CLINICAL AND INSTRUMENTAL EVALUATION OF THE FACE BIOREVITALIZATION EFFECT WITH THE MEDICAL DEVICE

FOLIAGE HYDROFIL

Clinical Investigation Plan (CIP) code: *FOLIAGE - 2022*

Version 1, 02/02/2022

Sponsor

Phitogen Holding S.p.A.
Via Valtellina, 19/21/23
63074 San Benedetto del Tronto (AP)
Italy
Phone: +39 0735-762020
Fax +39 0735-758350

Principal Investigator

Dr. Tiziana Lazzari, MD
Poliambulatorio di Medicina Estetica
Casa della Salute
Largo XII Ottobre 62 - Genova
Phone: +39 010-8078631

Sponsor's Approval Page

***Technical Director*:** Dott. Stefano Chahine

Signature & Date: _____

Quality Assurance: Dott.sa Giada Iachini

Signature & Date: _____

Principal Investigator's Approval Page

I understand that all information concerning the medical device Foliage Hydrofil supplied by Phitogen Holding SpA and/or Latis Srl in connection with this study protocol are confidential information. This information include: CIP, Case Report Form, product documents.

I understand that any change in this study protocol must be approved in writing by Phitogen Holding SpA the Co-ordinating Investigator and the Ethics Committee before implementation, except where necessary to eliminate apparent immediate hazard to subjects.

I confirm that I will conduct the study according to this protocol (except when mutually agreed to in writing with Phitogen Holding SpA or its delegate Latis Srl), the Good Clinical Practice (GCP), the UNI EN ISO 14155, the Declaration of Helsinki current edition and laws and regulations in the Country where the study is to be conducted.

I confirm that I will record and report all adverse events occurring during the study, according to this protocol.

I confirm that I am informed about the need of data records retention, according to current regulations and that no data can be destroyed without the written consent of Phitogen Holding SpA.

I confirm that I will transfer adequate ownership of my responsibilities for the clinical investigation and will inform the Sponsor, in case I retire from my PI role.

I confirm that in case the Trial Center File is stolen or anyhow damaged, I will promptly inform the Sponsor and declare it to the Competent Authorities.

Principal Investigator: ……

Signature & Date: _____

General information

Sponsor Representative Contact	*Technical Director* – Dr. Stefano Chahine Phone. +39 0735/762020 E-mail. farmacovigilanza@phitogenspa.it *Quality Assurance – Dr.ssa Giada Iachini* *P*hone. +39 0735/762020 E-mail. quality@phitogenspa.it
Clinical CRO	Latis Srl – Viale Sauli 39/5 – 16121 Genova Phone. +39 010 562234 Fax. +39 010 540699 E-mail. info@latiscro.it
Contacts for safety reporting	
CRO Contact for Serious Adverse Events (SAEs) and Reportable Events	Latis Srl – Viale Sauli 39/5 – 16121 Genova Phone. +39 010 562234 Fax. +39 010 540699 Laura Michellini *Scientific Director* Mobile. +39 347 4785898 E-mail. michellini@latiscro.it Phitogen Holding S.p.A. - Via Valtellina, 19/21/23 63074 San Benedetto del Tronto (AP) Dr Stefano Chahine Phone. +39 0735 762020 E-mail. farmacovigilanza@phitogenspa.it

TABLE OF CONTENTS

1. SYNOPSIS OF THE CLINICAL INVESTIGATION PLAN

CIP Title	CLINICAL AND INSTRUMENTAL EVALUATION OF THE FACE BIOREVITALIZATION EFFECT WITH THE MEDICAL DEVICE FOLIAGE HYDROFIL
CIP Code	FOLIAGE - 2022
Sponsor	Phitogen Holding S.p.A.
Countries and Sites	Italy – 1 Clinical Site
Study Design	Post market, prospective, single arm, clinical investigation.
Objectives	*Primary objective* To evaluate the clinical performance of Foliage Hydrofil in the improvement of the skin hydration in the treated area (face) at Day 98, from baseline. *Secondary objectives* ▪ To evaluate the clinical performance of Foliage Hydrofil in the improvement of the skin hydration in the treated area at Day 21 and 42, from baseline; ▪ To evaluate the improvement of the skin texture, skin tone, smoothing of fine lines and wrinkles in the treated area at Day 21, 42 and 98, from baseline; ▪ To evaluate the aesthetic change from baseline as judged by the Investigator at Day 21, 42 and 98; ▪ To evaluate the aesthetic change from baseline as judged by the subject at Day 21, 42 and 98; ▪ To evaluate the subject's satisfaction at Day 21, 42 and 98. *Safety objectives* ▪ To assess the local safety at injection site (pain, erythema, edema, bruising) of the treatment with Foliage Hydrofil; ▪ To assess the systemic safety of the treatment with Foliage Hydrofil.

Primary study endpoint of clinical performance	Evaluation of the skin hydration modification from baseline to Day 98. Changes will be evaluated through corneometer skin examination.
Secondary study endpoints of clinical perfomance	- Evaluation of the skin hydration modification from baseline to Day 21 and 42, through corneometer examination - To evaluate the number of responders to Foliage Hydrofil treatment at Day 98; - Evaluation of the of the skin texture, skin tone, smoothing of fine lines and wrinkles modification from baseline in the treated area at Day 21, 42 and 98; - Investigator's aesthetic change assessment using the Global Aesthetic Improvement Scale (GAIS) on Day 21, 42 and 98. GAIS is a categorical scale with 5-points, from worse than before treatment to "optimal improvement"; - Subject's aesthetic change assessment using the Patient's Aesthetic Improvement Scale (PAIS) on Day 21, 42 and 98. PAIS is a categorical scale with 5-points, from "worse" to "very much improved"; - The overall subject's satisfaction at on the treatment at Day 21, 42 and 98, through a 5-points scale (very much satisfied, satisfied, not satisfied nor unsatisfied, unsatisfied, very unsatisfied).
Safety endpoints	Number, typology, severity of local and systemic adverse events. Particular attention will be paid to local adverse events like pain, erythema, edema, bruising.
Study Treatment	Foliage Hydrofil is a CE marked, class III, resorbable medical device (sterile, non-pyrogenic and physiological gel) to be used for the hydration of the skin and for the correction of superficial skin imperfections of the face and body and in the process of repairing the dermal tissue. The main component of Foliage Hydrofil is HA sodium salt of non-animal origin, produced by bacterial fermentation. Each subject will receive three treatments, one vial 2mL (1.6% HA formulation) each, with Foliage Hydrofil, 3 weeks apart. Foliage Hydrofil will be injected with needles 30G 4mm only. The area treated with the

	study treatment will be the face. The device administration could be done after an anesthetic cream application, according to Investigator's judgement.
Study Duration	For each subject the clinical investigation will be of about 14 weeks (3,5 months). The enrolment period will be of 4-6 months; therefore, the overall project duration will be of maximum 10 months.
Number of Subjects	24 females and males planned.
Target Study Population	Caucasian women and man of 30-55 years, with signs of skin aging in the face.
Selection Criteria	*Inclusion Criteria* Subjects presenting all the following inclusion criteria will be eligible to enter the study: 1. Generally healthy man and women, aged between 30 and 55 years (inclusive), looking for aesthetic procedures to improve the aspect of their face. 2. Caucasian race (skin type from I to IV as per Fitzpatrick Skin Type scale) 3. Visible signs of skin aging in the face (e.g. reduced skin elasticity, reduced turgor, actinic elastosis, smaller and larger wrinkles) according to the Investigators opinion; 4. Corneometer Capacitance of the area treated $\leq$ 80 5. Subjects wishing not to modify their lifestyle during the study period; 6. Subjects with cooperative attitude, able to comprehend the full nature and the purpose of the investigation, including possible risks, side effects and discomforts and willing to adhere to the study schedule (including attendance to the planned visits) and study prescriptions; 7. Written informed consent release prior to any study-related procedures.

