NCT04206293

Study ID: CMO-MA-FAS-0617

Title: Prospective, Open-Label Study, to Evaluate The Impact on Skin Quality Attributes by Juvederm® Volite Injection on Healthy Volunteers

Statistical Analysis Plan Date: 16Jan2020

STATISTICAL ANALYSIS PLAN

PROSPECTIVE, OPEN-LABEL STUDY, TO EVALUATE THE IMPACT ON SKIN QUALITY ATTRIBUTES BY JUVEDERM® VOLITE INJECTION ON HEALTHY VOLUNTEERS

Protocol #: 18E2581

Investigational product:	**Juvederm® VOLITE**
Forms:	**Sterile gel**
Administration	**Intradermal injection**
CRO	**DERMSCAN - Pharmascan** 114 Boulevard du 11 Novembre 1918 69100 VILLEURBANNE FRANCE
Study centres	**Dermscan Poland** Sp. z o.o. ul. Matuszewskiego 12 80-288 GDANSK POLAND
Principal Investigators:	[redacted]
Sponsor:	**Allergan Pharmaceuticals International Limited** Clonshaugh Industrial Estate Coolock Dublin 17 Ireland
Date and version:	**Final Version 1.0** 16/01/2020
Author of the report	**Biostatistician:** [redacted]

TABLE OF CONTENTS

LIST OF TABLES

[redacted]

LIST OF ABBREVIATIONS AND TERMS

A.U	Arbitrary Unite
ADE	Adverse Device Effect
AE	Adverse Event
CI	Confidence interval
D	Day
GU	Glossymeter Units
IP	Investigational product
ISR	Injection Site Reaction
max	Maximum value
min	Minimum value
miss	Number of missing data
mm	Millimeter
N	Number of valid data
NRS	Numerial Rating Scale
PT	Preferred Term
Q1	First quartile
Q3	Third quartile
SADE	Serious Adverse Device Effect
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard deviation
SE	Standard error of estimated mean
SEM	Standard error of the mean
SOC	System Organ Class
TDC	Tissue Dielectric Constant

1. OBJECTIVES

This statistical analysis plan (SAP) provides a detailed description of the statistics that will be performed on the efficacy and safety parameters and other data of the clinical study "PROSPECTIVE, OPEN-LABEL STUDY, TO EVALUATE THE IMPACT ON SKIN QUALITY ATTRIBUTES BY JUVEDERM® VOLITE INJECTION ON HEALTHY VOLUNTEERS" with the reference 18E2581PL – CMO-MA-FAS-0617

2. SYNOPSIS OF THE PROTOCOL

Clinical investigation plan #:	18E2581PL – CMO-MA-FAS-0617
Title of the Clinical Investigation:	**PROSPECTIVE, OPEN-LABEL STUDY, TO EVALUATE THE IMPACT ON SKIN QUALITY ATTRIBUTES BY JUVEDERM® VOLITE INJECTION ON HEALTHY VOLUNTEERS**
Sponsor:	**Allergan Pharmaceuticals International Limited** Clonshaugh Industrial Estate Coolock Dublin 17 Ireland
Objectives:	To evaluate the impact on skin quality attributes, including physical measurements [redacted] following administration of Juvéderm® VOLITE in the volar forearms of healthy volunteers
Design:	Prospective, single-center, open study
Planned Sample Size:	11 subjects included for at least 10 subjects analyzed
Number of centres:	One center
Inclusion criteria:	1. Healthy subject 2. Sex: male and female 3. Age: between 30 and 50 years old at the time of the written consent 4. Subjects with Fitzpatrick skin type II or III. 5. Subject willing to receive Juvéderm® VOLITE in the forearms and agrees to complete all study required procedures, including having 6 punch biopsies taken in the forearms and blood drawn (HIV, B and C hepatitis analysis at screening). [redacted] 9. Written informed consent and data privacy consent obtained.
Exclusion criteria:	**<u>In terms of population:</u>**

1. Pregnant or nursing woman or planning a pregnancy during the study.

[redacted]

4. Subject participating to another research on human beings or being in an exclusion period for a previous study.
5. Intensive exposure to sunlight or UV-rays within the previous month and foreseen during the study.
6. Subject having other resorbable filling product injections, a laser treatment, an ultrasound-based treatment, radiation treatment, a dermabrasion, a surgery, a deep chemical peeling or other ablative procedure on the studied zones within the past 12 months prior to study start.
7. Subject with subcutaneous retaining structure on the studied zones (meshing, threads, gold strand).
8. Subject having received injections of permanent or semi-permanent filling products in the studied zones.

