CLINICAL STUDY PROTOCOL

Complife Italia Study no: IT0000145/26
Study code: H.E.HU.TE.NHL00.060.08.00

"RANDOMIZED PLACEBO-CONTROLLED CLINICAL TRIAL FOR THE ASSESSMENT OF THE EFFICACY OF A FOOD SUPPLEMENT IN IMPROVING HAIR APPEARANCE"

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Co-investigator:	Dr Valentina Cortale, Efficacy Clinical Trial Technician senior Dr. Arianna Ottone, Efficacy Clinical Trial Technician Dr. Aurora De Pascalis, Efficacy Clinical Trial Technician junior Dr. Camilla Garavaglia, Efficacy Clinical Trial Technician junior Dr. Simona Cedro, In Vivo Safety and Efficacy Technician
Sponsor:	BIOS LINE S.P.A.
Project manager:	Dr Francesca De Gennaro, Biologist
Study site:	Complife Italia srl Via Mons. Angelini, 21 27028 San Martino Siccomario (PV)

VERSION N° 01 – 29th January 2026

GENERAL INFORMATION

TITLE:		
Randomized placebo-controlled clinical trial for the assessment of the efficacy of a food supplement in improving hair appearance		
STUDY CODE/STUDY NO.		
H.E.HU.TE.NHL00.060.08.00_ IT0000145/26		
PROTOCOL NO. AND VERSION		
IT0002564/2025 Rev.1 by 29/12/2025		
SPONSOR:		
NAME AND ADDRESS:	**PERSONS AUTHORIZED TO SIGN THE PROTOCOL AND ITS AMENDMENTS:**	**MEDICAL STAFF:**
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OTHER LABORATORIES		
None		
OTHER DEPARTMENTS		
Not applicable		

PROTOCOL APPROVAL

I have read the protocol **IT0002564/2025 Rev.1 by 29/12/2025 - Study code: H.E.HU.TE.NHL00.060.08.00, Study n°: IT0000145/26** titled **"Randomized placebo-controlled clinical trial for the assessment of the efficacy of a food supplement in improving hair appearance"** 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

Cristiano Maggiato
BIOS LINE S.p.A.
R&D Department

Date 29/01/2026

Principal Investigator

Signature

Dr.ssa Roveda Gloria
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Study Director and Quality Control

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Biologist

Clinical Trial Reporter

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Dr Anna Pelizzola
Biotechnologist

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
	00	16/01/2026	Gloria Roveda Francesca De Gennaro Anna Pelizzola	First drafting
	01	29/01/2026	Gloria Roveda Francesca De Gennaro Anna Pelizzola	Added INCI information

2. BACKGROUND

The scalp is a highly specialized and well-vascularized area of the skin where a large number of hair follicles, known as bulbs, are present. From these follicles hair originates; hair is mainly composed of keratin, lipids, minerals, and pigments. Keratin is a protein made up of 18 amino acids, among which cysteine is the most representative. The scalp also contains sebaceous and sweat glands whose function is to maintain the hydrolipidic film, allow perspiration, and support thermoregulation through sebum and sweat.

The scalp, being composed of skin like all other areas of the body, is subject to problems such as alterations, anomalies, disorders, or true pathological conditions. Dry and fine hair that appears weak and fragile and tends to fray and break easily is among the most common issues. In these situations, hair may appear dull and lacking shine. The causes can be varied: poor nutrition, hormonal fluctuations, stress, living in polluted environments, and aggressive cosmetic treatments. All these factors can lead to inflammation of the hair bulb, with a reduced hydrolipidic film, making the hair more vulnerable and exposed to external aggressions.

The aim of the study is to evaluate the effectiveness of a supplement containing specific ingredients designed to counteract inflammation and free radicals that develop at the level of the hair bulb as a result of the above-mentioned factors, to nourish it in a targeted way, promoting the growth of stronger, healthier, and shinier hair while simultaneously restoring the hydrolipidic film.

