Title	A randomized, single-center, prospective biopsy study comparing a poly L-lactic acid biostimulator and a calcium hydroxylapatite semi-permanent filler
Protocol number:	GLI.04.US.SL.020
Sponsor name and address:	Galderma Laboratories, L.P. 14501 North Freeway Fort Worth, TX 76177 USA
Study treatment products:	Sculptra® Radiesse® (+)
Investigator agreement:	I have read the clinical study described herein, recognize its confidentiality, and agree to conduct the described study in compliance with Good Clinical Practices (GCP), the ethical principles contained within the Declaration of Helsinki, this protocol, and all applicable regulatory requirements.
Principle Investigator:	
	Signature Date
Investigator name:	Jill S. Waibel, MD
Address:	Miami Dermatology & Laser Institute 7800 SW 87th Avenue, Suite B200 Miami, FL 33173 USA Telephone: (305) 279-6060

A RANDOMIZED, SINGLE-CENTER, PROSPECTIVE BIOPSY STUDY COMPARING A POLY L-LACTIC ACID-BASED BIOSTIMULATOR AND A CALCIUM HYDROXYLAPATITE-BASED SEMI-PERMANENT FILLER

APPROVAL SIGNATURE PAGE

Jill S. Waibel, MD Principle Investigator Miami Dermatology & Laser Institute	Date
Thu Q. Nguyen, PhD Medical Manager Galderma Laboratories, L.P.	Date
Jennifer Le, PharmD, RPh Medical Affair Advisor Galderma Laboratories, L.P.	Date
Matthew Meckfessel, PhD Director, Medical Affairs Galderma Laboratories, L.P.	Date

1. SYNOPSIS

Primary Study Objective	To compare gene expression stimulated by a semi-permanent filler and a biostimulator via punch biopsy
Secondary Study Objective	To assess and compare volume change in the nasolabial fold via clinical photography
Methodology	Randomized, single-center, comparative study
Number of Subjects	20 subjects to complete (10 subjects per treatment product group)
Study duration	13 weeks total
Study treatment products	Sculptra® - poly L-lactic acid (PLLA) biostimulator Radiesse® (+) – calcium hydroxylapatite (CaHA) semi-permanent filler
Conditions of use	Per on-label instruction
Study visits	Visit 1 (Day 0/baseline), visit 2 (day 5), visit 3 (day 28/week 4), visit 4 (day 84/week 12), and visit 5 (day 89).
Specific Inclusion Criteria:	○ **Demographics and study skin conditions** - Any race and ethnicity (to be recorded) - Fitzpatrick skin type (to be recorded) - Women or men - Age: 22-50 years old - Subject with a minimum of shallow nasolabial fold (NLF) contour deficiencies as assessed via the wrinkle assessment scale (WAS) - Subject with identical WAS scores on both NLFs - Subject who in the opinion of the treating Investigator would only need 2 treatments for optimal clinical outcomes - Subject with healthy immune systems ○ **Administrative** - Ability of giving consent for participation in the study - Agreement to have skin biopsies on NLFs

	- Agreement to adhere to the procedures and requirements of the study and to report to the institute on the day(s) and at the time(s) scheduled for the assessments
Specific Exclusion Criteria:	○ **Skin conditions** - Significant NLF asymmetry, or different WAS score on each NLF - Pregnant, planning pregnancy during the course of the study or breastfeeding - History of allergy or hypersensitivity to any ingredient of the treatment products - History of allergy or hypersensitivity to anesthetics or lidocaine - Have been diagnosed with a bleeding disorder - Have experienced excessive bleeding after other medical procedures - Currently taking blood-thinning medications (e.g., aspirin, aspirin-containing medications, warfarin or heparin), prescription facial wrinkle therapies or topical steroid applied to the NLF, immunosuppressive medications or systemic steroids. - Currently taking supplements or homeopathic medications - History of skin infections, such as impetigo - History of keloid formation or hypertrophic scarring - Previous permanent or semi-permanent implant (including polylactic acid, polymethyl methacrylate and silicone) in proposed treatment area - Previous resorbable and permanent fillers, hyaluronic acid or *botulinum toxin* injections in the proposed treatment area within 12 months prior to the baseline visit - History of other facial treatment/procedure in the previous 12 months that would potentially interfere with study outcome (e.g., facial surgery, oral surgery, resurfacing, mesotherapy, lipolytic injections) - History of facial nerve palsy - Subject who is a smoker
Procedure	○ **Visit 1 (D0/Baseline)** - Subjects will be screened on the basis of the selection criteria for study qualification. - Subjects will be randomized to each treatment group and biopsy side.

	- Subjects will report to the site as D0 visit, will be given an informed consent form, HIPAA form, photography release form, and medical history form to complete. - 3D standardized photography will be performed on full global face. - A 3-mm punch biopsy will be performed on one side of enrolled subject's nasolabial fold accordingly pre-determined randomization. - Investigator will inject the assigned treatment product to both of the subject's nasolabial folds, based on the predetermined randomization and the treatment products' instruction to achieve the optimum correction. o **Visit 2 (D5)** - Subjects will return to the site to remove surgical stitch at the biopsy site. o **Visit 3 (D28/W4)** - All subjects to return to the site for follow-up. - Photography will be performed similarly to D0. - Subjects in PLLA group to have an additional treatment to both nasolabial folds. - Subjects in CaHA group to have an optional touch-up based on the Investigator's assessment. o **Visit 4 (D84/W12)** - All subjects to return to the site for follow-up. - Photography will be performed similarly to D0. - A 3-mm punch biopsy will be performed on the opposite of the subject's nasolabial fold from D0. o **Visit 5 (D89)** - All subjects will return to the site to remove surgical stitch at the biopsy site. - Investigator will assess the biopsy areas and offer an optional laser treatment (one or both sides) to minimize scar formation upon study completion.
Biopsy analysis	- Gene expression assessing the following biomarkers: scar tissue formation, collagen, elastin, extracellular matrix integrity, epidermal barrier, anti-aging, antioxidant, cell renewal/regeneration, inflammation, growth factor, hydration.
Statistical analysis	Comparison of gene expression and imaging analysis between the treatment products.

