Hylase® „Dessau“

Phase II pilot study: Determination of an effective and tolerable dose of Hylase® "Dessau" (bovine hyaluronidase) in the treatment of hyaluronic acid filler-induced overinjections by injection into defined hyaluronic acid filler-injected skin areas of healthy volunteers

Hylase® treatment of hyaluronic acid filler-induced overinjections

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Ethics committee(s):	Lead EC: EC of Cottbus, Germany EC of Hamburg, Germany

1. Title page

Study Title	Phase II pilot study: Determination of an effective and tolerable dose of Hylase® "Dessau" (bovine hyaluronidase) in the treatment of hyaluronic acid filler-induced overinjections by injection into defined hyaluronic acid filler-injected skin areas of healthy volunteers
Study Title German	Phase II Pilotstudie: Ermitteln einer wirksamen und verträglichen Dosis von Hylase® „Dessau“ (bovine Hyaluronidase) in der Behandlung von durch Hyaluronsäurefiller verursachten Überspritzungen durch Injektion in definiert mit Hyaluronsäurefiller überspritzte Hautareale gesunder Probanden
Short Title	Hylase® treatment of hyaluronic acid filler-induced overinjections
Protocol No.	CTU148N
EudraCT Nr	2019-001151-40
Name of test drug/product	Hylase® "Dessau" 150 I.U. (bovine hyaluronidase)
Comparator	Not applicable
Dosage	Subcutaneous (s.c.) injection of 0,5 mL Hylase® „Dessau“ 150 I.U., diluted to 15, 25, 50, and 75 IU
Indication	Hyaluronic acid filler-induced overinjections
Design	Prospective, multicenter, randomized, single-blind, five-arm pilot study
Development phase	Phase II
Sponsor	RIEMSER Pharma GmbH, Greifswald, Insel Riems, Germany
Principal Investigator	Dr. Tanja Fischer, Haut- & Lasercentrum Potsdam, Germany
Author of report	Dr. Sigrun Niemitz
Study initiation date	09/July/2019
Study completion date	March 31, 2020
Version and date of report	V2.0 19-November-2020

This study was performed in compliance with Good Clinical Practices (GCP), including the archiving of essential documents.

2. Synopsis

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Name of Finished Product: Hylase® Dessau		
Name of Active Ingredient: Bovine hyaluronidase		

Title of study:
Phase II pilot study: Determination of an effective and tolerable dose of Hylase® "Dessau" (bovine hyaluronidase) in the treatment of hyaluronic acid filler-induced overinjections by injection into defined hyaluronic acid filler-injected skin areas of healthy volunteers

Principal Investigator:
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Study center(s):
Two study centers in Germany participated in this study.

Publication (reference):
Not applicable

Studied period (years): 2019 to 2020	**Phase of development**: Phase II

Objectives:
Primary objective:

Determination of a tolerable and effective dose of Hylase® "Dessau" (bovine hyaluronidase) as an antidote for the treatment of overcorrection (blue lines, persistent swelling, vascular involvement) by hyaluronic acid (HA) fillers based on the treatment of artificially induced overinjections on the hands.

24 h after Hylase injection the response of the Hylase dosages was determined. A response is defined as a volume reduction of 90%, which was detected by ultrasound, and a nodule, which is hardly or no longer tactile by palpation.

Secondary objectives:

- Evaluation of the volume reduction by means of a 3D camera
- Evaluation of the safety and tolerability of Hylase® "Dessau" in clinical practice used as antidote for HA fillers
- Number of adverse events (AE) per dose and HA filler
- Number of expected adverse drug reactions (ADR) per dose and HA filler
- Number of unexpected ADRs per dose and HA filler
- Detection of AEs and ADRs after treatment with HA fillers

Study Design and Methods

The aim of this prospective, multicenter, randomized, observer-blinded, five-arm phase II pilot study was to find an effective and tolerable dose of Hylase® "Dessau" (bovine hyaluronidase) as

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an antidote in the treatment of overcorrection with hyaluronic acid (so-called 'filler'). For this purpose, HA fillers were injected artificially into the back of the hands of healthy volunteers.