Principium Hair Beauty features a formulation designed to provide specific nutrients, including Cotinus (*Cotinus coggygria Scop.)*, better known as the "smoke tree" because of the appearance of its feathery inflorescences, which give it a smoky aura. Its bark is rich in fisetin, a flavonol also found in other edible plants such as strawberries and apples, persimmons, grapes, onions, and kiwifruit. Fisetin has recently attracted attention as an antioxidant and anti-aging agent and has been shown to modulate multiple signaling pathways, including PI3K/AKT/mTOR, Wnt/β-catenin, NF-κB, and TRAIL/TRAIL-R pathways. Fisetin has been shown to promote a process known as TERT (telomerase reverse transcriptase), which triggers the onset of a new phase of hair follicle growth and promotes hair regeneration.

In one study, the usefulness of fisetin as an agent to counteract skin aging was suggested by evaluating its effects on models of senescent dermal fibroblasts and on chimeric mice with full-thickness skin samples obtained from elderly humans. This study highlighted that the substance could be useful in counteracting cellular senescence and also demonstrated its ability to oppose alopecia and, therefore, hair loss.

Supporting the activity of fisetin-rich Cotinus is cystine, a sulfur-containing amino acid that contributes to the structure of hair, as it plays a key role in keratin formation, where it accounts for about 17%. A deficiency in cystine leads to fragility of hair, nails, and skin. An experimental study showed that cystine is able to counteract drug-induced alopecia caused by medications such as doxorubicin. A meta-analysis confirmed the therapeutic efficacy of cystine in reducing diffuse hair loss by increasing the anagen growth rate, reflecting a normalization of hair cycle disorders. This amino acid protects against oxidative stress because it participates in the synthesis of glutathione, a highly antioxidant substance produced by the body.

In scenarios where hair is dry and fragile, a fundamental contribution to the formulation rationale is the presence of ceramides, lipid substances normally found in the body in various tissues such as the brain, nails, and hair, as well as in epidermal cells and the intercellular spaces between corneocytes. Ceramides help form the barrier function and promote skin hydration and elasticity. In this regard, several studies demonstrate the benefits of ceramide supplementation at the skin level. A clinical study conducted on women aged between 18 and 65 observed a significant increase in the number of hairs in the anagen phase, along with a reduction in the telogen effluvium phase.

Other elements included in the formulation that contribute to the product's functionality are vitamin C, which aids in the hydroxylation of proline and lysine for collagen formation, a key component of the skin; zinc, which is a cofactor involved in important functional activities within the hair follicle; and finally vitamins B9 (folic acid) and B12, which are essential for red blood cell formation and consequently contribute to nourishment of the hair bulb.

References

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2. Maher P (2021). Preventing and treating neurological Disorders with the Flavonol Fisetin. Brain Plast 6:155–166. https://doi.org/10.3233/BPL-200104
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4. Chisato kubo et al. (2020). Fisetin Promotes Hair Growth by Augmenting TERT Expression. Frontiers in Cell and Developmental Biology https://doi:10.3389/fcell.2020.566617

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6. D'Agostini F. et al. (2013). Chemoprevention of doxorubicin-induced alopecia in mice by dietary administration of L-cystine and vitamin B6. Arch Dermatol Res.;305(1):25-34. https://doi:10.1007/s00403-012-1253-1
7. Stéphanie Dudonné et al. (2023). Efficacy of a wheat polar lipid complex in reducing hair loss and improving hair growth in healthy women: A randomized, double-blind, placebo-controlled clinical study. Int J Cosmet Sci.; 00:1–13.

3. OBJECTIVES

The study is aimed to assess the efficacy of a food supplement (tested in different concentrations) in improving hair conditions in particular improving hair strength and radiance and reducing hair breakage compared to a placebo product.
In order to reach this goal a randomized, placebo controlled, parallel group (3 arms), clinical trial* is carried out on 60 (66 included**) healthy female subjects enrolled according to specific inclusion and non-inclusion criteria (see section 5). In particular, female subjects aged between 18 and 60 (+ 2) years old, with thin, brittle hair that breaks easily.
According to a predisposed randomization list, the enrolled subjects will be divided in 3 groups as follows:

- G1: 22 subjects will take 2 cpr/die of the active food supplement and 2 cpr/die of placebo food supplement (for a total of 4 cpr/die);
- G2: 22 subjects will take 4 cpr/die of the active food supplement;
- G3: 22 subjects will take 4 cpr/die of the placebo food supplement