	Exclusion criteria Subjects presenting one or more of the following exclusion criteria will not be eligible to enter the study: 1. Pregnancy or breastfeeding woman or woman of childbearing potential not practicing adequate contraception. 2. Subjects with Fitzpatrick skin type from V to VI 3. Subjects with known allergy/hypersensitivity to product components. 4. Extensively photo damaged and aged skin according to the Investigators opinion. 5. Active skin disease, inflammation or related conditions, such as infection, perioral dermatitis, seborrheic eczema and rosacea in the face, hands or décolletage. 6. Immune system disorders 7. History of or active collagenosis (e.g. systemic lupus, erythematosis, rheumatic arthritis, skin or systemic sclerosis). 8. Concomitant therapy with thrombolytics or anticoagulants, or have taken inhibitors of platelet aggregation, within 2 weeks before the first product administration. 9. History of cancerous or pre-cancerous lesions in the face, neck and décolletage. 10. Tattoo/s on the skin around the treatment site 11. Previous tissue augmenting therapy with non-permanent filler or treatment with botulinum toxin, aesthetic surgical therapy, laser treatment, mesotherapy, or any form of peeling in the face, hands or décolletage within 12 months prior to the baseline visit. 12. Use of injectable revitalization preparations within 12 months prior to the baseline visit. 13. Use of retinoic acid within 6 months prior to the baseline visit. 14. Previous or concomitant treatment with chemotherapy, immunosuppressive agents or corticoids. 15. History of treatment with permanent filling materials.

	16. Participation in any other clinical study within 30 days prior to the first visit or plan to participate in another clinical study during this study period. 17. Planned or ongoing weight reduction program during the study. 18. Known history of drug or alcohol abuse within 6 months prior to the baseline visit. 19. Known hypersensitivity to one or more components of anesthetic creams 20. Nicotine use during the study or stopped within 12 months before the first visit. 21. Any medical condition that in the opinion of the Investigator makes the subject unsuitable for inclusion (e.g. severe chronic disease, malignancy, bleeding disorder, skin diseases etc). 22. Other condition preventing the subject to entering the study in the Investigator's opinion e.g. subjects anticipated to be unreliable, unable to return for the follow-up visits, not likely to avoid other prohibited treatments or procedures or incapable of understanding the information or instructions.
Previous and Concomitant Treatments	*Non permitted pharmacologic and non-pharmacologic treatments*: ▪ any tissue augmenting therapy with non-permanent filler or treatment with botulinum toxin, aesthetic surgical therapy, laser treatment, mesotherapy, or any form of peeling in the face, or décolléte; ▪ use of injectable revitalization preparations; ▪ use of retinoic acid; ▪ treatment with permanent filling materials ▪ chemotherapy, immunosuppressive agents or corticoids; ▪ thrombolytics, anticoagulants, inhibitors of platelet aggregation ▪ sun exposure or tanning booths or UV sources (SPF 50+ should applied in case sun exposure cannot be avoided).

	To be avoided close to the treatment, the intake of any treatment (aspirin, N.S.A.I.D., Vitamin E) acting on the fluidity of the blood, to minimize the possibility of bruising or bleeding of the injected area.
Sample size determination	24 males and females will be enrolled. Assuming an average difference in the skin hydration evaluated through corneometer skin examination between baseline and Day 98 of 7.5, with a standard deviation of 8, 20 subjects will allow to appreciate this difference as statistically significant at an alpha = 0.05 and a 95% power. Considering the above and a possible drop-out rate of 20%, 24 subjects will be recruited.
Statistical Analysis	Descriptive statistical analysis of all relevant variables will be performed. Continuous variables will be summarized by the number of subjects (N), mean, standard deviation, median, minimum, maximum. Categorical variables will be summarized by the number (N) and the proportion of subjects (%). Where appropriate, 95% confidence intervals for the target variables will be estimated. The significance level of statistical tests will be set at 0.05. Parametric tests (e.g., paired t-test) will be used to analyze continuous variables; when continuous variables are not normally distributed, the corresponding non-parametric tests (e.g. Wilcoxon signed-sum rank test) will also be performed. Details of statistical analysis are provided in the following paragraphs. The statistical analysis will be performed using SAS 9.4 for Windows (SAS Institute Inc., Cary, NC, USA).
Primary performance endpoint Analysis	Paired t-test/Wilcoxon signed rank test will be used to assess a significant change in skin hydration, evaluated through corneometer skin examination, from baseline to Day 98.
Statistical Analysis – Secondary performance endpoints	Paired t-test/Wilcoxon signed rank test will be used to assess a significant change in skin hydration, evaluated through corneometer skin examination, from baseline to Day 21 and Day 42. Subjects will be considered responders when skin hydration increase is at least 10% between baseline and Day 98 and non-responders when

	there is not any increase in skin hydration. The number (N) and proportion of subjects (%) for each category will be reported. The skin texture, skin tone, smoothing of fine lines and wrinkles will be summarized at all time-points in terms of number (N) and percentage (%). Subjects will be considered responders when Investigator's evaluation of skin texture, skin tone and smoothing of fine lines increases at least one category on the assessment scale; otherwise, subjects will be considered non-responders. Subjects will be considered responders when Investigator's evaluation of wrinkles decreases at least two grades on the severity rating scale; otherwise, subjects will be considered non-responders. Subjects whose grade on the severity rating scale of Investigator's evaluation of wrinkles decreases from 2 "Slight" to 1 "Absent" will also be considered responders. The Global Aesthetic Improvement Scale (GAIS) filled-in by the investigator and the Patient's Aesthetic Improvement Scale (PAIS) filled-in by the subject will be summarized at all time-points in terms of number (N) and percentage (%). Subjects will be considered responders when the evaluation increases at least one level on the categorical scale; otherwise, subjects will be considered non-responders The overall subject's evaluation of satisfaction with the treatment at Day 98, performed by means of the 5-item scale, will be summarized through number (N) and proportion of subjects (%) for each item.
Statistical Analysis – Safety endpoints	The number of subjects who experienced at least one adverse event (AE) or adverse device event (ADE), study product-related AE or ADE, serious AE or ADE, severe AE or ADE and the number of subjects withdrawn due to AE will be summarized. For each system/organ class term (SOC) and preferred term (PT), summaries will be made with respect to the proportion of subjects having at least one occurrence of that event during the study and the total number of events.

	Local tolerability at the site of administration will be reported and summarized (with particular attention to pain, erythema, edema, bruising).
Population	Safety analysis set (SAF): all subjects enrolled who sign informed consent and receive the treatment. Full analysis set (FAS): all subjects of the SAF who have performed the baseline assessments and have at least one post-baseline assessment of any performance endpoint (primary or secondary). Per-Protocol analysis set (PPAS): all subjects of the FAS who also meet all inclusion/exclusion criteria and who do not have any major protocol deviation.