2. TABLE OF CONTENTS

3. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse Event
°C	Degrees Celsius
CaHA	Calcium Hydroxylapatite
CRF	Case Report Form
etc.	et cetera
e.g.	for example (Latin; exempla gratia)
°F	Degrees Fahrenheit
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICH	International Conference on Harmonization
ICT	Information and communication technologies
i.e.	that is (Latin; id est)
IEC	Independent Ethics Committee
IFU	Instruction for Use
IRB	Institutional Review Board
N or n	Number
NLF	Nasolabial fold
PLLA	Poly L-lactic acid
%	percent
SAE	Serious Adverse Event
SD	Standard deviation
SOP	Standard Operating Procedure
SWFI	Sterile water for injection
UPT	Urine Pregnancy Test

4. BACKGROUND AND RATIONALE

During aging, the skin undergoes significant changes that are often characterized by dermal thinning, redistribution of fat, and loss of skin elasticity and collagen.[1, 2] Various factors can considerably accelerate this aging process, including environmental exposure, leading to rhytids, wrinkles, and skin laxity. There has been an increase in popularity of soft-tissue injectables as they are non-invasive and are effective for restoring lost volume and for correcting contour deficiencies to the aging face.[3] Among them are two well-known injectable agents: Sculptra® (Galderma, New Jersey, USA) and Radiesse® (+) (Merz Pharmaceutical, Wisconsin, USA). Sculptra® is a poly-L-lactic acid (PLLA) biostimulator and Radiesse® (+) is a calcium hydroxylapatite (CaHA) filler. Both treatments are currently FDA-approved in the United States for correction of facial wrinkles and folds. Both provide a delayed but progressive volumizing effect by stimulating the regeneration of endogenous collagen overtime.[4, 5]

As such, a similar mechanism of action of these injectables represents an interesting line of examination, particularly in the context of cellular activities occurring at the genomic level that contributes to neocollagenesis and potentially other structural integrity of the extracellular matrix. This study is conducted to examine the effect of PLLA and CaHA injectables in the treatment of nasolabial folds (NLFs).

5. STUDY OBJECTIVES AND CLINICAL HYPOTHESIS

5.1 Study Objectives

The primary objective of this study is to compare gene expression stimulated by a semi-permanent filler and a biostimulator via punch biopsy.

The secondary objective of this study is to assess and compare volume change in the NLF via clinical photography.

5.2 Clinical Hypothesis

When each treatment is injected to the skin, it triggers a series of biochemical reactions and inflammatory responses that contribute to the production of collagen and other protein structures in the extracellular matrix. The result is an aesthetic increase in skin volume at the treated facial area.

6. SELECTION AND DISPOSITION OF STUDY POPULATION

6.1 Number of Subjects

An appropriate number of subjects meeting inclusion/exclusion criteria listed below will be enrolled on the study to achieve maximum 20 subjects who complete the study as planned.

6.2 Study Population Characteristics

Males and females of any skin type with diagnosis of NLF contour deficiency.

6.3 Inclusion Criteria

1. Subjects of 22 to 50 years of age.
2. Subjects clinically diagnosed with a minimum of shallow NLF contour deficiency based on the wrinkle assessment scale (WAS, see section 10.1).

3. Subjects with identical WAS scores on both NLFs (see section 10.1).
4. Subjects who in the opinion of the Investigator would only need 2 treatments for optimal clinical outcomes.
5. Subjects of any gender.
6. Subjects of any race and ethnicity.
7. Subjects of any Fitzpatrick skin type.
8. Subjects with healthy immune systems.
9. Subjects who are able and willing to provide written informed consent prior to any study related procedures.
10. Subjects who agree to be photographed at each visit.
11. Subjects who agree to have a 3-mm punch biopsy on each NLF.
12. Subjects apprised of the Health Insurance Portability and Accountability Act of 1996 (HIPAA) and applicable state Bill of Rights.
13. Subjects who agree to adhere to the procedures and requirements of the study, to report to the institute on the day(s) and at the time(s) scheduled for the assessments, and to complete all required visits.

6.4 Exclusion Criteria

1. Subjects with significant NLF asymmetry, or different WAS score on each NLF (see section 10.1)
2. Subjects who are current smokers or consume nicotine (e.g., cigarettes, e-cigarettes, vaping device with pre-filled pods, vapor tank or mod, chewing tobacco).
3. Subjects with any diseases, condition or presentation that, in the opinion of the Investigator, may put the subject at risk, may confound study results, or may interfere with participation in the study.
4. Subjects who are pregnant or breast-feeding, or who plan to become pregnant or breast feed during the course of the trial, confirmed by urine pregnancy test (UPT).
5. Subjects that are relatives of the Investigator or are themselves or a relative of any study staff or any Galderma employee.
6. Subjects who have participated in an investigational study within 30 days of enrollment; participated in biologic investigational studies within 90 days of enrollment, or subjects planning to participate in any other interventional clinical research study while enrolled in this trial.
7. Subjects with an active skin infection, such as impetigo.
8. Subjects with history of keloid formation or hypertrophic scarring.
9. Subjects with history of facial nerve palsy.
10. Subjects with history of epilepsy.
11. Subjects with history of migraine.
12. Subjects with history of allergy or hypersensitivity to lidocaine and/or any ingredient in the treatment products (PLLA, sodium carboxymethylcellulose, non-pyrogenic mannitol, CaHA, glycerin, and sterile water for injection (SWFI)).
13. Subjects with previous permanent or semi-permanent implant (including polylactic acid, polymethyl methacrylate and silicone) below the level of zygomatic arch.
14. Subjects resorbable and permanent fillers, hyaluronic acid or *botulinum toxin* injections below the level of zygomatic arch within 12 months prior to Visit 1.
15. Subjects with history of other facial treatment/procedure at the study area (NLF) in the previous 12 months that would potentially interfere with study injections (e.g., facial surgery, oral surgery, resurfacing, mesotherapy, lipolytic injections).
16. Subjects with diagnosis of a bleeding disorder.