**The study will be carried out under dermatological control in accordance with the ethical principles applicable to medical research, based on the following protocol; the study protocol and informed consent will be submitted to the approval of an Independent Ethical Committee.*

***6 more subjects will be included for a total of 66 subjects, considering a 10% of drop-out.*

3.1. Primary objectives

The primary objective of the study is to assess the efficacy of the active product (taken in different concentration) in comparison with a placebo as evaluated through the following end-points:

- hair elasticity (hair elongation) by means dynamometer and hair radiance by means of spectrophotometer/colorimeter
- hair breakage by means of the count of the broken hair after a standardized brushing procedure
- overall hair appearance by means clinical internal scale

3.2. Secondary objectives

The secondary objectives of the study are to assess:

- subject's satisfaction of the tested product (self-evaluation questionnaire);
- incidence and nature of Adverse Event (AE) and Serious Adverse Event (SAE).

4. STUDY DESIGN

A randomized, placebo-controlled parallel clinical study will be carried out on 3 groups, consisting of 22 subjects each. The study will provide 84 days of products intake, and the evaluations will be performed at baseline (T0±2), after 28 (T28±2) and 84 days (T84±2) of product intake.

In order to check volunteers' compliance at each time point the number of capsule intakes will be noted

4.1. Population characteristics

The study is planning to enrol a total of 66 healthy female subjects aged between 18 and 60 (+ 2) years with thin, brittle hair that breaks easily. If the subjects satisfy all inclusion/non-inclusion criteria, they will be enrolled and randomly attributed to one of the three treatment groups.

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, in Via Signorelli, 159 – 20024 - Garbagnate Milanese (MI), Italy, in Corso S. Maurizio, 25 – 13900 Biella (BI), Italy, Nutratech S.r.l., Via Francesco Todaro, 20/22 – 87036 Rende (CS), Italy.
The principal investigator is Dr. Gloria Roveda (MD, Specialist in Dermatology and Venereology). The principal co-investigator is Dr. Valentina Cortale (Efficacy Clinical Trial Technician Senior). The in-site Study Director is Dr. De Gennaro Francesca (Clinical Trial Project Manager).

5. STUDY POPULATION

A total of 66 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
- ✓ Age between 18 and 60 (+ 2) years old
- ✓ Subjects with thin, brittle hair that breaks easily
- ✓ Subjects agreeing not to take any treatment (oral or topic) able to interfere with the hair strength during the whole study duration
- ✓ Willingness not to dye/blench hair during the 2 weeks preceding each visit
- ✓ Willingness not to cut hair for all the study length
- ✓ Subjects registered with health social security or health social insurance
- ✓ Subjects having signed their written Informed Consent form (ICF) and Privacy Policy for their participation in the study and a photograph authorization
- ✓ Subjects able to understand the language used in the investigation centre and the information given
- ✓ Subjects able to comply with the protocol and follow protocol constraints and specific requirements
- ✓ Willingness to take during all the study period only the product to be tested
- ✓ Willingness not to use/take similar products/food supplement that could interfere with the product to be tested
- ✓ Willingness to not vary the normal daily routine (i.e. lifestyle, physical activity, diet etc.)
- ✓ Subjects under effective contraception (oral/not oral) if women of childbearing potential; not expected to be changed during the trial

5.2. Non-inclusion criteria

- ■ Subjects who do not meet the inclusion criteria
- ■ Subject is taking part or planning to participate to another clinical study in the same or in another investigation centre
- ■ Subject who is deprived of freedom by administrative or legal decision or under guardianship
- ■ Subject admitted in a sanitary or social facilities
- ■ Subject who is planning a hospitalization during the study
- ■ Subjects under treatment with food supplements which could interfere with the functionality of the product under study
- ■ Subject has participated in another clinical study with anti-hair loss product or treatment within the last 24 weeks before the inclusion visit
- ■ Subject breastfeeding, pregnant or not willing to take necessary precautions to avoid pregnancy during the study (if women of childbearing potential)
- ■ Subject has started or changed oestrogen-progesterone contraception or hormonal treatment, within the 3 months prior to the study or foreseeing it for the duration of the study
- ■ Subject having an acute, chronic or progressive illness liable to interfere with the study data or considered by the Investigator hazardous for the subject or incompatible with the study requirements
- ■ Subjects under radiotherapy, chemotherapy at any time
- ■ Subjects under locally pharmacological/non-pharmacological treatment applied on the area of interest monitored during the test
- ■ Subject having food disorders
- ■ Subject who has any other hair disorder or hair disease (female pattern hair loss, any type of alopecia...)
- ■ Subject having excessive and/or fluctuating hair shedding for more than 6 months
- ■ History or clinical signs of hyperandrogenaemia

- Any hair care product applied on the scalp between the last shampoo and the inclusion visit (e.g. gel, hairspray, wax, foam...)
- Scalp surgery (hair transplants, laser) at any time.