On the back of both hands, 0.5 mL aliquots of HA fillers were injected into each hand (artificial overinjection). The injection of the HA filler was done per injection site after a single puncture of the skin into the dermis (injection depth according to the manufacturer's instructions) in one pass, so that the complete 0.5 mL aliquot was injected at one site. After one or 2 days (maximum), the saline solution (control) and four different concentrations of Hylase were injected randomly at 5 injection sites per hand into the over-injected skin areas (following the 4-eye principle). Study evaluation was performed using imaging techniques (ultrasound and 3D photography) and palpation at several time points (Table 1) assessing the presence of artificially administered HA at the injection site.

Number of participants:	**planned**: 57 **screened**: 28	**randomized**:28 **completed**: 28	**analyzed efficacy**:28 **analyzed safety**: 28

Diagnosis and main criteria for inclusion and exclusion:

Inclusion criteria:

1. Male and female adults, age ≥ 18 - ≤65 years at the time of enrollment
2. Signed informed consent and ability to understand and follow the study instructions
3. Study participants were healthy and are in good physical condition
4. Heart frequency of > 50 - < 90 beats per minute and systolic blood pressure (BP) > 90 - < 140; diastolic BP > 50 - < 90 mmHg

Exclusion criteria:

1. Known hypersensitivity against hyaluronidase, bovine protein and/or gelatin
2. Pregnancy or breast-feeding during the whole study
3. Women of childbearing potential who did not use contraceptive methods with a Pearl Index ≤ 1, e.g. "pill", implant, depot injection, sterilization (exception: menopause since ≥1 year)
4. Subjects with congenital heart defects, venous congestion or shock symptoms
5. Known allergies to venom
6. Subjects with infections, wounds and skin diseases and/or swelling caused by stitches or bites in the treatment area
7. Alcohol and/or drug dependency
8. Subjects who were simultaneously taking drugs containing antihistamines, heparin, morphine, chondroitin sulfate B, cholic acid, dicumarol, vitamin C, flavonoids, sulfonate detergents, salicylic acid derivatives and heavy metal ions (Fe, Mn, Cu, Zn, Hg), salicylic acid derivatives, histamine, calcium or phosphates These substances can inhibit or enhance the effect of hylase.
9. Subjects with cancer and autoimmune diseases (e.g. psoriasis)
10. Subjects who have received or had received a plasma infusion, in case the serum protein levels of the subject were < 55 g/L
11. Subjects with known immunodeficiency
12. Tendency to keloid formation and/or hypertrophic scarring
13. Participation in another clinical trial within the last 30 days
14. Subjects unwilling or unable to follow the requirements of the study protocol

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15. Employees/relatives of the sponsor or employees/relatives of the principal investigator (PI)

Test product, dose and mode of administration, batch number (LOT):
Hylase® „Dessau“ 150 I.U.,0.5 mL, single dose of 15, 25, 50, and 75 I.U., subcutaneous (s.c.) injection. One repeated injection at visit 6 and 7, on ‘as needed’ basis
batch number (LOT): 2471117
Sodium chloride solution (0.9%) was used as placebo control .

Duration of treatment:
Single injection; repeated injection on ‘as needed’ basis

Reference therapy, dose and mode of administration, batch number (LOT):
Required to test Hylase® „Dessau”:
Stratum 1: Allergan HA Filler Voluma® 10 mg/0,5 mL, s.c. injection (LOT: VB20A80852)
Stratum 2: Merz HA Filler, Volume®; 13 mg/0.5mL, s.c. injection (LOT: 547331/1)
Stratum 3: Galderma HA Filler Restylane Volyme®, 10 mg/0.5 mL, s.c. injection (LOT 16848-1):

Criteria for evaluation:
Efficacy:

24 h after Hylase injection the response of the Hylase dosages was determined. A response is defined as a volume reduction of 90%, which was detected by ultrasound, and a nodule, which is hardly or no longer tactile by palpation.