17. Subjects who take prescription facial wrinkle therapies or topical steroid applied in the NLF within 4 weeks of Visit 1.
18. Subjects who take immunosuppressive medications or systemic steroids (i.e., oral prednisone) within 6 months of Visit 1. Intranasal or inhaled steroids are acceptable.
19. Subjects who, at the discretion of the Investigator, have experienced excessive bleeding after other medical procedures.
20. Subjects who are currently taking concomitant medications that have the potential to prolong bleeding times such as anticoagulants or inhibitors of platelet aggregation (e.g., aspirin, aspirin-containing medications, warfarin, heparin, other nonsteroidal anti-inflammatory drugs, vitamin E, estrogen preparations, herbal supplements or homeopathic medications known to affect coagulation).

6.5 Concomitant Therapies

All treatments and therapies used 30 days prior to enrollment (visit 1/Day 0) or 90 days prior to enrollment for biologics and all treatments or therapies used during the course of the study must be recorded in the Case Report Form (CRF) or electronic Case Report Form (eCRF).

6.5.1. Authorized Therapies

Unless listed under the exclusion criteria (Section 6.4) or in Prohibited Therapies (Section 6.5.2), other therapies to treat ongoing conditions are authorized.

6.5.2. Prohibited Therapies

None other than as specified in the Inclusion/Exclusion criteria.

The decision to administer a prohibited medication/treatment should be made with the safety of the subject being the primary consideration. Whenever possible, Galderma Laboratories, L.P. should be notified before the prohibited medication/treatment is administered to discuss possible alternatives.

If a subject receives prohibited therapy during the study, the subject may be allowed (at the discretion of the Investigator / Galderma Laboratories, L.P.) to continue in the study for safety evaluation purposes, only.

7. STUDY TREATMENT

The term “study treatment” refers to the study products (see Section 7.1)

7.1 Study product Identification and Use

Study product: Sculptra®	
Form	Lyophilized PLLA with sodium carboxymethylcellulose, non-pyrogenic mannitol.
Mode of administration	Injection following reconstitution
How supplied	Sterile freeze-dried preparation in a clear glass vial, which is sealed by a penetrable stopper, covered by an aluminum seal with a flip-off cap.
Lot numbers	To be added upon study completion

Storage and handling	Store under controlled room temperature conditions, up to 30°C (86°F). DO NOT FREEZE. Refrigeration is not required.

Study product: Radiesse® (+)	
Form	Opaque, sterile, non-pyrogenic, semi-solid, cohesive implant whose component is synthetic CaHA suspended in a gel carrier of glycerin, sodium carboxymethylcellulose, 0.3% lidocaine hydrochloride, and sterile water.
Mode of administration	Injection
How supplied	Individual treatment syringe with needle
Lot numbers	To be added upon study completion
Storage and handling	Store at controlled room temperature between 15°C and 32°C (59°F and 90°F).

7.2 Additional Products and Materials

The Sponsor will provide Sculptra® and UPTs for the study. The study overhead will cover any additional materials or supplies.

7.3 Study Product Accountability

Upon receipt of the study products, the Investigator or designee will conduct an inventory. In accordance with federal regulations, the Investigator must agree to keep all test article in a secure location with restricted access. Designated study personnel will provide the test article to the subjects in accordance with the protocol.

During the study, the Investigator must maintain records of study treatment dispensation and collection for each subject. This record must be made available to the study monitor for the purposes of verifying the accounting of clinical supplies. Any discrepancies and/or deficiencies between the observed disposition and the written account must be recorded along with an explanation. At the conclusion of the study, the Investigator will be responsible for returning all unused study product (i.e., Sculptra®) unless otherwise instructed by the Sponsor. Shipping label and cost will be provided to the Investigator by the Sponsor.

7.4 Study Product Preparation

Each PLLA vial will be reconstituted by adding 8 mL of SWFI and 1 mL of 2% lidocaine to obtain a total of 9 mL of injectable preparation. Reconstitution description can be found in the Instruction for Use (IFU).

CaHA is supplied in sterile individual treatment syringes packaged with fine gauge needles. Reconstitution is not needed.

7.5 Method of Treatment Assignment

Before the start of the study, a randomization list will be prepared by the Sponsor utilizing a computer-generated software. This will randomly assign the study product to each enrolled subject and the NLF side for punch biopsy.

7.6 Treatment Dosage and Mode of Administration

The injection technique (skin disinfection material, type of syringe, type and gauge of the intradermal needle, penetration angle, depth) will be the technique customarily used by the Investigator. The type of gauge of the injection needle will be recorded in the CRFs.

PLLA: two treatment sessions at 4-week interval with the first treatment at Visit 1 and the second treatment at Visit 3. Each treatment session will be consisted of multiple deep dermal injections, up to 9 mL/session (or up to 4.5 mL/NLF/session), into the left and right NLFs of each subject according to the IFU. The treatment amount for each PLLA subject will be determined at the Investigator discretion to achieve an optimal correction. Within each PLLA subject, the amount of each treatment needs to be equal at the left and right NLFs because of the identical WAS scores. The treatment volume injected during each treatment session for each subject will be recorded in the CRFs.

CaHA: up to two treatment sessions at 4-week interval with the required first treatment at Visit 1 and an optional second treatment at Visit 3 to achieve an optimal cosmetic correction. Each treatment session will be consisted of multiple subdermal injections, up to 1.5 mL/session (or up to 0.75 mL/NLF/session), into the left and right NLFs of each subject according to the IFU. The second treatment is optional and will be determined based on the Investigator assessment. If the subject needs a second treatment, an appropriate amount will be determined at the Investigator discretion to achieve an optimal outcome. The amount of each treatment needs to be equal at the left and right NLFs because of the identical WAS scores. The treatment volume injected during each treatment session for each subject will be recorded in the CRFs.