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 data collection sheet. 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 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 supplement.
The potential benefits associated with the use of the product are related to an improvement of hair conditions in particular improving hair length, improving hair density and reducing hair breakage.

6. STUDY FLOW CHART

The duration of the study will be 84±2 days. Clinical visits are planned at baseline days (T0±2), after 28 days (T28±2) and 84 days (T84±2) days of products intake.

6.1. Study schedule

Study schedule is as follows:

Study phases	Initial visit (T0±2)	Follow-up visit (T28±2)	Final visit (T84±2)
Signed Informed Consent and Privacy Policy	X	--	--
Subject eligibility*	X	X	X
Products distribution	X	--	--
Recording of the subject demographic data and medical history	X	--	--
Product collection and counting	X	X	X

Hair elongation	X	X	X
Hair radiance	X	X	X
Assessment of the number of broken hair	X	X	X
Digital macrophotography	X	X	X
Clinical evaluation	X	X	X
Digital macrophotography	X	X	X
Self-assessment questionnaire supplying and collection	--	--	X
AE and local tolerance assessment	--	X	X

**The experimenter checks at each visit the compliance of the subjects with all inclusion/exclusion criteria*

6.1.1. Screening – Initial visit (T0±2)

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 the date for the screening visit will be planned;

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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 are carried out:

- signature of the Informed Consent Form and the Privacy Policy
- recording of the subject demographic data
- checking of the current and previous subject's medical history and concomitant therapies
- checking of subject eligibility
- in order to check the compliance to the product intake, the products are previously counted
- supplying of the product/placebo in accordance with the randomization list
- instrumental evaluation: hair elongation, hair radiance, assessment of number of broken hair, digital macrophotography
- supplying of the brush and subjects are asked to brush their hair according to a standard procedure and indicate the number of broken hair found during hair brushing;
- fixing the date of the follow-up visit after 28 days of treatment.

6.1.2. Intermediate visit (T28±2)

The following procedures are carried out:

- checking of subject eligibility
- product collection and counting to check the compliance to the treatment
- instrumental evaluation: hair elongation, hair radiance, assessment of number of broken hair, digital macrophotography
- clinical evaluation of the overall hair appearance
- recording reactions (AE, SAE) and assessment of product tolerability
- fixing the date of the final visit after 84 days of treatment.

6.1.3. Final visit (T84±2)

The following procedures are carried out:

- checking of subject eligibility
- product collection and counting to check the compliance to the treatment
- instrumental evaluation: hair elongation, hair radiance, assessment of number of broken hair, digital macrophotography
- clinical evaluation of the overall hair appearance
- supplying and collecting of the self-assessment questionnaire

7. TREATMENT

7.1. Products

7.1.1. Active product description

Active product - Principium Hair Beauty:

- **G1:** The food supplement is based on L-cystine, vitamin C, ceramides and Cotinus bark, zinc, folic acid, and vitamin B12 and C. The product is in tablet form and is taken at a dosage of two tablets per day.
- **G2**: The food supplement is based on L-cystine, vitamin C, ceramides and Cotinus bark, zinc, folic acid, and vitamin B12 and C. The product is in tablet form and is taken at a dosage of four tablets per day.