Figure 1: *Study design*

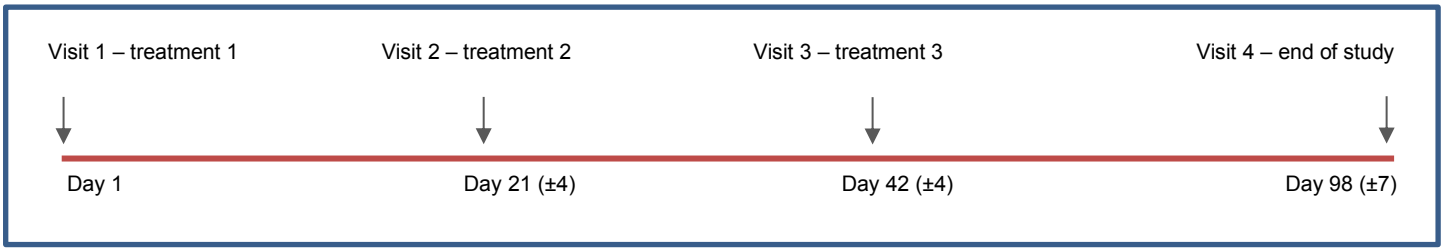

Table 1: *Study Flow-Chart*

Visit number	**V1**	**V2**	**V3**	**V4**
Time	**Day 1**	**Day 21** (allowed window: ±4 days)	**Day 42** (allowed window: ±4 days)	**Day 98** (allowed window: ±7 days)
Phase	**Screening, baseline, Treatment 1**	**Treatment 2**	**Treatment 3**	**Follow-up / End of study**
Informed consent	X			
Inclusion/exclusion criteria	X			
Demography	X			
Medical and surgical history	X			
Previous/Concomitant treatments	X	X	X	X
Physical examination	X	X	X	X
Pregnancy test (urine dipstick, where applicable)	X			
FOLIAGE HYDROFIL ADMINISTRATION	**X**	**X**	**X**	
Photos of the treated areas	X*	X*	X*	X
Corneometer analysis of treated areas	X*	X*	X*	X
Skin surface characteristics (texture, tone etc.)	X*	X*	X*	X
PAIS (Subject)		X	X	X
GAIS (Investigator)		X	X	X
Overall satisfaction by subject				X
AEs collection	X	X	X	X

*before the administration of the study product.

2. Identification and Description of the Investigational Device

Foliage Hydrofil is a CE marked, class III, resorbable medical device (sterile, non-pyrogenic and physiological gel) to be used for the hydration of the skin and for the correction of superficial skin imperfections of the face and the body and in the process of repairing the dermal tissue. The main component is HA sodium salt of non-animal origin, produced by bacterial fermentation, with a molecular weight similar to the human HA. Foliage Hydrofil does not contain human tissues or their derivatives.

Foliage Hydrofil is indicated for superficial cutaneous blemishes of the face or other body areas.

The manufacturer, Phitogen Holding S.p.A. is also the Sponsor of this post-market clinical follow-up study.

3. Justification for the Design of the Clinical Investigation

The slowing of aging process is interested by numerous researches; more figures (plastic surgeon, cosmetic surgeon etc) pay attention to aging of face in modern society [1]. Skin aging process could be viewed as an alteration occurring in collagen, elastin and, mainly, in HA content [2, 3]. HA degradation, together with the bone resorption, plays a major role in the formation of wrinkles and other signs of aging, and structural proteins are essential to skin health and youthfulness [4, 5]. The increasing concern to maintain a youthful appearance has driven the growth of new dermatological procedures for treatment of skin aging. So, in recent years there has been an increasing emphasis on minimally invasive treatments and techniques designed to treat volume loss, wrinkles, and skin photodamage [6].

Among treatments today available, bio-revitalization is a process which triggers the skin's own, natural collagen production and cell rejuvenation through the injection of hyaluronic acid serums. Hyaluronic acid, (HA) is a naturally occurring molecule in the body which is found in the skin, joints, cartilage and other areas, providing natural lubrication. This substance is hydrophilic, drawing many times its weight in water. As we age, we have less naturally produced HA in our tissues and skin dehydration becomes a telltale sign of aging. The HA owns a great hydrating power, due to this property is widely used in the biorevitalization treatments. Its use results in supple, younger-looking skin with reduced wrinkles and improved elasticity [7, 8].

In bio-revitalization procedures that involve intradermal micro-injections of natural HA, with a molecular weight close to the endogenous one, carried out in correspondence with the imperfections, the viscoelastic properties of HA allow to rehydrate the tissues, allowing to prevent and contrast the aging process skin and promote their remodeling.
Although the procedure can be performed on almost any area of the skin, the most popular treatment areas are the face, neck and décolleté area and the back of the hands. These areas of the body tend to be more susceptible to sun damage and as a result people often notice aging in these areas first.
Today, intradermal injections of biological substances able to induce a revitalization of the dermis can stimulate qualitative and quantitative improvements in aging skin alterations. The most frequently used substance is natural noncross-linked hyaluronic acid (HA), which is the main ingredient of the medical device Foliage Hydrofil.
The aim of this study is to confirm the clinical performance and safety of the medical device Foliage Hydrofil on the face bio-revitalizing treatment. To this purpose no. 24 adults (males and females) searching for bio-revitalization treatment will be enrolled and treated with three injective sessions apart 3 weeks each other. All the subjects will receive the same treatment in an open label fashioned study.

4. Benefits and Risks of the Investigational Device, Clinical Procedures and Clinical Investigation

Foliage Hydrofil belongs to a class of minimally invasive treatments used to maintain a youthful appearance. All the subjects will be treated in the face sites agreed with the physician.
The product will be injected through micro-injections in the derma. Injections, even if done with micro needles could generate some risks and a certain level of discomfort to the subject. In order to avoid risks, the Investigator will duly disinfect the injection sites before treatments. If necessary, an anesthetic cream could be used before injections.
Potential side effects due to the treatment are mainly pain, erythema, edema, bruising, but they are usually of mild intensity and transient. In such cases the Investigator will suggest the appropriate treatment to manage any side effect.

No tests or invasive examinations are foreseen in this study that can increase the risk for participants.
In the light of the above, no additional risk arising from the participation to this clinical investigation can be envisioned.

5. Objectives and Hypotheses of the Clinical Investigation

5.1 Primary Objective and Endpoint

The primary objective of this clinical investigation is to evaluate and confirm the clinical performance of Foliage Hydrofil in the improvement of the skin hydration in the treated area at Day 98, from baseline.

The primary endpoint of the study is the change in skin hydration from baseline to Day 98, evaluated through corneometer skin examination.

5.2 Secondary Objectives and Endpoints

The secondary objectives of this clinical investigation are:

- to evaluate the clinical performance of Foliage Hydrofil la in the improvement of the skin hydration in the treated area at Day 21 and 42, from baseline;
- to evaluate the improvement of the skin texture, skin tone, smoothing of fine lines and wrinkles in the treated area at Day 21, 42 and 98, from baseline;
- to evaluate the aesthetic change from baseline as judged by the Investigator at Day 21, 42 and 98;
- to evaluate the aesthetic change from baseline as judged by the subject at Day 21, 42 and 98;
- to evaluate the subject's satisfaction at Day 21, 42 and 98.