8. TREATMENT OF SUBJECTS

8.1 Informed Consent Form

An IRB-approved informed consent form (ICF) will be given to each prospective subject before participation in any study procedures. Prospective subjects will be given as much time as needed to read the ICF and will have the opportunity to have any study-related questions answered to their satisfaction prior to signing the ICF. If further questions exist, prospective subjects will be given sufficient time during the visit to have questions regarding the study and/or the ICF answered by the Investigator or study coordinator prior to signing.

8.2 Subject Identification

Enrolled subjects will be assigned a number that will uniquely identity every subject on the study. The numbers will remain with the subject throughout the study and should be used in all references to the individual in this study.

8.3 Subject Instructions for the Study

Subjects must show up to the study site with clean face without any makeup. Male subjects with facial hair must shave prior to any study visit.

Throughout the study, subjects must avoid extended periods of sun exposure and use of tanning beds or sunless tanning products on the face. Extra care should be taken to wear sunscreen and accessories (i.e., hat) and avoid sun exposure from 10 am to 3 pm.

All subjects must follow a provided post-care after biopsy procedure (see Appendix I: Biopsy Procedure and Post-Care).

Subjects in PLLA group needs to massage the injection areas for 5 minutes, 5 times a day for 5 consecutive days after the treatment. Subjects will complete a daily diary, recording time and number of massages to ensure treatment compliance.

9. STUDY PROCEDURES

There will be 5 visits during the course of the study:

1. Visit 1 – Screening/Day 0/Baseline
2. Visit 2 – Day 5
3. Visit 3 – Day 28 (Week 4)
4. Visit 4 – Day 84 (Week 12)
5. Visit 5 – Day 89

Test design / flow chart	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**
Informed consent	X				
Photo consent	X				
Demographics/Med History	X				
Concomitant Meds.	X				
Inclusion and exclusion criteria	X				
UPT	X		X		
Randomization	X				
Case report form (CRF)	X	X	X	X	X
3D clinical photography	X		X	X	
Punch biopsy	X			X	
Treatment product injection	X		X*		
Stitch removal		X			X
AE Reporting	X	X	X	X	X

*PLLA group required for a second treatment. CaHA group for an optional touch-up.

9.1 Visits and Examinations

9.1.1. Visit 1 (Screening/D0/Baseline)

1. Subjects will report to the testing clinic at D0 for baseline screening.
2. Subjects will be screened and qualified on the basis of the subject inclusion and exclusion criteria. Subjects failing to meet criteria will be dismissed from the study.

3. Prior to beginning of any study related activities, subjects will be informed about the purpose and nature of the study, the expected post-treatment events, and the potential risks involved with the treatments.
4. Subjects will be given an informed consent form, HIPAA form, and photography release form to read.
5. Once subjects have completed reading, they will be interviewed to ensure their understanding of the aforementioned forms and be given the opportunity to ask any study related questions.
6. Subjects who agree to sign the aforementioned forms will be asked to complete a demographic information, medical history form, and concomitant medication form. Subjects declining to sign any of the forms will be dismissed from the study.
7. Female subjects will need to perform UPT to confirm childbearing potential.
8. Subjects will be assigned to a treatment group and side of punch biopsy at the NLF based on the predetermined computer-generated randomization.
9. Subjects will participate in the following procedures completed by the Investigator or a trained clinic staff:
    a. 3D clinical photography by Cherry Imaging of full face as described in section 10.2
    b. A 3-mm punch biopsy at the assigned side of the NLF as described in section 10.3
10. Investigator will then instruct the subjects on the assigned treatment administration procedure and perform the treatment into both left and right NLFs.
11. Investigator or clinic staff will record the details of the administration, amount of each treatment used for each NLF and any subsequent adverse events (AEs) in the CRF.
12. Subjects will be instructed on any standard care post-biopsy and post-treatment, and when to contact the Investigator in case of emerging AEs.
13. Subjects will be scheduled to return to the clinic in 5 days (±1 day) and dismissed from the clinic.

Note: Whenever possible, the same Investigator/staff that perform the baseline assessments and treatments should perform these assessments for each individual subject for the entire duration of the study. In the event of a change in the assigned Investigator/staff for a given subject, the reason for change must be documented.

9.1.2. Visit 2 (D5 ±1 day)

1. Clinic staff or Investigator will record any AEs that are observed or reported.
2. Investigator will examine the wound and remove any suture from the biopsy site.
3. Subjects will be scheduled for a follow-up visit at Day 28 or Week 4 (±3 days) and be dismissed from the clinic.

9.1.3. Visit 3 (D28/W4 ±3 days)

1. Clinic staff or Investigator will record any AEs that are observed or reported.
2. Female subjects will need to perform UPT to confirm childbearing potential.
3. All subjects will have 3D clinical photography by Cherry Imaging taken of full face as described in section 10.2.
4. For PLLA group, subjects will have a second treatment into both left and right NLFs performed by the Investigator.
5. For CaHA group, Investigator will assess and perform an optional touch-up on both NLFs to reach the optimal aesthetic result.

   a. If touch-up is needed, the same study treatment and type of injection technique for the baseline treatment need to be used for this step. The amount of treatment can differ compared to Visit 1; however, it needs to be the same for the left and right NLFs.
6. Investigator or Clinic staff will record the amount of the treatment used for the NLFs in the CRFs.
7. Once completed, subjects will be scheduled for a follow-up visit at Day 84 or Week 12 (±3 days) and be dismissed from the Clinic.

9.1.4. Visit 4 (D84/W12 ±3 days)

1. Clinic staff or Investigator will record any AEs that are observed or reported.
2. Subjects will participate in the following procedures completed by the Investigator or a trained clinic staff:
   a. 3D clinical photography by Cherry Imaging of full face as described in section 10.2.
   b. A 3-mm punch biopsy at the other side of the NLF as described in section 10.3.
3. Subjects will be scheduled for a follow-up visit at Day 89 (±1 day) and be dismissed from the clinic.
4. Once completed, subjects will be dismissed from the Clinic.