[redacted]

Related to previous or ongoing treatment

	24. Subject under anti-coagulant treatment or treatment liable to interfere with the healing process or hemostasis, during the previous month and during the study. 25. Subject receiving or is planning to receive anti-inflammatory drugs (oral/injectable corticosteroids or NSAIDs, e.g., aspirin, ibuprofen), or other substances known to increase coagulation time (herbal supplements with garlic or ginkgo biloba, etc) for 10 days prior to study treatment and 3 days after. 26. Subject under immunosuppressive therapy. [redacted]
Investigational device: **Name / code:** **Galenic form:** **Dosage:** **Administration route:**	Juvéderm® VOLITE Sterile gel in disposable syringe On Day 0 (D0) the specialist injector will treat the study area with an appropriate volume based on his/her clinical experience and according to Instructions for Use. Intra-dermal injection in forearms
Evaluation criteria/Endpoints:	**Performance exploratory Endpoints** Changes in skin quality parameters, one and three months after Juvéderm® VOLITE injection in the forearms, in comparison with before treatment (D0). Evaluated skin parameters and instruments used are summarized in the table below:

Instrument	Measurement of interest
MoistureMeter D® 0.5 mm	Hydration (epidermis + dermis)
MoistureMeter D® 1.5mm	Hydration (epidermis + dermis)
Corneometer®	Hydration (superficial layers of epidermis)
Cutometer®	Elasticity
Elastimeter®	Elasticity
Dermascan®	Skin thickness and density
Vivosight OCT®	Skin roughness, density and vascularity
Glossymeter®	Brightness
Spectrophotometer®	Colour

	Mexameter®	Melanin content
	[redacted]	
	Safety Endpoints - Injection Site Reactions (ISRs) evaluated by the investigator at each time point after injection. - Collection of adverse events and incidents at all visits including severity, action taken, and relationship to device.	
Study Procedures:	**Screening visit:** informed consent, medical examination, medical background and previous treatment, subject selection, blood sampling. [redacted]	

3. OVERALL STUDY CONSIDERATIONS

3.1. OBJECTIVES

To evaluate the impact on skin quality attributes, including physical measurements [redacted] following administration of Juvéderm® VOLITE in the volar forearms of healthy volunteers.

3.2. STUDY DESIGN

This study is a prospective, single-center, single arm, open label study in healthy subjects. Each subject will serve as its own control (intra-individual).

3.3. SAMPLE SIZE

This study is an exploratory study, no formal sample size calculation has been performed.
11 subjects will be included to have at least 10 subjects analysed (10% of drop-out rate expected).

3.4. BLINDING

This is an open label study.

3.5. RANDOMIZATION

Not applicable. This is a single-arm study.

[redacted]

3.7. STUDY OUTCOME

3.7.1. Performance outcomes

3.7.1.1. Summary of performance assessments

The table below is a summary of performance parameters.

Table 2. Summary of performance parameters

Instrument	Measurement of interest	Parameters studied
MoistureMeter D® 0.5 mm	**Hydration (epidermis+ dermis)**	Hydration rate (TDC)
MoistureMeter D® 1.5mm	**Hydration (epidermis + dermis)**	Hydration rate (TDC)
Corneometer®	**Hydration (superficial layers of epidermis)**	Hydration rate (A.U.)
Cutometer®	**Elasticity**	Uf (R0), Ur, Ua (R8), Uf – Ua (R1), Ua/Uf (R2), Ur/Ue (R5), Uv/Ue (R6), Ur/Uf (R7) (mm), Q1, Q2, Q3
Elastimeter®	**Elasticity**	ISE (N/m)
Dermascan®	**Skin thickness and density**	% of non-echogenic surface (skin density), skin thickness (mm)
Vivosight OCT®	**Skin roughness, density and vascularity**	For roughness: Ra, Rz For density: epidermal thickness, Optical Attenuation Coefficient (OAC) For vascularity: plexus depth, vessel diameter, vessel density, density @300 µm
Glossymeter®	**Brightness**	Brightness Index (GU)
Spectrophotometer®	**Colour**	L*, a*, b* (A.U.), ITA°(°)
Mexameter®	**Melanin content**	Melanin Index (A.U.)