Primary endpoint
Response to treatment was assessed by

- Ultrasound; the elevation of the injection site decreased by more than 90%
- Palpation, rating of 0 or 1, according to the palpation rating scale

Secondary endpoints:

- Evaluation of the volume reduction by means of a 3D camera
- **Safety**:
  - Number of adverse events (AE) per dose and HA filler
  - Number of expected ADRs per dose and HA filler
  - Number of unexpected ADRs per dose and HA filler
  - Detection of AEs and ADRs after treatment with HA fillers

Statistical methods:

Population-related data were evaluated descriptively. Categorical variables were presented in form of frequency tables using absolute values and percentages. Percentages are calculated using one decimal place.
Continuous variables were presented on the basis of the number of subjects, the number of subjects without missing examinations (N), mean, standard deviation (SD), median, minimum (min), maximum (max), and of the 1st and 3rd quartiles. As baseline, all measurements at visit 2 (day 1) 10 minutes (±5 minutes) before injection of Hylase® "Dessau" were used.

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All efficacy analyses were performed for the ITT and PP set and separately for hyaluronic acid filler.
The four different doses of Hylase® "Dessau" were tested within a hierarchical analysis. The saline solution was tested as a fifth dose (placebo control) as part of the randomisation. The analysis was based on the assumption that a higher Hylase concentration causes a higher response rate. Thus, there was no loss of power due to parallel testing of the doses. The following null hypothesis was tested:
Null hypothesis: π_Response=80%
Alternative hypothesis: π_Response>80%, i.e. if the lower limit of the 95% confidence interval (CI) of the response estimate was above 80%, the null hypothesis could be rejected.
If the null hypothesis for the highest dose could be rejected, the next highest dose at the same significance level could be tested. This was done until the smallest dose was tested. If, however, no significance could be achieved at one of these hierarchical levels, the test stopped and all subsequent levels were also assessed as not significant. This strict hierarchy ensured strict compliance with the first type of error in terms of multiple testing.

Due to the premature end of recruitment, the targeted number of participants could not be reached. Even if the assumed response rate of 90% had been achieved, the lower limit of 80% could not have been exceeded significantly. In order to discuss the results of all doses, the response rates for all HA fillers and doses including their 95% CI (probability of error not adjusted) were given exploratively for all HA fillers and doses.

Sensitivity analysis related to HA filler
The primary analysis of the efficacy of Hylase was carried out exclusively with regard to the HA filler, as these can be split by hyaluronidase with varying effectiveness. As a general effect of the Hylase "Dessau", however, a sensitivity analysis was performed to determine whether the response rate per dose was also estimated similarly independent of the filler.
Since each subject was treated with two HA fillers, dependent data were available for each dose, so that a generalized mixed model was used for estimating the response rate 24 hours after Hylase injection, in which the subject identification number was also estimated as a random effect, and thus subject-related dependencies could be taken into account. In the same way, the center affiliation was modelled as a random effect. The hierarchical testing was performed as already described.
The sensitivity analysis based on imputed data (if more than 5% of the test persons were no-responders), was not done due to premature termination of recruitment.
In addition, AEs as well as expected and unexpected ADRs of Hylase® "Dessau" and of HA fillers were analyzed descriptively. The analysis was performed depending on the dose per HA filler.

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Summary – Conclusion:

Efficacy results:

It has to be noted, that due to the premature end of recruitment (due to the coronavirus pandemic), the target number of participants could not be reached. Even if the assumed response rate of 90% had been achieved, it had not been possible to achieve any statistical significancies.

Baseline characteristics:

Twenty-two females and 6 males were included in the study (ITT population). In the PP population there were 18 females and 5 males. The mean age of the subjects of the ITT population was 49.5 years, range 20.0 – 65.0 years. The mean age in females was 50.5 years, range 21.0 – 65.0 years, and the mean age in males was 46.0 years, range 20.0 – 64.0 years.

The mean age of the subjects of the PP population was 50.0 years, range 20.0 – 65.0 years. The mean age in females was 52.1 years, range 21.0 – 65.0 years, and the mean age in males was 42.4 years, range 20.0 – 65.0 years.

Baseline characteristics were similar in the ITT as well as in the PP population.