9.1.5. Visit 5 (D89 ±1 day)

1. Clinic staff or Investigator will record any AEs that are observed or reported.
2. Investigator will examine the wound and remove any suture from the biopsy site.
3. Investigator will assess both biopsy sites on scarring potential. If needed, the Investigator will offer a subsequent laser treatment session to minimize scars.
4. If laser treatment is not needed, subjects complete the study and will be dismissed from the clinic.

9.2 Discontinued Subjects

Any subject is free to discontinue his/her participation in this study at any time and for whatever reason, specified or unspecified, and without prejudice.

An Investigator may decide to discontinue a subject from the study for safety reasons or when it is in the best interest of the subject. Galderma Laboratories, L.P. may also decide to prematurely terminate or suspend the study or the participation of a subject in the study. All data gathered on the subject prior to termination should be made available to Galderma Laboratories, L.P.

Criteria for the discontinuation of a subject during the study will include the following:

- Adverse Event
- Lack of Effect
- Pregnancy
- Subject Request
- Protocol Violation
- Lost to Follow-up
- Any unmanageable factor, in the Investigator’s opinion, that may significantly interfere with the protocol or interpretation of results.

10. STUDY ASSESSMENTS

All images taken from the study will be saved and shared to the Sponsor via a data-protected platform.

10.1 Wrinkle Assessment Scale

Clinical grading of NLF will be performed at baseline for screening purpose. The grading will be based on a 6-point Wrinkle Assessment Scale[6] according to the numerical definitions (describe in Figure 1 below).

0 = no visible, minimal wrinkles
1 = just perceptible wrinkles
2 = shallow, visible wrinkles with a slight indentation
3 = moderately deep wrinkles
4 = deep wrinkles, well-defined folds
5 = very deep wrinkles, redundant folds

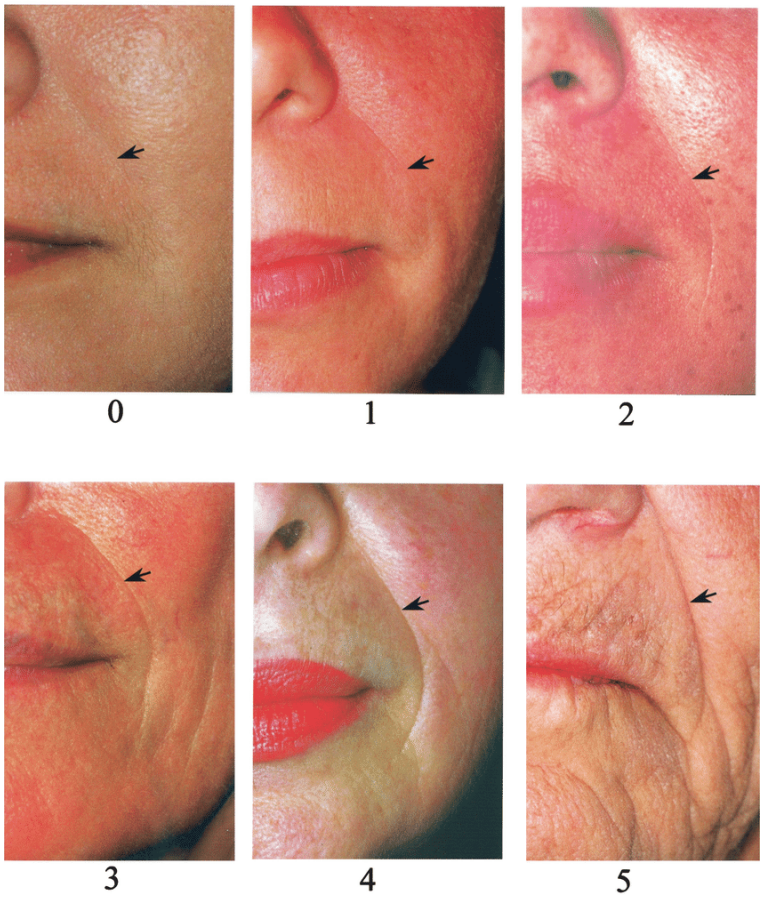

Figure 1. Wrinkle Assessment Scale of nasolabial folds

10.2 Clinical Photography (Cherry Imaging)

Three (3) dimensional images of the subject's face will be taken at baseline/day 0 (pre-biopsy and pre-treatment), day 28 (pre-treatment), and day 84 (pre-biopsy).

Clinic staff needs to ensure subjects to have a clean face with no makeup, no facial hair, and to remove any jewelry from the area to be photographed. Subjects will be provided with a headband to keep hair away from the face. Subjects will be instructed to adopt neutral, nonsmiling expression with their eyes gently closed, and in a relaxed positioned for each scan.

A total of three 3D images will be taken of each subject's face using the Cherry Imaging device (Cherry Imaging, Israel). The Cherry Imaging relies on multi-directional illumination and computer-aided reconstruction of the skin surface in 3D.

Change in volume on each NLF will be analyzed by the Cherry Imaging software.

10.3 Punch Biopsy Collection and Analysis

At baseline/day 0 and day 84, subjects will participate in a 3-mm punch biopsy procedure (post-photography and pre-treatment). Each subject will have 1 biopsy at one side of the NLF (based on the pre-determined randomization) at baseline and 1 biopsy at the other side of the NLF at day 84; total of 2 biopsies per subject at both timepoints.

Biopsies will be obtained using standard sterile technique after an intradermal local anesthesia and treated with topical antibiotic and sterile dressing in routine fashion (see Appendix I: Biopsy Procedure and Post-Care).

The biopsy specimens will be immediately transferred into RNAlater solution and stored at room temperature for 4 hours and then moved to 4°C. Collected biopsy samples will be sent to the following address for gene expression analysis on icepack (4°C).

The biopsy sample will contain the subject number/identification (baseline and day 84), product information, and any other relevant information needed to identify the samples post-analysis.

Attn: Rishabh Kala, PhD
Genemarkers, LLC
126 E South Street
Kalamazoo, Michigan 49007
Phone: (844) 220-6231

Gene expression analysis will be performed via qPCR processing using a panel of biomarkers related to scar tissue formation, collagen, elastin, extracellular matrix integrity, epidermal barrier, anti-aging, antioxidant, cell renewal/regeneration, inflammation, growth factor, and hydration among others.