[redacted]

3.7.1.2. Biopsies analysis

[redacted]

[redacted]

3.7.2. Safety outcomes

3.7.2.1. Injection Site Reactions

Immediately after injection, one and three months after injection, the investigator will evaluate the injection site reactions of the tested device with the following 4-point numerical rating scale (NRS):

Table 3. List of ISR

Parameter	None	Mild	Moderate	Severe
Redness / Erythema	☐ 0	☐ 1	☐ 2	☐ 3
Pain / Tenderness	☐ 0	☐ 1	☐ 2	☐ 3
Induration	☐ 0	☐ 1	☐ 2	☐ 3
Swelling / Oedema	☐ 0	☐ 1	☐ 2	☐ 3
Lumps / Bumps	☐ 0	☐ 1	☐ 2	☐ 3
Bruising / Hematoma	☐ 0	☐ 1	☐ 2	☐ 3
Itching	☐ 0	☐ 1	☐ 2	☐ 3
Discoloration/ pigmentation	☐ 0	☐ 1	☐ 2	☐ 3

Each local reaction will be described by the intensity as follows:

Table 4. Description of ISR

	Qualification	Definition
0	**None**	
1	**Mild**	Discomfort noted, but no disruption to normal daily activities. No treatment considered
2	**Moderate**	Discomfort enough to reduce or affect normal daily activities; treatment may be needed.
3	**Severe**	Inability to work or to carry out normal daily activities; treatment is required.

Moreover, the subjects will be given a daily log in order to grade each ISR described above, each day during 28 days after injection. The ISR will not be reported in the Adverse Event Form excepted if still present at D28, if they require a medical treatment of if judged abnormal by the investigator.

3.7.2.2. Other adverse events

Collection of adverse events by the investigator.

4. STATISTICAL METHODOLOGY

4.1. DESCRIPTIVE ANALYSIS

The quantitative data will be summarized by time point using descriptive statistics:

- Number of values
- Mean
- Median
- Standard deviation (SD)
- Standard error of the mean (SEM)
- First quartile (Q1) and third quartile (Q3)
- Minimum value
- Maximum value

The categorial data will be summarized in frequency (N) and percentage (%).

4.2. LEVEL OF SIGNIFICANCE

All statistical tests will be 2-sided and assessed at $\alpha = 5\%$ level of significance when applicable.

4.3. STATISTICAL PASS/FAIL CRITERIA TO BE APPLIED

Not applicable, pilot study.

4.4. CRITERIA FOR THE TERMINATION OF THE CLINICAL INVESTIGATION ON STATISTICAL GROUNDS

No statistical criteria have been defined concerning a premature study end.

4.5. DEVIATION(S) FROM THE ORIGINAL STATISTICAL PLAN

Any modification to the statistical analysis plan will be documented as an amendment and will be described in the final study report if applicable.

4.6. ADJUSTMENTS FOR COVARIATES

No covariate is identified for this study, therefore no adjustment for covariates in efficacy analyses is envisaged.

4.7. HANDLING OF DROPOUTS AND MISSING DATA

No strategy for taking in charge missing data has been defined. Data that are not valid or missing will be considered and treated as missing data.

4.8. INTERIM ANALYSES

An analysis will be planned after D94 visit for safety and performance endpoints. A final clinical report will be written after this analysis.
Only a descriptive analysis will be done after the M9 follow-up phone call and a follow-up report will be written.

4.9. MULTIPLE COMPARISONS / MULTIPLICITY

No adjustment for study wise type I error rates will be done.