7.1.2. Qualitative and Quantitative formula

1) PRODUCT 1:

ACTIVE PRODUCT - Principium Hair Beauty

L-CISTINA, AGENTE DI CARICA: MICROCELLULOSA CRITALLINA, MALTODESTRINE, VITAMINA C (ACIDO L-ASCORBICO), INULINA DA CICORIA (*CICHHORIUM INTYBUS L.*) RADICE, SCOTANO (*COTINUS COGGYGRIA SCOP.*) CORTECCIA ESTRATTO TITOLATO SECCO AL 20% IN FISETINA, SPIRULINA (*SPIRULINA PLATENIS (GOMONT) GAITLER*) TALLO POLVERE, AGENTE ANTIAGGLOMERANTE: BIOSSIDO DI SILICIO, STABILIZZANTE: IDROSSIPROPILMETILCELLULOSA, AGENTE ANTIAGGLOMERANTE: SALI DI MAGNESIO DEGLI ACIDI GRASSI, SOLFATO DI ZINCO, GRANO (*TRITICUM SATIVUM LAM.*) FRUTTO ESTRATTO SECCO TITOLATO AL 50% IN CERAMIDI, ACIDO FOLICO (ACIDO PTEROILMONOGLUTAMMICO), VITAMINA B12 (METILCOBALAMINA).

The product does not contain allergens; the product is gluten-free.

2) PRODUCT 2:

PLACEBO PRODUCT

SORBITOLO, CELLULOSA MICROCRISTALLINA, ESTRATTO DI CARTAMO, SPIRULINA BIOSSIDO DI SILICIO, MAGNESIO STEARATO, OSSIDO DI FERRO NERO.

The product does not contain allergens.

7.1.3. Products dosage and way of use

- G1: 22 subjects will take 2 cpr/die of the active food supplement and 2 cpr/die of placebo food supplement (for a total of 4 cpr/die);
- G2: 22 subjects will take 4 cpr/die of the active food supplement;
- G3: 22 subjects will take 4 cpr/die of the placebo food supplement

7.1.4. Product supply, labeling, storage and accountability

a) 7.1.4.1. 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. Eleonora Spartà - T. +39 0382 25504

b) 7.1.4.2. Labeling

Product will be supplied with an anonymous packaging and Complife will affix on each product the following label.

Figure 1. Product 1 label.

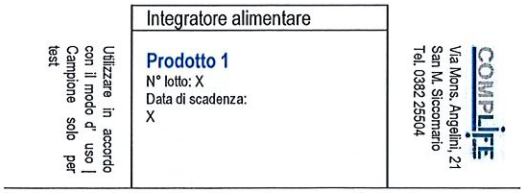


Figure 2. Product 2 label.

Figure 3. Product 3 label.

AVVERTENZE:

Legend. Product 1 and 2 - Active product
Product 3 - Placebo product.

c) 7.1.4.3. Storage

All products are stored at room temperature (between 15 and 25°C) at COMPLIFE ITALIA Srl, protected from direct light, heat and source of water safe place with restricted access.

d) 7.1.344. 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.
The returned product at the end of the study will be destroyed according to the current internal procedures.

e) 7.1.4.5. Compliance to treatment

At the beginning of the study (T0) subjects will receive one bottle containing product samples sufficient for all the study duration. The compliance to treatment is assessed by the principal investigator by counting and recording the remaining pills in the bottle after 28 days and 84 days of treatment.
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.
Compliance to treatment will be calculated by product accountability, as follows:

$$\text{Compliace to treatment} = \frac{\text{number of intake product}}{\text{number of product to intake}} \text{x100}$$

The average of overall compliance shall be ≥ 80%.

7.1.5. Randomization

A restricted randomization lists are generated by the in-site Study Director using an appropriate statistic algorithm ("Wey's urn"). For each subject participating in the study, an envelope containing the information on the product tested is prepared. 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.

1. EFFICACY ENDPOINTS AND EVALUATIONS

In this section the instrumental evaluations to assess the efficacy parameters employed in the study are reported. All the study procedures are carried out under controlled ambient conditions (T = 18-26°C and RH = 40-60%). Subjects are left to acclimatize to ambient condition for 15-20 minutes before the check visit.

1.1. 60-seconds Hair count

The subjects are issued identical combs (having the teeth separated by 1 mm on one half of the comb and by 2 mm on the other half) to be used throughout the study. The subjects used their usual shampoo (without any claim related to hair repairing). Two days after the shampoo, they are instructed to comb their hair for 60 seconds before in the morning, starting at the vertex, and combing forward. The hair is combed over a towel or pillowcase of contrasting color so that any broken hair could be adequately visualized. The subjects then collected the broken hairs and recorded their number.