11. STATISTICAL ANALYSIS

Data of completing subjects will be included for all statistical analyses. Descriptive statistical summary will be performed including N, mean, median, standard deviations, minimum, and maximum of values at all applicable time points and for both treatments.

Comparison between the study treatments will be made in terms of changes from baseline. The null hypothesis, that the mean change from baseline is equal between the 2 study treatments at post-baseline time point, will be tested using methods described in the Statistical Analysis Plan table.

Evaluation	Change from Baseline	Comparisons between study treatments	Notes/Interpretation
Volume change by 3D imaging analysis	Paired t-test; If normality fails, a Wilcoxon signed-rank test will be used.	Wilcoxon rank-sum test or sample t-test	A higher value reflects an improvement in skin volume.

Statistical analysis for gene expression will be determined using the relative quantification method.

12. ADVERSE EVENTS

Throughout the course of the study, all adverse events will be monitored and reported on an adverse event CRF/eCRF without omitting any requested and known information. When AEs occur, the main concern is the safety of the study subjects. At time of the informed consent signature, each subject must be given the name and phone number of investigational site personnel for reporting AEs and medical emergencies.

At each visit, after the subject has had the opportunity to spontaneously mention any problems, the Investigator should inquire about AEs by asking the standard questions:

- “Have you had any health problems since your last study visit?”
- “Have there been any changes in the medicines you take since your last study visit?”

AEs should be reported for any clinically relevant change, as determined by the Investigator, in concomitant medication(s) that is the result of an untoward (unfavorable and unintended) change from baseline in a subject's medical health following exposure to the study treatment.

Changes from baseline in any protocol-specific parameter evaluated during the study are to be reviewed by the Investigator. In addition, the subject’s responses to any questionnaire utilized during the study are to be reviewed by the Investigator. Any untoward (unfavorable and unintended) change from baseline in a protocol-specific parameter or question response that is clinically relevant, in the opinion of the Investigator, is to be reported as an AE. These clinically relevant changes will be reported regardless of causality.

12.1 Definitions

12.1.1. Adverse Events (AE)

An adverse event (AE) is defined as any untoward medical occurrence in a subject taking part in the clinical study, and which does not necessarily require a causal relationship with the investigational product and/or a clinical trial procedure.

An AE can be any unfavourable and unintended sign (including an abnormal laboratory value), symptom, or disease temporally associated with the use of the investigational product, whether or not related to this product.

When an AE has a likely or very likely causal relationship with the investigational product and/or a clinical trial procedure, it is named undesirable effect or related AE (see Section 3).

12.1.2. Local tolerability signs and symptoms (only applicable for cosmetic safety studies)

In cosmetic studies, local skin tolerability includes some expected functional and/or physical signs on the application area, observed by the Investigator or reported by the subjects. Those signs are collected in the final report based on scales or a diary. If the severity of a local skin tolerability sign or symptom, is such that the product application is permanently discontinued and/or a corrective concomitant treatment (except moisturizer or emollient) is prescribed, it is recorded as an undesirable effect (related AE).

12.1.3. Serious Adverse Events (SAE) and serious undesirable effect/related SAE

A serious adverse event (SAE) involves a serious injury that is life threatening, results in permanent impairment of a body function or permanent damage to a body structure or necessitates medical or surgical intervention to preclude permanent impairment of a body function or permanent damage to a body structure, requires inpatient hospitalization or prolongation of an existing hospitalization.

Notes:

The term “immediate vital risk” refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it was more severe.

Inpatient hospitalization is considered to have occurred if the subject has had to stay for a night at the hospital. Hospitalization solely for the purpose of a diagnostic test (even if related to an AE), elective hospitalization for an intervention that was already planned before subject enrolment in the clinical trial, admission to a day-care facility, social admission (e.g., if the subject has no place to sleep), or administrative admission (e.g., for a yearly examination) should not be considered as a SAE.

A serious undesirable effect/related SAE is defined as any SAE which the Investigator classifies as having a reasonable possibility for a causal relationship with the investigational product and/or the clinical trial procedure.

12.2 Severity Assessment

For all AEs occurring during the clinical trial, the Investigator is to classify and report the intensity of AEs using the following definitions as a guideline:

- Mild: awareness of signs and symptoms, but easily tolerated
- Moderate: discomfort, enough to cause interference with usual activity
- Severe: incapacitating, with inability to work or perform usual activity.

12.3 Causality Assessment

The Investigator is to assess the causal relationship (causality) between an adverse event and the investigational product and/or the clinical trial procedure according to the following definitions (Decision of 25 November 2013 on Guideline on Annex I to Regulation (EC) No 1223/2009 (2013/674/EU) - Causality assessment of undesirable effect caused by cosmetic products):

- Very likely
- Likely
- Not clearly attributable

- Unlikely
- Excluded

Medical judgment should be used to determine the relationship, considering all relevant factors including the pattern of reaction, temporal relationships, positive de-challenge or re-challenge, relevant medical history, and confounding factors such as co-medication or concurrent diseases.

12.4 Collection, Management and Reporting Procedures

The period of collection of adverse events starts from the time of signature of the Informed Consent Form (ICF) by the subject *(and/or, for subjects who are minor, by the parents/legal representatives)* until the end of the subject's participation in the clinical study.

If a Serious Adverse Event (SAE) is on-going at the final clinical trial visit, it should be followed by the Investigator until it has resolved or has reached a stable condition.

After the subject completes the clinical study, the Investigator should also inform the Sponsor (see Sponsor's contact details below) if he/she becomes aware of an SAE involving a subject who has participated in the clinical study.

At each post-enrollment visit, the Investigator will question the subject about AEs using an open non-persuasive question to elicit reporting of AEs, for example "*Have you noticed any change in your health since the last visit?*" Direct questioning and examination will be performed when appropriate.