Performance secondary endpoints of this clinical investigation are:

- the evaluation of the skin hydration modification from baseline to Day 21 and 42, through corneometer examination.
- the evaluation of the of the skin texture, skin tone, smoothing of fine lines and wrinkles modification from baseline in the treated area at Day 21, 42 and 98;

- the Investigator's aesthetic change assessment using the Global Aesthetic Improvement Scale (GAIS) on Day 21, 42 and 98. GAIS is a categorical scale with 5-points: worse than before treatment, no change, minimal improvement, good improvement, optimal improvement;
- the subject's aesthetic change assessment using the Patient's Aesthetic Improvement Scale (PAIS) on Day 21, 42 and 98. PAIS is a categorical scale with 5-points: worse, no change, somewhat improved, moderately improved improved, very much improved;
- the evaluation of the overall subject's satisfaction at on the treatment at Day 21, 42 and 98, through a 5-points scale (very much satisfied, satisfied, not satisfied nor unsatisfied, unsatisfied, very unsatisfied).

5.3 Tolerability and Safety Objectives and Endpoints

To evaluate the local safety at injection site (pain, erythema, edema, bruising) and the systemic safety of the treatment with Foliage Hydrofil.

Safety endpoints are:

- the number, typology, severity of local and systemic adverse events. Particular attention will be paid to local adverse events like pain, erythema, edema, bruising.

6. Design of the Clinical Investigation

6.1 General

This is a monocenter, open label post-market clinical follow-up investigation.
Each subject will be treated with the study device.
The subject will start treatment on the day (Day 1) after the first visit and two further treatments at 3 weeks distance. Two months after the last treatment, a final visit will be planned to evaluate the final aesthetic result.
Each subject for whom written consent is obtained will be identified during the study by a "Subject Code", automatically generated and assigned by the e-CRF.

All screened subjects will receive the Subject Code regardless of whether they receive the treatment or not. If a subject discontinues from the study at any time, the Subject Code will not be re-used.
All subjects who will sign the informed consent and receive the Subject Code will be entered into a Subject's Register, containing the name and surname of the subjects and their Subject Codes. The Subject's Register will be accessible for source data verification but will be preserved only at clinical site.
The Investigator will keep record of all enrolled subjects in the Subject's Screening/Enrolment Log: Subject Code, the date of consent, or eventually the reason for not actively entering the study or interrupting the study will be recorded.

6.2 Investigational device

6.2.1 Foliage Hydrofil

Foliage Hydrofil is a CE marked, class III, resorbable medical device (sterile, non-pyrogenic and physiological gel) to be used for the hydration of the skin and for the correction of superficial skin imperfections of the face and body and in the process of repairing the dermal tissue. The main component is HA sodium salt of non-animal origin, produced by bacterial fermentation, with a molecular weight similar to the human HA. Foliage Hydrofil does not contain human tissues or their derivatives.
Foliage Hydrofil is indicated for superficial cutaneous blemishes of the face or other body areas.
The medical device is formulated as injectable vials of 2 mL (1.6% HA formulation). In this clinical investigation each subject will receive three treatments, one vial 2mL each, with Foliage Hydrofil, 3 weeks apart. Foliage Hydrofil will be injected with 30G 4mm only. The area treated will be the face. The device administration could be done after an anesthetic cream application, according to Investigator's judgement.

6.2.1.1 Packaging and labelling

The Foliage Hydrofil will be provided for the study by the Sponsor of this clinical investigation.
The device shipment to the study site will be done only after the completion of Ethics and administrative procedures.

The market packaging and labelling will be used. Only and additional label will be added to the outer box, specific for the clinical investigation.
Each package of treatment will contain:
• Instruction for use (IFU)
• 1 sealed blister containing 1 single-dose / single-use sterile pre-filled syringe (1x2 ml)
• adhesive labels to be applied on the clinical chart/patient card for product tracking
• 1 needle

6.2.1.2 Study device instructions for use

Foliage Hydrofil is intended for injection in the derma, for the hydration of the skin and the correction of skin blemishes, thanks to its HA content.
The device will be administered at clinical site by a qualified physician, already experienced with the use of similar devices. In any case specific instructions for the correct management of the study product will be provided by the Sponsor before the enrolment start. Product instructions for use (IFU) shall be fully complied.
Each treatment session will involve multiple microinjections, with needles 30G 4mm, in the face (external corner of the eye and cheek). The amount of product injected will be up to 2 mL, according to Investigator's judgement; the microinjections will be performed at a distance of 1–2 cm from each other.
An anesthetic cream could be used before injections, at Investigator's discretion.

The study product shall be carefully stored at the study site, in a safe area and separately from other devices/drugs/products. It shall be stored at room temperature (Min 2°C; Max 25°C), possibly in a dry and cool place, away from direct sunlight or heat.

6.2.1.3 Study product accountability and compliance

The Investigator is responsible for ensuring the accountability of the study product.
Accountability records will include:

- confirmation of product delivery and receipt to/at the clinical site
- records concerning the product administration to each subject
- the return to the Sponsor or alternative disposition of unused products.

The Investigator should maintain records that adequately document:

- that the subjects were treated with the quantities specified by the clinical investigation plan/amendment(s)
- that all study products provided by the Sponsor were fully reconciled.

Unused products must not be discarded or used for any purpose other than the present clinical investigation.

Subjects will be reminded of the importance of strictly complying with the instructions received from the Investigator.

Compliance to the treatment will be done through Investigator's records and vials accountability.

6.2.2 Concomitant Medications/Treatments

Any medication/treatment (other than those excluded by the clinical investigation plan) that is considered necessary for the subjects' well-being and does not interfere with the study product can be given/administered/prescribed at the Investigator's discretion.

The following prior and concomitant medications shall be avoided, as far as possible, during the study participation:

- any tissue augmenting therapy with non-permanent filler or treatment with botulinum toxin, aesthetic surgical therapy, laser treatment, mesotherapy, or any form of peeling in the face, or décolléte;
- use of injectable revitalization preparations;
- use of retinoic acid;
- treatment with permanent filling materials
- chemotherapy, immunosuppressive agents or corticoids;
- thrombolytics, anticoagulants, inhibitors of platelet aggregation
- sun exposure or tanning booths or UV sources (SPF 50+ should applied in case sun exposure cannot be avoided).

To be avoided close to the treatment, the intake of any treatment (aspirin, N.S.A.I.D., Vitamin E) acting on the fluidity of the blood, to minimize the possibility of bruising or bleeding of the injected area.

Any concomitant medication/treatment shall be recorded in the appropriate section of the eCRF.

6.3 Subjects

In this clinical investigation 24 caucasian man and women of 30-55 years, with signs of skin aging in the face and fulfilling the following eligibility criteria will be enrolled.
Some eligibility criteria reflect the device contraindications/precautions and other criteria have the purpose of avoiding confounding factors that could interfere with study results.

6.3.1 Inclusion criteria

Subjects presenting all the following inclusion criteria will be eligible to enter the study:

1. Generally healthy man and women, aged between 30 and 55 years (inclusive), looking for aesthetic procedures to improve the aspect of their face.
2. Caucasian race (skin type from I to IV as per Fitzpatrick Skin Type scale);
3. Visible signs of skin aging in the face (e.g. reduced skin elasticity, reduced turgor, actinic elastosis, smaller and larger wrinkles) according to the Investigators opinion;
4. Corneometer Capacitance of the area treated $\leq$ 80
5. Subjects wishing not to modify their lifestyle during the study period;
6. Subjects with cooperative attitude, able to comprehend the full nature and the purpose of the investigation, including possible risks, side effects and discomforts and willing to adhere to the study schedule (including attendance to the planned visits) and study prescriptions;
7. Written informed consent release prior to any study-related procedures.