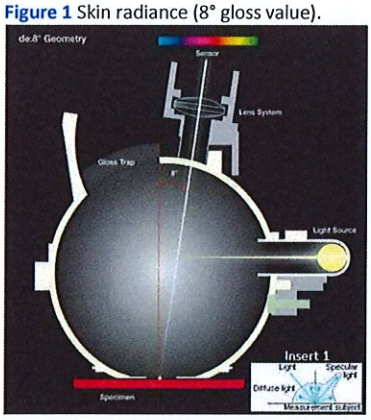
1.2. Hair elasticity (hair elongation)

Hair elasticity is measured using a dynamometer (C610 Autotensile Tester, Labthink.) in accordance with UNI EN ISO 5079:1998 standard procedure. Hair elasticity is calculated as the maximum elongation before hair breakage.

1.3. Hair radiance

Hair radiance (ability to reflect the light) is measured using a spectrophotometer/colorimeter CM 700D (Konica Minolta) by means of the 8° gloss value (Fig. 1).

Figure 1 Skin radiance (8° gloss value).



When light reach a surface it is reflected at the equal but opposite angle from the light source; this is called specularly reflected light. This specular component is reflected as if reflected by a mirror. The light that is not specularly reflected, but scattered in many directions, is called diffuse reflectance (insert 1). The sum of the specular reflectance plus the diffuse reflectance is called the total reflectance. For objects with shiny surfaces, the specularly reflected light is relatively strong and the diffused light is weaker. On rough surfaces with a low gloss, the specular component is weak and the diffused light is stronger. The measuring geometry d:8° features an optical device which provides diffuse illumination (Ulbricht sphere). The light (Xenon lamp) is projected into a sphere. The interior of the sphere is coated with a white highly reflecting substance (barium sulphate, ceramic, special plastic) which reflects the light manifold. A shutter, an optical element inside the sphere, prevents the directional rays from reaching the measuring sample directly. The sample is positioned at an opening of the sphere and is illuminated from all directions with a close to perfect diffuse light. Through an opening at the top of the sphere the sensor is viewing the surface being measured with an angle of 8° to the vertical. In order to prevent reflection of specular light from the sample surface, the instrument feature a gloss trap. When the trap which is arranged with an angle of -8° to the viewing opening, is open, the light which would otherwise be reflected from the interior wall of the sphere, will be eliminated and can therefore not illuminate the sample. The relation between directional and diffuse reflection allows calculating the gloss component. The measuring system including gloss is named di:8° whilst the measuring system excluding gloss is described as de:8°.

1.4. Expert scoring

The experimenter will evaluate according to an internal clinical scale (on digital pictures) the following parameters:

- **OVERALL HAIR ASPECT**

Box 1. Clinical classification of overall hair aspect at T28 and T84	Score
No variation	1
Slight improved	2
Moderately improved	3
Remarkably improved	4

1.5. Digital macrophotography

Digital pictures are taken, under standard lighting conditions, using a professional digital reflex camera. Two images per subject are taken: one frontal view and one image at vertex level. Images will be taken under standard reproducible lighting conditions.

The 4 best cases (2 for each active groups) will be inserted in the final report.

1.6. Self-assessment questionnaire (T84)

At the end of the study (T84) subjects are asked to express their personal opinion on the tested treatment by answering to a questionnaire about products acceptability and effects. *The self-assessment questionnaire is reported in the Annex I (English and Italian versions).*

2. 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 (contact at Complife: Gloria Roveda T: +39 0382 25504). If a subject reports a reaction, the principal investigator has to decide if it is related to the investigational product use. If yes, she reports it as 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).

2.1. 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 unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a tested 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 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 (Cristiano Meggiato - cmeggiato@biosline.com) with a copy to the project manager. SAE must be sent within 24 hours after the observation. Reactions related to the product must be reported as soon as possible. If pictures 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.

2.2. Tolerance assessment

For each sign, intensity, location, duration (hours, minutes), and 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 is recorded. 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 use, way of use), iii) description of the reaction (functional and physical signs, intensity of the signs, location, date/time of onset, timeframe between product use 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 (use 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 is done in conjunction with clinical expertise, knowledge of the product (type of product, conditions of use...), identification of concomitant events.