4.10. EXAMINATION OF SUBGROUPS

No examination of subgroups of patients is planned.

4.11. STATISTICAL SOFTWARE

SAS® 9.4.

5. STATISTICAL ANALYSIS

The study analysis will be performed after the database has been cleaned and judged valid after the blind review meeting. Then, the database will be locked.

5.1. DEFINITION OF ANALYSIS POPULATION

The following analysis populations will be studied and will be considered for the statistical analysis:

- Safety population: any subject having used the tested device.
- FAS (Full Analysis Set): Any subject included in the study with at least a post-basal value.

The analysis of the safety/tolerance parameters will be performed on the “safety” population. The analysis of the performance parameters will be performed on the FAS.

5.2. DESCRIPTIVE STATISTICS

Descriptive statistics will be presented for each parameter. Categorical variables will be summarized with frequency and relative frequency. Continuous variables will be summarized by number of subjects, mean, median, standard deviation, minimum, and maximum. Where appropriate, 2-sided 95% CIs for population mean, or population proportion, will be provided as part of the descriptive summary.

5.3. PATIENT ACCOUNTABILITY

A flow diagram will be drawn with the number of patients enrolled, the number and % of screen failures and reason for exclusion, the number and % of patients receiving the medical device, the number and % of patients dropping out before and reason for dropping out, and the number and % of patients completing the study.

5.4. DEMOGRAPHIC DATA AND OTHER BASELINE CHARACTERISTICS

The study population will be described on the demographic and injection parameters recorded at the screening and inclusion visits.
The demographic data will be:

- Sex
- Age (variable calculated from birth date and screening visit date)
- Fitzpatrick skin phototype
- Women with childbearing potential
- Contraceptive method

The injection parameters will be:

- Volume injected
- Injection technique

The listing of medical history and previous and concomitant treatments will also be provided.

5.5. PERFORMANCE EVALUATION

5.5.1. Skin quality measurements

All parameters listed in Table 2 of paragraph 3.7.1.1 ([redacted]) will be analysed using a mixed ANOVA model for repeated measures (*MIXED procedure of SAS®*) fitted to raw data.

The following factors will be included in the model:

- Zone as fixed (2 levels: treated and control)
- Time as fixed (3 levels: D0, D28 and D84)
- Zone by time interaction
- Subject as random

To capture the correlation between data obtained from a same subject, an unstructured variance -covariance matrix is set.

The following comparisons on adjusted means obtained from the mixed linear model will be performed:

1) D28 versus D0 for treated zone
2) D84 versus D0 for treated zone
3) D84 versus D28 for treated zone

4) D28 versus D0 for non-treated zone
5) D84 versus D0 for non-treated zone
6) D84 vs D28 for non-treated zone

7) $(D28\text{-}D0)_{\text{treated zone}}$ versus $(D28\text{-}D0)_{\text{non-treated zone}}$
8) $(D84\text{-}D0)_{\text{treated zone}}$ versus $(D84\text{-}D0)_{\text{non-treated zone}}$
9) $(D84\text{-}D28)_{\text{treated zone}}$ versus $(D84\text{-}D28)_{\text{non-treated zone}}$

All adjusted means differences will be tabulated associated with their 95% CI.

The underlying assumptions (residual normality and homoscedasticity) will be analyzed with Shapiro-Wilk test (□=0.01) and graphs to judge the model validity. In case of strong deviation, a non-parametric approach (Wilcoxon signed rank test) will be carried out.

[redacted]

5.6. SAFETY EVALUATION

For each parameter of ISR evaluation, the score distribution will be summarized in frequency and percentage by time point.

In addition, for each parameter of ISR evaluation, the proportion of the subjects presenting the sign will be also computed and summarized in frequency and percentage.

At M9, for the safety follow-up phone call, any remarks from the investigator or the subject will be listed.

The individual listing of AE will be provided.
AEs, ADEs, SAEs and SADEs will be summarized by presenting the number and percentage of patients having any sign or symptom. AEs will be classified by System Organ Class (SOCs) and Preferred Term (PTs) using MedDRA.