6.3.2 Exclusion criteria

Subjects presenting one or more of the following exclusion criteria will not be eligible to enter the study:

1. Pregnancy or breastfeeding woman or woman of childbearing potential not practicing adequate contraception.
2. Subjects with Fitzpatrick skin type from V to VI
3. Subjects with known allergy/hypersensitivity to product components.

4. Extensively photo damaged and aged skin according to the Investigators opinion.
5. Active skin disease, inflammation or related conditions, such as infection, perioral dermatitis, seborrheic eczema and rosacea in the face, hands or décolletage.
6. Immune system disorders
7. History of or active collagenosis (e.g. systemic lupus, erythematosis, rheumatic arthritis, skin or systemic sclerosis).
8. Concomitant therapy with thrombolytics or anticoagulants, or have taken inhibitors of platelet aggregation, within 2 weeks before the first product administration.
9. Tattoo/s on the skin around the treatment site
10. History of cancerous or pre-cancerous lesions in the face, neck and décolletage.
11. Previous tissue augmenting therapy with non-permanent filler or treatment with botulinum toxin, aesthetic surgical therapy, laser treatment, mesotherapy, or any form of peeling in the face, hands or décolletage within 12 months prior to the baseline visit.
12. Use of injectable revitalization preparations within 12 months prior to the baseline visit.
13. Use of retinoic acid within 6 months prior to the baseline visit.
14. Previous or concomitant treatment with chemotherapy, immunosuppressive agents or corticoids.
15. History of treatment with permanent filling materials.
16. Participation in any other clinical study within 30 days prior to the first visit or plan to participate in another clinical study during this study period.
17. Planned or ongoing weight reduction program during the study.
18. Known history of drug or alcohol abuse within 6 months prior to the baseline visit.
19. Known hypersensitivity to one or more components of anesthetic creams
20. Nicotine use during the study or stopped within 12 months before the first visit.

21. Any medical condition that in the opinion of the Investigator makes the subject unsuitable for inclusion (e.g. severe chronic disease, malignancy, bleeding disorder, skin diseases etc).
22. Other condition preventing the subject to entering the study in the Investigator's opinion e.g. subjects anticipated to be unreliable, unable to return for the follow-up visits, not likely to avoid other prohibited treatments or procedures or incapable of understanding the information or instructions.

6.4 Procedures

6.4.1 Study Visits and Assessments

Visit 1 screening – baseline- treatment start (day 1):

Subjects will be evaluated with regard to the inclusion and exclusion criteria that will allow their participation into the study.

Before any study specific evaluation is carried out, subjects will receive all the information about the study by the Investigator and will sign an informed consent form.

The following activities will be performed at first visit:

- Collection of demographic information
- Medical and surgical history
- Physical examination (focused on the skin)
- Previous (in the 30 days before) and concomitant medications / treatments recording
- Urine pregnancy test (where applicable)
- Inclusion/exclusion criteria assessment
- Photos of the treated area (before injections)
- Corneometer examination of treated area (before injections)
- Skin surface characteristics, texture, tone, fine lines and wrinkles (before injections)
- First treatment
- Local and systemic adverse events after the first treatment.

The following treatment session will be scheduled three weeks later, with a window of ± 4 days. The subject will be solicited to contact the Investigator for any problem between visits.

Visit 2 (Day 21 ± 4) and Visit 3 (Day 42 ± 4):

The following activities will be done at Visit 2, and 3:

- Physical examination (focused on the skin)
- Change in concomitant medications / treatments recording
- Photos of the treated area (before second/third injection session)
- Corneometer examination of treated area (before second/third injection session)
- Skin surface characteristics, texture, tone, fine lines and wrinkles (before second/third injection session)
- PAIS Subject
- GAIS Investigator
- Second/third treatment
- Adverse events (occurred since previous visit) recording
- Local and systemic adverse events after the second/third treatment.

The second and third treatment sessions will be scheduled three weeks apart from each other, with a window of ± 4 days. The subject will be solicited to contact the Investigator for any problem between visits.

The final visit will be scheduled about 2 months after last treatment session.

Visit 4 – End of study (Day 98 ±7):

The following activities will be done at Visit 4:

- Physical examination (focused on the skin)
- Change in concomitant medications / treatments recording
- Photos of the treated area
- Corneometer examination of treated area
- Skin surface characteristics, texture, tone, fine lines and wrinkles
- PAIS Subject
- GAIS Investigator
- Overall satisfaction by subject
- Adverse events (occurred since previous visit) recording.

Should the subject interrupt or conclude in advance the clinical study, for any reason, a Visit 4 will be completed.

6.4.2 Corneometer assessment

Corneometer is one of the most popular measurement instrument to reproducibly and accurately determine the hydration level of the skin surface (stratum corneum).

The corneometer indicates the hydration level of the superficial layers of the skin via measurement of skin dielectric properties.

The measurements are performed by the application of a probe to the skin surface. Upon contact, an electric field passes through the stratum corneum and the dielectric constant is obtained.

The value of the dielectric constant (in arbitrary units) is directly proportional to the level of skin hydration.

On the probe head there is a fine piece of glass to ensure that only the capacitance changes due to water content are identified. Even small changes in water can be detected. The measurement time is short at only 1 second minimising occlusion effects.

The depth of the measurement is 10-20µm, therefore limited to the stratum corneum, ensuring that deeper skin layers do not influence the measurement.

The measuring surface is around 49mm^2, so even small areas can be accurately measured.

6.4.3 Skin texture, skin tone, fine lines and wrinkles assessment

The Investigator will evaluate at each visit the skin texture, skin tone, smoothing of fine lines and wrinkles modification in the treated area and will score each item as follows:

Skin texture	Poor	Good	Very good
	☐	☐	☐
Fine lines	Poor	Good	Very good
	☐	☐	☐

Skin tone	Poor	Sufficient	Middle	Good
	☐	☐	☐	☐

Wrinkles will be assessed through the severity rating scale:

Grade	Description
1	Absent
2	Slight
3	Moderate
4	Severe
5	Extreme

6.4.4 Global Aesthetic Improvement Scale (GAIS) by the Investigator

GAIS is a categorical scale with five levels:

Grade	Description
1	worse than before treatment
2	no change
3	minimal improvement
4	good improvement
5	optimal improvement

The Investigator will be requested to judge the aesthetic improvement of the treated area at all visits following the first one.

6.4.5 Patient's Aesthetic Improvement Scale (PAIS)

PAIS is a categorical scale with five levels:

Grade	Description
1	worse
2	no change
3	somewhat improved
4	moderately improved
5	very much improved

The subject will be requested to judge the aesthetic improvement of the treated area at all visits following the first one.

6.4.5 Subject Overall Satisfaction

Overall subject's satisfaction on the treatment received will be defined by means of a 5-items scale at the end of the study (Visit 4).

The subject will be asked “Please express your degree of satisfaction with the treatment received”

Grade	Description
1	very much satisfied
2	satisfied
3	not satisfied nor unsatisfied
4	unsatisfied
5	very unsatisfied

6.5 Monitoring Plan

The study will be monitored on a regular basis by the CRO’s adequately qualified and trained clinical Monitors throughout the study period to ensure the proper conduct of the clinical Investigation.

The purposes of study monitoring are to verify that the rights and well-being of study subjects are protected, that the reported study data are accurate, complete and verifiable against the source documents, and that the study is conducted in accordance with the current clinical investigation plan, Good Clinical Practice guideline (UNI EN ISO 14155) and applicable regulatory requirements.