The Investigator will obtain and maintain in the subject's files all pertinent medical records, and (if applicable) information and medical judgment from colleagues who assisted in the treatment and follow-up of the subject. If necessary, the Investigator will contact the subject's personal physician or hospital staff to obtain further details.

As a minimum, Investigators are requested to report in the Case Report Form (CRF) and in the report all <u>related</u> adverse events (i.e. undesirable effects) and all Serious Adverse Events, <u>whether related or not.</u>

12.4.1. Management and reporting procedures for undesirable effects (i.e. related adverse events

Undesirable effects should be recorded in the CRF and summarized in the report in a summary table with at minimum the subject number, AE number, AE diagnosis or signs and symptoms, location, date of onset, seriousness, severity, action taken, relationship, date of resolution and concomitant treatment associated as well as a detailed narrative of the event.

In addition, based on his/her medical judgment, the Investigator will assess whether an undesirable effect requires immediate (i.e. within 24 hours) reporting to the Sponsor. In such cases, the summary table will be sent to the Sponsor, along with the AE narrative and any other relevant information (concomitant treatments, product weighing, …).

All undesirable effects should be appropriately documented, i.e. any relevant information such as demographics, medical history and concomitant therapies should be recorded in the CRF.

The Investigator is to monitor and record the progress of the adverse effect until the last subject's study visit.

The Investigator is to update the AE narrative as appropriate, each time follow-up information is collected and when the final outcome of the adverse effect is known.

12.4.2. Management and reporting procedures for Serious Adverse Events

The Investigator is to take the following steps:

1. Take prompt and appropriate medical action, if necessary. The safety of clinical trial subjects is the first priority
2. Ensure the AE is classified as an SAE. Immediately inform the Sponsor's representative of the event by email or fax (see both contact details below) and discuss further actions to be taken:

   Global PV email: safety.g-med@galderma.com
   US PV email: pharmacovigilance.USDFW@galderma.com
3. Complete the Serious Adverse Event (SAE) form provided by the Sponsor's representative **Within 24 hours**, fax or send by e-mail **to the Sponsor's representative** the completed SAE form, accompanied any other relevant information (e.g. test results or medical records).
4. Monitor, record and send to Sponsor's representative the progress of the event until it resolves or reaches a stable outcome, with or without sequelae (send the updated SAE form with follow-up information and any other relevant information to Sponsor's representative).
5. Obtain and maintain in the subject's file all pertinent medical records, information, and medical judgments from colleagues who assisted in the treatment and follow-up of the subject. If necessary, contact the subject's personal physician or hospital staff to obtain further details.
6. If applicable, comply with the regulatory requirement(s) related to the reporting of SAEs to the Institutional Review Board (IRB) / Independent Ethics Committee (IEC).

12.5 Pregnancy

Pregnancy itself is not to be considered as an adverse event. If a pregnancy occurs during the clinical trial, **the product application should be stopped immediately**, the subject should be withdrawn from the clinical study and Sponsor's representative (see Sponsor's contact details above) should be informed **within 24 hours.**

Pregnancy must be recorded as a reason for discontinuation in the exit form of the CRF.

No specific follow-up of pregnancy is required, except if it is a regulatory requirement in the country(ies) where the clinical trial is conducted.

13. ETHICAL AND REGULATORY PROCEDURES

13.1 Research Standards/Good Clinical Practice

This study will be conducted in accordance with all applicable guidelines for the protection of human subjects for research as outlined in 21 CFR 50 the accepted standards for Good Clinical Practice (GCP), and the standard practices of SGS Stephens in accordance with the protocol and amendment(s) as applicable.

13.2 Quality Assurance/Audit/Inspection

To ensure compliance with GCP and all applicable regulatory requirements, Galderma Laboratories, L.P. may conduct a quality assurance audit of the site records, and the regulatory agencies may conduct a regulatory inspection at any time during or after completion of the study. The Investigator must agree to grant the auditor(s) and inspector(s) direct access to all relevant documents and to allocate their time and the time of their staff to discuss any findings/relevant issues.

13.3 Institutional Review Board

This study (protocol, ICF and all addenda) will be reviewed and approved by Sterling IRB. The study will not be activated and subjects will not be consented, receive any study products, or participate in any study procedures until the IRB has approved the protocol and the ICF. In addition, the IRB will review the study before any significant change in the protocol is initiated. After each review, the IRB's approval will be documented in a letter to the Investigator and a copy of the IRB approval letter will be forwarded to the Sponsor.

14. STUDY CONDUCT CONDISERATIONS

14.1 Clinical Monitoring

The conduct of the study will be closely monitored by representatives of Galderma Laboratories, L.P. following GCP, ICH guidelines, applicable SOPs, guidelines, and all local regulations. The clinical investigation will be monitored to ensure that: the rights and well-being of the subjects are protected; the reported data are accurate, complete and verifiable from applicable source documents; and the study is conducted in accordance with the currently approved protocol and any other study agreements, GCP, and all applicable regulatory requirements. The Investigator will allow the Galderma Laboratories, L.P. representatives to have access to all study records, CRF/eCRFs, corresponding subject medical records, and any other documents considered source documentation. The Investigator also agrees to assist the representatives, if required, which can include AE reporting. All study monitoring details will be detailed in the Clinical Monitoring Plan.

14.2 Data Collection

Investigators must keep accurate records of all subjects' visits and all procedures done, being sure to include all pertinent study related information from which CRF/eCRF data will be recorded. Data for this study may be recorded in the subject's chart (e.g. source documents / electronic records) or if approved by the Galderma Laboratories, L.P. directly into CRF/eCRFs. If electronic records are maintained, the method of verification must be determined in advance of starting the study. The process of administering the informed consent must also be documented. Any and all side effects and AEs with the concomitant therapies associated must be thoroughly documented. Results of any diagnostic tests conducted during the study should be included in the source documentation. Pertinent telephone conversations with the subjects and/or Galderma Laboratories, L.P. concerning the study will be documented and kept on file.

It is required that the author of an entry in the source documents be identifiable. Direct access to all source documentation (medical records) must be allowed for the purpose of verifying that the data recorded in the CRF/eCRF are consistent with the original source.

