno (il mattino) su tutto il viso.**

**TENERE AL RIPARO DA FONTI DI LUCE E DI CALORE, TENERE AL DI FUORI DALLA PORTATA DEI BAMBINI.**

#### 7.1.3.3. Storage

All products are 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 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 are 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 are generated by the in site Study Director using an appropriate statistic algorithm ("Wey's urn"). For each subject participating in the study are prepared an envelope containing the information on the product tested. Both the randomization list and the subjects envelopes are 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. Evaluation of skin sebum

The sebum measurement is based on the internationally recognized Sebumeter® method (Sebumeter 815, Courage+Khazaka GmbH). The measurement principle is the photometric method, the grease spot photometer. According to this method the sebum on skin is collected on a 64 $mm^2$ mat synthetic tape contained in a cassette, then the measuring head of the cassette is inserted into the aperture of the device, where a photocell measures the transparency. The light transmission represents the sebum content on the surface of the measuring area. A microprocessor calculates the result, which is shown on the display in µg sebum/$cm^2$ of the skin.

### 8.2. Evaluation of skin moisturising

The measurement of skin moisturisation 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).

**8.3. Evaluation of skin pH**

The used instrument is the SKIN pH-METER 905®, Courage + Khazaka GmbH. The measure is based on a combined electrode of high quality, in which both the glass electrode sensitive to H+ and the additional reference electrode are placed in the same site. It is connected to an handle probe containing the measurement electronics.
Before the measurements, the SKIN pH-meter® 905 (Courage + Khazaka electronic GmbH) is calibrated using two buffer solutions with known pH (pH 4.01 and 1.7) as reference.
Measurement range: 0 to 12
Accuracy: ± 0.1 pH

**8.4. Evaluation of the number of acneic lesions**

The investigator visually assesses (and by palpation if necessary) the facial skin counting the acneic lesions. The assessment is mainly based on the evaluation of the number of papules, pustoles, open comedones (blackheads) and closed comedones (whiteheads) on the face of the subjects.

**8.5. Evaluation of skin complexion evenness**

Clinical evaluation of skin complexion evenness is performed by the Dermatologist according to the clinical scores reported in the tables below.

| Clinical classification of skin complexion at T0 (basal evaluation) | Score | Product effect classification | Score |
|---|---|---|---|
| Skin complexion is uneven, imperfections are all over the face | **1** | No variation | **1** |
| Skin complexion is slightly uneven, imperfections are in some parts of the face | **2** | Slight variation | **2** |
| Skin complexion is quite uniform | **3** | Moderate variation | **3** |
| Skin complexion is uniform | **4** | Evident variation | **4** |

**8.6. Evaluation of the improvement of the skin inflammatory status of the area interested by acne lesions**

The effect of the product on the improvement of the skin inflammatory status of the area interested by acneic lesions is evaluated on the digital pictures acquired in standard conditions with cross-polarized filters. The images are evaluated by the Dermatologist according to the score reported in table below by comparing the images acquired at T0 with those acquired at each following time.

| Product effect classification | Score |
|---|---|
| No variation | **1** |
| Slight variation | **2** |
| Moderate variation | **3** |
| Evident variation | **4** |

**8.7. Digital macrophotography**

Digital pictures of the face are acquired at each experimental time using a reflex digital camera (NIKON D300 digital camera, Nikon Corporation Tokyo, Japan) equipped with macro-objective (AF-S Micro NIKKOR 60mm f/2.8G ED, Nikon Corporation Tokyo, Japan), a flash system (Kit R1C1, Nikon Corporation Tokyo, Japan) and cross-/parallel-polarized filters.

**8.8. Self-assessment questionnaire**

At the end of the study subjects are asked to express their personal opinion on the tested treatment by answering to a questionnaire about products acceptability and effects.

**9. SAFETY ENDPOINTS AND EVALUATIONS**

Tolerability of the treatment are closely followed by the study principal investigator during the course of the study. Subjects 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 reports it as a cosmetic or an adverse event.
Any unexpected related side effect judged as severe by the principal investigator is reported to the Sponsor. Upon investigator judgment, the subject may be withdrawn from the study and the side effect is 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 is closely followed by the study principal investigator and her collaborator during the study period. Subjects 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 reports it as an intolerance. Any unexpected related side effect judged as severe by the principal investigator is reported to the Sponsor. Upon investigator judgment, the subject may be withdrawn from the study and the side effect is 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.

**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.

**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.

**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 are enrolled in the study, and randomly assigned to the treatments under study.

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 are 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 is 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.) are reported. Data are 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 clinical data are submitted to Friedman's 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 Mann Whitney U Test.

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 four study groups at all experimental times.

Results of the safety evaluation are 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., are clearly marked and permit easy identification of a subject's participation in the specified clinical trial. The principal investigator records 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 allows direct access to all relevant files (for all subjects) for the purpose of verifying entries made in the data collecting sheet, and assists 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 is 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, explains 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 is 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, is retained by the investigator. The investigator supplies 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) are 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 is 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 are forwarded to the Sponsor of the study or to Sponsor partners in France or abroad. In each case, data are anonymized and are 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 remain strictly confidential and are not 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 contains 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 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 covers 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 are 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 maintains an updated list which is available on request. The definitive list of all Centre and investigators is provided with the final report.