During the monitoring visits, Monitors will verify the following, including but not limited to: subject informed consent, subject’s eligibility, safety data and reporting, quality of source documents and CRF data against subject’s medical records. If inconsistencies are found, the corresponding corrections to the CRF data will have to be made by the Investigator or designated person. Monitors will also check subject compliance, accrual, delegation of responsibilities within the Investigator’s team, relevant communications with family doctors, if any, ancillary equipment and facilities, etc. The Investigator and other site staff involved in the study must allocate enough time to the Monitor at these visits.

Given the current COVID-19 pandemic situation also proper approaches will be implemented remotely to allow the performance of the monitoring activities, in line with applicable guidelines of competent authorities and best practices.

Upon request by the Sponsor, on-site study audits may be conducted in order to ensure the study is in compliance with GCP, applicable regulatory requirements, and the clinical investigation plan. The auditing activities may also be conducted after study completion.

The Investigator agrees to allow Sponsor/auditors/CRO monitors to have direct access to his/her study records for review, being understood that they are bound by professional secrecy, and as such will not disclose any personal identity or personal medical information.
The Investigator will take all appropriate measures required by the Sponsor to implement corrective actions for all problems found during audits.

7. Statistical Design and Analysis

This section summarizes the statistical principles and methods planned to analyze the data for this clinical investigation.

7.1 Sample Size Determination

Assuming an average difference in the skin hydration evaluated through corneometer skin examination (capacitance) between baseline and Day 98 of 7.5, with a standard deviation of 8, 20 subjects will allow to appreciate this difference as statistically significant at an alpha = 0.05 and a 95% power. Considering the above and a possible drop-out rate of 20%, 24 subjects will be recruited.

7.2 Definition of Study Populations for Analysis

The following populations are defined for this investigation:

- Safety analysis set (SAF): all subjects enrolled who sign informed consent and receive at least one administration of the investigational device.
- Full analysis set (FAS): all subjects of the SAF who have performed the baseline assessments and have at least one post-baseline assessment of any performance endpoint (primary or secondary).
- Per-Protocol analysis set (PPAS): all subjects of the FAS who also meet all inclusion/exclusion criteria and who do not have any major protocol deviation.

The analysis of safety endpoints will be performed in the SAF population. Analysis of performance endpoints will be performed on the FAS population. Additionally, analysis of primary endpoint will also be performed on the PPAS population.

7.3 Statistical Analysis

Descriptive statistical analysis of all relevant variables will be performed. Continuous variables will be summarized by the number of subjects (N), mean, standard deviation, median, minimum, maximum. Categorical variables will be summarized by the number (N) and the proportion of subjects (%). Where appropriate, 95% confidence intervals for the target variables will be estimated.

The significance level of statistical tests will be set at 0.05. Parametric tests (e.g., paired t-test) will be used to analyze continuous variables; when continuous variables are not normally distributed, the corresponding non-parametric tests (e.g., Wilcoxon signed rank test) will also be performed. Details of statistical analysis are provided in the following paragraphs.

The statistical analysis will be performed using SAS 9.4 for Windows (SAS Institute Inc., Cary, NC, USA).

7.3.1 Missing Data

Missing data will not be replaced in any statistical analysis.

7.3.2 Multiplicity

No adjustment is needed.

7.3.3 Covariates, Interactions and Subgroups

There are no planned subgroup analyses.

7.3.4 Analysis of Demographics and Baseline Variables

Demographic (gender, age) and baseline characteristics will be summarized using mean, median, standard deviation, minimum and maximum for continuous variables and frequencies and percentages for categorical variables.

7.3.5 Efficacy analysis

7.3.5.1 Primary endpoint

Paired t-test, when the skin hydration is normally distributed, or the corresponding non-parametric Wilcoxon signed rank test will be used to assess a significant change in skin hydration, evaluated through corneometer skin examination (capacitance), from baseline to Day 98.

7.3.5.2 Secondary endpoints

Paired t-test, when the skin hydration is normally distributed, or the corresponding non-parametric Wilcoxon signed rank test will be used to assess a significant change in skin hydration, evaluated through corneometer skin examination (capacitance), from baseline to Day 21 and Day 42.

Subjects will be considered responders when skin hydration increase is at least 10% between baseline and Day 98 and non-responders when there is not any increase in skin hydration. The number (N) and proportion of subjects (%) for each category will be reported.

The skin texture, skin tone, smoothing of fine lines and wrinkles will be summarized at all time-points in terms of number (N) and percentage (%). Subjects will be considered responders when Investigator's evaluation of skin texture, skin tone and smoothing of fine lines increases at least one category on the assessment scale, from baseline; otherwise, subjects will be considered non-responders. Subjects will be considered responders when Investigator's evaluation of wrinkles decreases at least two grades on the severity rating scale, from baseline; otherwise, subjects will be considered non-responders. Subjects whose grade on the severity rating scale of Investigator's evaluation of wrinkles decreases from 2 "Slight" to 1 "Absent" will also be considered responders.

The Global Aesthetic Improvement Scale (GAIS) filled-in by the investigator and the Patient's Aesthetic Improvement Scale (PAIS) filled-in by the subject will be summarized at all time-points in terms of number (N) and percentage (%). Subjects will be considered responders when the evaluation increases at least one level on the categorical scale, from baseline; otherwise, subjects will be considered non-responders

The overall subject's evaluation of satisfaction with the treatment at Day 98, performed by means of the 5-item scale, will be summarized through number (N) and proportion of subjects (%) for each item.

7.3.6 Safety Analysis

All enrolled subjects receiving at least one treatment injection will be included in the safety analysis.

Adverse events (AEs) and Adverse Device Events (ADEs) will be coded using the last updated version of the Medical Dictionary for Regulatory Activities (MedDRA) dictionary to give a preferred term (PT) and a system/organ class term (SOC) for each event. The number of subjects who experienced at least one AE or ADE, study product-related AE or ADE, serious AE or ADE, severe AE or ADE and the number of subjects withdrawn due to AE will be summarized.

For each SOC and preferred term, summaries will be made with respect to the proportion of subjects having at least one occurrence of that event during the study and the total number of events. The incidence of AEs and ADEs will be presented overall, by SOC and preferred term, and additional grouping by severity and relationship to the study treatment.

Local tolerability at the site of administration will be reported and summarized.

7.4 Planned Interim Analysis

No interim analysis is planned.

8. Data Management

During each study visit, the study Investigator (or designee) will collect and report study data in the relevant subject's chart, documenting all significant observations.

Any contact with the subject via telephone or other means that provides significant clinical information shall be documented in the source data.

An Electronic Case Report Form (e-CRF) will be used to record subject's study data.

All data relating to the study will be documented in the electronic CRF (e-CRF). This CRF is developed to record the data requested by the protocol.

The Investigator will maintain a list of all persons authorized to make entries and/or corrections on the e-CRFs. Each authorized person will be provided with a user-specific

ID protected by a renewable password. Data entries and corrections will be made only by the authorized persons. The e-CRF system will record date and time of any entry and /or correction and the user ID of the person making the entry/correction. The system will keep track of all old and new values (audit trail). It is the responsibility of the Investigator to ensure that the CRFs are properly and completely filled in. The CRFs must be completed for all subjects who have been included in the study. The Investigator will review all CRFs and electronically sign and date them for each subject, verifying that the information is complete, true and correct. All fields on the CRF must be completed as applicable.