Only designated individuals may complete the CRF/eCRFs. The principal Investigator will review the reported data and certify that the CRF/eCRFs are accurate and complete.

After monitoring has occurred at the clinical site(s) and the CRF/eCRFs have been reviewed, additional data clarifications and/or additions may be needed including AE reporting. Data clarifications and/or additions are documented and are part of each subject's CRF/eCRFs.

14.3 Record Retention

The Investigator is required to maintain up-to-date, complete regulatory documentation as indicated by Galderma Laboratories, L.P. and the Investigator's files will be reviewed as part of the ongoing study monitoring. The records must be easily accessible when needed (e.g., for a Galderma Laboratories L.P.'s audit or regulatory inspection) and must be available for review in conjunction with assessment of the facility, supporting systems, and relevant site personnel. Financial information is not subject to regulatory inspection and should be kept separately.

Galderma Laboratories, L.P. will inform the Investigator of the time period for retaining the site records in order to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to a particular site, as dictated by local laws/regulations, Galderma Laboratories, L.P. SOPs, and/or institutional requirements.

The Investigator should take measures to prevent accidental or premature destruction of these documents. If the Investigator retires, relocates, or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility. Galderma Laboratories, L.P. must be notified in writing of the name and address of the new custodian.

14.4 Changes in Study Conduct/Amendments

No amendment will be done for modification(s) due to change in logistical or administrative aspect of the study (e.g., change in monitors, change of telephone numbers). In such a case, the appropriate institution(s) and/or person(s) will be notified of the changes.

Modification of the protocol is prohibited without prior written agreement in the form of a protocol amendment. All amendments will be created by Galderma Laboratories, L.P. and must be approved by the IRB prior to implementation except when required to mitigate immediate safety risks or when the changes involve only logistical or administrative revisions.

Amendments may necessitate that the informed consent and other study-related material be revised. If the consent form is revised, all Subjects/subjects currently enrolled in the study may be required by the IRB to sign the approved, revised informed consent form.

14.5 Confidentiality

All the data provided to the Investigator and his/her staff and all data obtained through this Galderma Laboratories, L.P. protocol will be regarded as confidential and proprietary in nature and should not be disclosed to any third party without Galderma Laboratories, L.P.'s written consent"

15. REFERENCES

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5. Berlin AL, Hussain M, Goldberg DJ. Calcium hydroxylapatite filler for facial rejuvenation: a histologic and immunohistochemical analysis. *Dermatol Surg*. 2008;34(Suppl 1):S64-67.
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APPENDIX I: BIOPSY PROCEDURE AND POST-CARE

1. **Biopsy Visits**
    a. Prepare the Area to be Biopsied
        i. After the patient, guardian, and/or power of attorney have been consented, initials are completed the area of skin where biopsy will be performed needs to be identified and circled with surgical marker. Once area is identified – a photo of area being biopsy is mandatory. Next, the area of skin where the biopsy is taking place will be prepared with an alcohol swab to ensure sterile conditions prior to procedure.
    b. Anesthetize the Skin
        i. Once the skin has been identified, photo is taken, and prepared with alcohol swab - the next step is to anesthetize the area to be biopsied by injecting a solution of Lidocaine 1% and Epinephrine or Lidocaine 1% with no Epinephrine (if patient is allergic to Epinephrine or breast feeding) just under the epidermis (sub-epidermally) using a Syringe. The slight burning will quickly subside and the site will become numb. Once area has been anesthetized, it is critical for area to be tested to make sure the area of skin being biopsy is indeed numbed.
    c. Biopsy the Skin
        i. After the area to be biopsied is anesthetized, the biopsy continues. Using a sterile 3 mm skin punch, the study investigator applies pressure and twisting in a “drilling” motion until the blade of the skin punch has pierced the epidermis of the skin.
        ii. After the blade has sufficiently “cored” or carved out a 3mm cylinder of skin the punch is removed.
        iii. Nurse assists with hemostasis- gauze, gloves, etc. for bleeding purposes. Once specimen is removed by provider, 1-3 sutures will follow.

2. **Post-Biopsy Procedure**
    a. Keep the initial dressing/bandage in place and dry for up to 24 hours.
    b. Change the dressings 2 times a day using the following method.
        i. Wash your hands before and after changing the dressings.
        ii. Clean the affected area gently with mild soap. If you have stitches, you may use cotton tipped applicators (Q-tips) around the stitches. It is okay if the stitches get wet in the shower after the first 24 hours.
        iii. Gently pat dry the area.
        iv. Apply antibiotic ointment (Polysporin, Bactroban, Mupirocin, Gentamicin, Neosporin, Aquaphor, Vaseline) to the affected area.
        v. Cover with Band-Aid or telfa pad (non-stick) dressing and tape.
    c. Repeat this process daily until the wound is healed (usually 5-7 days) or the stitches are removed.
    d. If your wound becomes red, warm, painful, begins to drain or you develop a fever of 100 or greater please contact your provider immediately.
    e. Bleeding may occasionally occur as the local anesthetic wears off. Apply firm direct pressure to the bandage continuously for fifteen minutes. If the bleeding persists after fifteen minutes call your provider.
    f. Return to your provider in 5-14 days for suture removal (Depending on area being biopsied) and/or follow up.

3. **Suture Removal Visits**
    a. Patient will come into office for suture removal visit.
    b. Biopsied area will be cleansed with alcohol swab prior to suture removal.
    c. Sutures will be removed from the biopsy sites.
    d. Once sutures are removed, patient will be instructed to apply antibiotic ointment (Polysporin, Bactroban, Mupirocin, Gentamicin, Neosporin, Aquaphor, Vaseline) to the affected area. Also, patient will be instructed to keep area covered with a Band-Aid or telfa pad dressing and tape.
    e. Repeat this process daily until the wound is healed (usually 5-7 days).
    f. If you wound becomes red, warm, painful, begins to drain or develop a fever of 100 or greater please contact your provider immediately.