6. SIGNATURES

SPONSOR : **Allergan Pharmaceuticals International Limited**

CRO: **Eurofins / Pharmascan**

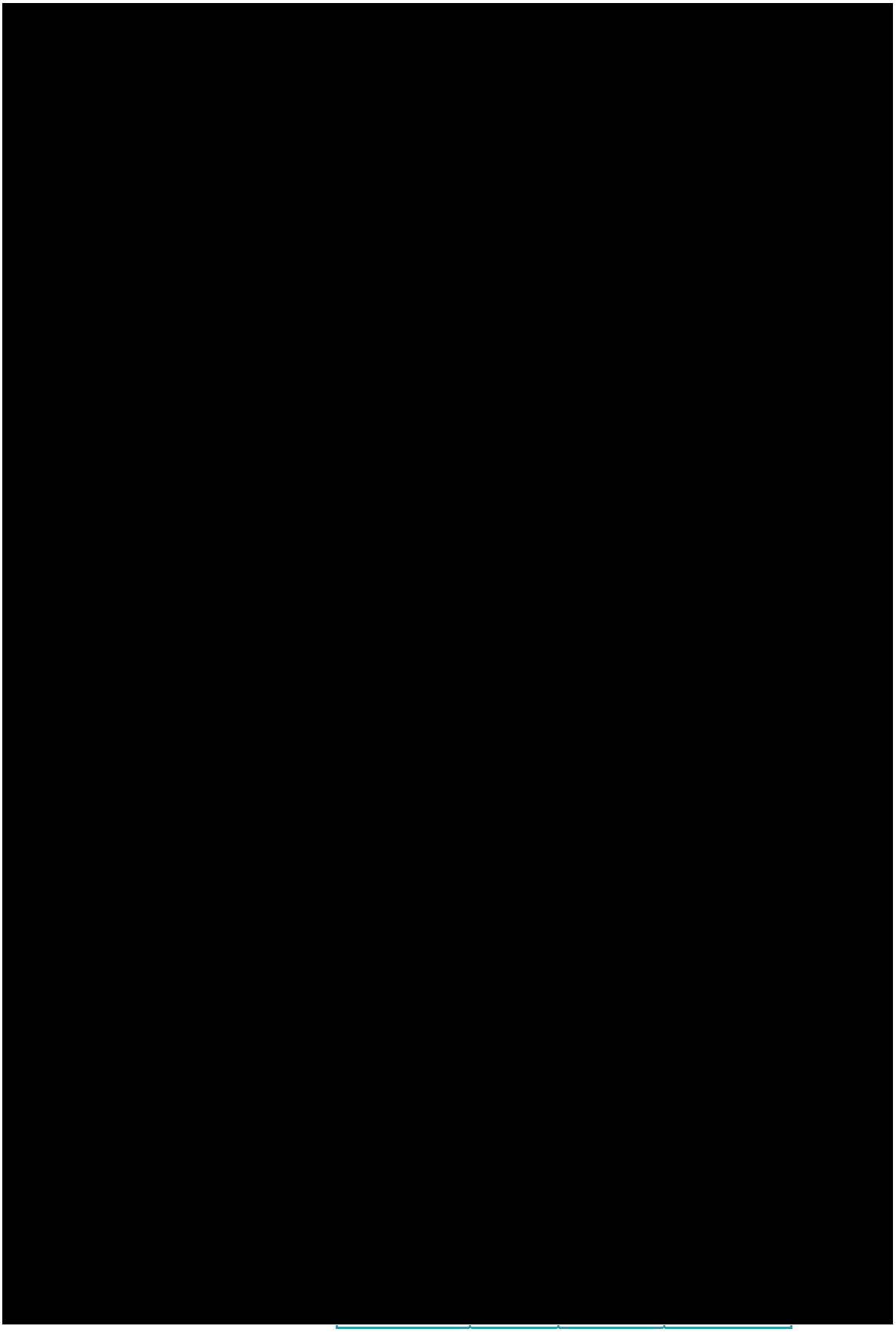
[redacted]

7. APPENDICES : STRUCTURE OF TABLES

[redacted]

[redacted]

[redacted]



[redacted]

[redacted]