Checks to assist during the data entry and to assess the appropriateness and consistency of data will be developed on the e-CRF system. E-CRF pages will be reviewed both on site by the monitor of the center and remotely, by the data management staff of the CRO. Data Clarification Sheets (DCS) will be generated through the e-CRF system, both automatically, through edit checks, and manually, by CRAs and/or data managers, and the Investigator will have to check and solve them. The Investigator is responsible for the review and approval of all query resolutions.

Subjects will be provided with paper questionnaires/scales. Such documents will be filled by the subjects during the study visits, to record data concerning their opinion on the aesthetic result of the treatment and the consequent satisfaction with the treatment received.

It is responsibility of the Investigators to instruct the study participants on how to fill in questionnaires in a clear way and preferably in black ball-point pen. The questionnaires/scales will be anonymous, each subject is identified through the Subject Code. Questionnaires/scales data will be reported on the e-CRF by the Investigator (or designee). Questionnaires will be considered source data.

At the beginning of the study, a Study Master File will be established according to Sponsor or CRO SOPs, in order to maintain the study documents as specified in the UNI EN ISO 14155 Guideline and as required by the applicable regulatory requirements. The CRO will take measures to prevent accidental or premature destruction of these documents.

Clinical sites will permit study-related monitoring, audits, and regulatory inspection providing direct access to source data / documents.

Essential documents of the clinical investigation, including the subject identification list, source data and case report forms will be retained for 10 years after the completion or discontinuation of the clinical investigation at clinical sites. The sponsor will retain the study master file for 10 years after the CE mark for the study product has been discontinued in all countries.

9. Amendments to the CIP

Changes to the clinical investigation plan may only be made by means of a written amendment, which has to be approved and signed by the authorized representatives of the Sponsor, and by the Investigator.

Exhaustive justifications that motivate the amendment to the clinical investigation plan should clearly be addressed in the document.

All substantial Clinical Investigation Plan amendments must be submitted for approval to IEC and notified to the Regulatory Authority. In case of non-substantial amendment the IEC and the Regulatory Authority (when applicable) will be only notified of the amendment without the request to review and approve it.

The Investigator, the Sponsor and IEC, separately or together, should decide whether the subject's informed consent form needs to be changed.

10. Deviations from Clinical Investigation Plan

The Investigator is to conduct the study in accordance with the relevant, current clinical investigation plan and will only deviate when necessary to protect the safety, rights and welfare of the subjects. In the event that an isolated, unforeseen instance occurs resulting in a clinical investigation plan deviation, the Investigator is to document this deviation and notify the CRO or the Sponsor as soon as possible. In no instance should this increase the subject's risk or affect the validity of the study.

11. Device Accountability

The device will be administered only at clinical site under the responsibility of the Investigator. The Investigator is responsible of ensuring the correct device administration and for registering data of the treatment administered.
The study product will be provided to the clinical site once ethical authorization is obtained.

12. Statement of Compliance

The study will be conducted in compliance with the current version of the Declaration of Helsinki (Appendix 2), with the clinical investigation plan, the MDR 745/2017, the UNI EN ISO 14155, the Italian laws in force and the principles of the Good Clinical Practice. The clinical investigation will start at clinical site only after obtaining the approval of the relevant Ethics Committee.

13. Informed Consent Process

The Investigator is responsible for and will obtain informed consent from each subject, in accordance with the UNI EN ISO 14155, and the current version of the Declaration of Helsinki.
All subjects invited to participate in the study are entitled to make their voluntary decision based on all current available information provided to them by the Investigator/designee. In addition, they will be given a document in native language written in clear concise lay language for review and consideration. The document will previously have been approved by the relevant independent Ethics Committee (IEC) and may further be updated as new important information becomes available that may affect subject's willingness to participate or continue in the study.
The subject must be made aware that he/she may refuse to join the study or may withdraw his/her consent at any time without prejudicing further medical care and that he/she is covered by the Sponsor's indemnity insurance in the event of a study related injury. Subjects must also know that their personal medical records may be reviewed in confidence by the Sponsor's staff or representatives and by Regulatory Authority and

IEC and that personal information will be collected and retained in a confidential database. Consent will always be given in writing after the subject has had adequate time to review the information and ask questions, if need be.

14. Adverse Events, Adverse Device Effects and Devices Deficiencies

14.1 Definitions

Adverse Event (AE)

Any untoward medical occurrence, unintended disease or injury or any untoward clinical signs, including an abnormal laboratory finding, in subjects, users or other persons, in the context of a clinical investigation, whether or not related to the investigational device.

Adverse Device Effect (ADE)

Any untoward and unintended response to a medical device. This includes any event resulting from insufficiencies or inadequacies in the instructions for use or the deployment of the device and any event that is a result of a user error.

Device Deficiency

Any inadequacy in the identity, quality, durability, reliability, safety or performance of an investigational device, including malfunction, use errors or inadequacy in information supplied by the manufacturer.

Serious Adverse Event (SAE)

Any adverse event that led to any of the following:

a) death,

b) serious deterioration in the health of the subject, that resulted in any of the following:

i. life-threatening illness or injury,

ii. permanent impairment of a body structure or a body function,

iii. hospitalisation or prolongation of patient hospitalisation,

iv. medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function,

v. chronic disease

c) foetal distress, foetal death or a congenital physical or mental impairment or birth defect.

Serious Adverse Device Effect (SADE)

Adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event or that might have led to any of these consequences if suitable action had not been taken or intervention had not been made or if circumstances had been less opportune.

Unanticipated Serious Adverse Device Effect (USADE)

Serious adverse device effect that by its nature, incidence, severity or outcome has not been identified in the risk analysis report.

Incident

Any malfunction or deterioration in the characteristics and/or performance of a device, as well as any lack of labelling or instructions for use which, directly or indirectly, may cause or have caused death or serious deterioration in the health of the patient or of a user or of other persons.

14.2 Adverse Event Intensity/Causality

The Investigator, based on his direct observation or on subjects' report, will record the event according to the current version of CTCAE:

Description	Definition
Grade 1 (Mild):	asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2 (Moderate):	minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental daily life activities.
Grade 3 (**Severe):**	medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self -care daily life activities.
Grade 4 (Life-threatening consequences):	urgent intervention indicated.
Grade 5:	death related to AE

The relationship of any AE to the product will be classified by the Investigator as follows:

Description	Definition
Not related	Relationship to the device, comparator or procedures can be excluded.
Probable	The relationship with the use of the investigational device or comparator, or the relationship with procedures, seems relevant and/or the event cannot be reasonably explained by another cause.
Possible	The relationship with the use of the investigational device or comparator, or the relationship with procedures, is weak but cannot be ruled out completely.
Causal relationship	The adverse event is associated with the investigational device, comparator or with procedures beyond reasonable doubt.

14.3 Adverse Event, Device Deficiency, Adverse Device Effect, reporting

The Investigator will record any AEs, Device Deficiency and ADEs occurring during the study. He/She will also co-operate with the Sponsor in connection with the reporting of any “reportable event” to the Competent Authority and to the Independent EC, if applicable.

All AEs regardless of severity occurring between recruitment and completion of the study by a subject must be recorded on the AE form provided with the CRF.

If there is a significant worsening of a medical condition that was present before starting the study, this should be considered as a new AE and a complete evaluation recorded.

Signs and symptoms considered as lack of efficacy and occurring during the study will not be recorded on the AEs Section of the CRF except on the condition that, in the Investigator’s opinion, these signs and symptoms are caused by any reason different from lack of efficacy of the study product or meet the definition of serious AE.