2.2.1. Causality assessment of local tolerance

Five 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/Ophthalmologist 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 are taken, and joined to results at the end of the study.

3. STATISTIC

3.1. Study population for analysis
A total of 66 subjects will be enrolled in the study. Efficacy analysis is based on the Per Protocol Population (for subjects with a compliance $\geq$80%). 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 one or more evaluation visit; or they do not used the product properly during the study period (as referred by the subject itself). Analysis of safety will be based on the Intent to Treat Population (IT) that is defined as all subjects that have been assigned a subject number and received at least one study treatment.

3.2. Descriptive analysis
Demographic variables (age) and randomization list will be shown in a Microsoft® Excel sheet. The quantitative demographic variable (age) will be described with i) the mean value, ii) the minimum value, iii) the maximum value, iv) the standard deviation.
Sample data of the endpoints will be reported for the PP population and will be described, represented, and summarised using common descriptive statistical techniques. They will be reported in a Microsoft® Excel sheet and will be described with i) the mean value and/or the median value, ii) the standard error and/or interquartile range, iii)

the individual variation or percentage variation (vs. basal value) if applicable, iv) the minimum variation or percentage variation (vs. basal value) if applicable, v) the maximum variation or percentage variation (vs. basal value) if applicable, vi) the mean variation or percentage variation (vs. basal value) if applicable.
The data on the self-evaluation questionnaire will be described with the frequency percentages of subjects that assigned a judgment (among those proposed).

3.3. Statistical analysis

To assess the efficacy of the product, inferential statistical analyses will be performed on the endpoints.
For each quantitative and qualitative endpoints (except self-evaluation questionnaire), appropriate parametric or non-parametric technique will be applied to compare active and placebo and to evaluate their effect over time. The threshold of significance, α level, is established at 0,05. Statistical analysis will be performed using NCSS 10 data analysis (Copyright © 1983-2015, NCSS, LLC.).

4. 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 initialled/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. 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.

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.

5. 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.

ANNEX 1
FINAL SELF-ASSESSMENT QUESTIONNAIRE – ENGLISH VERSION
At the end of the study (T84) subjects are asked to express their opinion on the tested products by answering to a questionnaire about products acceptability and effects.

No.	Question	Completely agree	Agree	Disagree	Completely disagree
01	My hair looks healthier	■	■	■	■
02	My hair has a natural bounce and is fuller	■	■	■	■
03	My hair looks shinier	■	■	■	■
04	My hair feels stronger and more resilient	■	■	■	■
05	My hair seems repaired	■	■	■	■
06	My hair seems thicker	■	■	■	■
07	My hair feels more flexible and less brittle	■	■	■	■
08	My hair feels silky and smooth	■	■	■	■
09	My hair feels more manageable and less frizzy	■	■	■	■
No.	**Question**	**Very satisfied**	**Satisfied**	**Unsatisfied**	**Very unsatisfied**
10	Are you satisfied with the product?	■	■	■	■
No.	**Question**	**Yes**	**No**	**--**	**--**
11	Was the product well tolerated?	■	■	--	--
12	Would you buy the product?	■	■	--	--

FINAL SELF-ASSESSMENT QUESTIONNAIRE – ITALIAN VERSION

No.	Domanda	Completamente d'accordo	D'accordo	In disaccordo	Completamente in disaccordo
01	I miei capelli appaiono più sani	■	■	■	■
02	I miei capelli hanno una naturale elasticità e sono più voluminosi	■	■	■	■
03	I miei capelli appaiono più lucenti	■	■	■	■
04	Percepisco i miei capelli più forti e resilienti	■	■	■	■
05	Percepisco i miei capelli riparati	■	■	■	■
06	I miei capelli appaiono più folti	■	■	■	■
07	Percepisco i miei capelli più flessibili e meno fragili	■	■	■	■
08	Percepisco i miei capelli setosi e lisci	■	■	■	■
09	I miei capelli sono più gestibili e meno crespi	■	■	■	■
No.	Domanda	Molto soddisfatta	Soddisfatta	Insoddisfatta	Molto insoddisfatta
10	Sei soddisfatto del prodotto?	■	■	■	■
No.	Domanda	Si	No	--	--
11	Il prodotto è stato ben tollerato?	■	■	--	--
12	Acquisteresti il prodotto?	■	■	--	--