In the event of a SAE (including a reportable event) the Investigator has to:

- Complete the relevant CRF pages and a reporting form with the available initial information,
- Immediately inform the Sponsor or the CRO by telephone,

- Fax the completed report form to the contact person at the Sponsor or the CRO as soon as possible but no later than 3 calendar days after the investigation site study personnel's awareness of the event.

Reporting contacts are on page 4 of this Clinical Investigation Plan.

The Investigator is responsible for ensuring the follow-up of any subject who experiences a serious adverse event (including any reportable event) during the study. The investigator must re-examine the subject at regular intervals until the symptoms have completely disappeared or stabilized.

The Sponsor or its delegate will be responsible for reporting the SAE/SADE/Serious Incident to the appropriate Authorities and EC, according to the local regulations.

15. Vulnerable Population

No vulnerable population will be included in this clinical investigation.

16. Suspension or Premature Termination of the Clinical Investigation

Unless premature interruption occurs, the end of the study will be the closure visit at clinical site.

16.1 Study Discontinuation

Subjects may be discontinued at any time from the study for any of the following reasons:

- An AE occurs that, in the opinion of the Investigator, makes it unsafe for the subject to continue in the study
- Lack of compliance of the subject to the study treatment or assessments
- The subject needs to use a medication/treatment not allowed
- The subject is lost to follow-up
- The subject dies
- The subject withdraws consent

- The Investigator, for any reason, terminates the entire study, or terminates the study for that subject or the attending physician requests that the subject be withdrawn for any medical reason
- The Sponsor or the Regulatory Authority or the Ethics Committee, for any reason, terminates the entire study or terminates the study for this site or for a particular subject.

If a subject is discontinued from the study, the Investigator will, as far as possible, complete the end of study visit (Visit 4) e-CRF fields. The Investigator should try to ascertain the reason(s) for withdrawal, while fully respecting the subject's rights.

16.2 Study Interruption

The Sponsor may consider study closure at the clinical site if the following occurs:

- serious and/or persistent non-compliance with the Clinical Investigation Plan
- inadequate collaboration of site personnel with CRO/Sponsor
- administrative reasons
- non-compliance with GCP, SOPs or regulatory requirements
- lack of confidentiality and/or non-compliance with the contract spread with the Sponsor.

17. Publication Policy

All information obtained as a result of the study will be regarded as confidential.

The results of the clinical study will be documented in an integrated clinical study report according to UNI EN ISO 14155.

The Sponsor and the Investigator agree that no publications presenting or discussing data and/or results from this clinical study sponsored by Phitogen Holding S.p.A. will take place until the participating center has completed the study, the data have been interpreted, and the final report has been issued.

As a rule, the Sponsor is free to use the data collected in the sponsored study for world-wide scientific product documentation, and for publication.

In general, the Sponsor has no objections if the Investigators publish the results of the study. However, the Investigator is requested to provide the Sponsor with a copy of the manuscript for review before submitting it to the publisher with a cover letter informing the Sponsor about the intention to publish the study results. When permission for

presentation or for publication is granted, Investigator, prior to submission of a manuscript or abstract to the publisher, shall forward a copy of said manuscript or abstract to the Sponsor who shall have 90 days to request any reasonable amendment thereto, which shall be taken into due account and consideration by the Investigators.
The Sponsor is entitled to include as authors of the publication all Sponsor's personnel who contributed substantially to the theoretical or experimental work and also to take part in the decision that establishes the order in which the authors' names will be given.
Costs for publication must be regulated by written agreement between the parties.
If publication of the results of the study, either in part or in full, is prepared by the Sponsor, the Investigator will be provided with a copy of the manuscript before the submission to the publisher and asked to give approval of the document. The Investigator will be asked in writing if they accept to be included as author of the publication. Answers should be sent in writing to the Sponsor within a reasonable time limit (30 days). If no answer is received, it is assumed that the Investigator agrees to the Sponsor's proposal.

18. Bibliography

1. Brandi C, Cuomo R, Nisi G, Grimaldi L, D'Aniello C. Face Rejuvenation: a new combinated protocol for biorevitalization. Acta Biomed. 2018 Oct 8;89(3):400-405.

2. Donejko M, Przylipiak A, Rysiak E, Miltyk W, Galicka E, Przylipiak J, et al. Hyaluronic acid abrogates ethanol-dependent inhibition of collagen biosynthesis in cultured human fibroblasts. *DrugDes Devel Ther*. 2015;24;9:6225–33.

3. Stellavato A, Corsuto L, D'Agostino A, La Gatta A, Diana P, Bernini P, De Rosa M, Schiraldi C. Hyaluronan Hybrid Cooperative Complexes as a Novel Frontier for Cellular Bioprocesses Re-Activation. PLoS One. 2016 Oct 10;11(10):e0163510. doi: 10.1371/journal.pone.0163510. PMID: 27723763; PMCID: PMC5056743.

4. Papakonstantinou E, Roth M, Karakiulakis G. Hyaluronic acid: A key molecule in skin aging.Dermato-endocrinol. 2012. 1;4(3):253–8. 10.4161

5. Croce MA, Dyne K, Boraldi F, Quaglino D Jr, Cetta G, Tiozzo Ret al.Hyaluronan affects proteinand collagen synthesis by in vitro human skin fibroblasts. *Tissue Cell*. 2001;33(4):326

6. Sparavigna A, Tenconi B, De Ponti I. Antiaging, photoprotective, and brightening activity in biorevitalization: a new solution for aging skin. Clin Cosmet Investig Dermatol. 2015 Feb 10;8:57-65.

7. Tedeschi A, Lacarrubba F, Micali G. Mesotherapy with an Intradermal Hyaluronic Acid Formulation for Skin Rejuvenation: An Intrapatient, Placebo-Controlled, Long-Term Trial Using High-Frequency Ultrasound. Aesthetic plastic surgery. 2015;39(1):129–33.

8. Gao F, Liu Y, He Y, et al. Hyaluronan oligosaccharides promote excisional wound healing through enhanced angiogenesis. Matrix biology: journal of the International Society for Matrix Biology.2010;29(2):107–16.

Appendix 1

List of Abbreviations

ADE	Adverse Device Effect
AE	Adverse Event
AIDS	Acquired Immune Deficiency Syndrome
CIP	Clinical Investigation Plan
CRF	Case Report Form
CRO	Contract Research Organization
CTCAE	Common Terminology Criteria for Adverse Events
FAS	Full Analysis Set
GAIS	Global Aesthetic Improvement Scale
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HA	Hyaluronic Acid
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonization
IFU	Instructions For Use
IEC	Indipendent Ethics Committee
MedDRA	Medical Dictionary for Regulatory Activities
ml	Milliliters
N	Number
PAIS	Patient's Aesthetic Improvement Scale
PI	Principal Investigator
PT	Preferred Term
QoL	Quality of Life
SADE	Serious Adverse Device Effect
SAF	Safety Analysis Set
SAE	Serious Adverse Event
SOC	System Organ Class
SOP	Standard Operating Procedure
USADE	Unanticipated Serious Adverse Device Effect

Appendix 2

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI
Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964
and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)
59th WMA General Assembly, Seoul, Republic of Korea, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

Preamble

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

3. The Declaration of Geneva of the WMA binds the physician with the words, “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient's best interest when providing medical care.”

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information

regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw

consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Dissemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available.

Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.