Efficacy: Responder Analysis

In the descriptive analysis there were most responders (n=11 out of 16 for the Allergan HA fillers, and n=13 out of 16 for the Galderma HA fillers) when using a Hylase dose of 50 I.U Using the Merz HA Fillers there were 13 responders out of 16 with a Hylase dose of 75 I.U.

Results of the hierarchical analysis - starting with the highest Hylase dose -showed that for none of the HA fillers tested the 0-hypothesis could be rejected. Response rates of the highest dose tested (75 I.U.) in the ITT population were as follows:

- 75.0% [53.8%; 96.2%] - Allergan HA-Filler Voluma
- 81.3% [62.1%; 100%] - Merz HA-Filler Volume und
- 56.3% [31.9%; 80.6%] - Galderma HA-Filler Restylane Volyme.

Their lower limit of the respective 95% CI was in no case above 80%, so that the hierarchical test procedure was terminated after the highest dose and all other Hylase® "Dessau" doses were also not considered significant. Thus, no (lowest) effective dose of Hylase could be determined.

However, it could be demonstrated clearly, that with each filler used the response was considerably better compared to the saline control with a response rate of 75.0%, 81.3% and 56.3% vs 6.3%, 0% and 18.8%, respectively.

Within the sensitivity analysis the response rates related to the concentration of Hylase “Dessau” as well as to the HA fillers were as follows:

- Hylase® „Dessau“ 75 I.U.: 71.3% [36.7%; 91.4%]
- Hylase® „Dessau“ 50 I.U.: 73.0% [37.7%; 92.4%]
- Hylase® „Dessau“ 25 I.U.: 60.1% [40.4%; 76.9%]
- Hylase® „Dessau“ 15 I.U.: 51.5% [34.9%; 67.8%]

Thus, even when considering the HA fillers in summary, neither the highest dose nor the lower dosages of Hylase® "Dessau" achieved a response rate above 80%.

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Looking at the response rates related to the sensitivity analysis due to the premature stop of recruitment the highest response rate of 90.9% [73.9%; 100%] after 24 hours was achieved with Allergan HA-Filler Voluma using Hylase® "Dessau" 50 I.U. At visit 6 a response was achieved in all subjects for all three HA fillers using Hylase® "Dessau" 75 I.U. and Hylase® "Dessau" 50 I.U.
In all analyses the results of the ITT population matched those of the PP population very well.

Follow-up injections: In the ITT population, the artificial overcorrected sites were not resolved at V6 in 17 subjects who received a total of 48 Hylase® “Dessau” follow-up injections; about half of the injection sites being those initial treated with saline solution.

In the PP population, the artificial overcorrected sites were not resolved in eight subjects at V6, and the follow-up injections were made exclusively at the injection sites that had been treated with saline solution.

Safety results:

All subjects received their injections according to the protocol but in 2 subjects the randomization scheme was not correctly applied (1 subject in the Allergan HA filler group and 1 subject in the Galderma HA filler group).

The safety population (SAF) comprised all of the 28 subjects who received Hylase® Dessau.

A total of 27 AEs were developed by 5 out of 28 subjects (17.9%); the remaining subjects had no AE.

All AEs were of mild intensity; there was no SAE during the study. All AEs were resolved until end of the study.

Eight AEs were assessed as not related to Hylase® “Dessau”, of wich five were reported directly after HA filler injection, and two were assigned to the injection of 0.9 % NaCl.

Seventeen AEs were assessed as related to the injection of Hylase® “Dessau” at V2 (ADRs). Those were injection site pain (n=8), peripheral swelling (n=2), application site hematoma (n=1), erythema (, n=4), nerve injury (n=1), and pain in extremity (n=1). Two of the ADRs, ulnar nerve injury and application site hematoma respectively, were unexpected.
Five ADRs required medicational treatment.

Two additional, expected, ADRs were reported after the follow-up injection of Hylase® “Dessau”.

Taken together, Hylase® “Dessau” treatment was well tolerated and safe.

Conclusion:

This study was performed to find an effective and safe dose of Hylase® “Dessau” to treat overinjections caused by HA fillers. The study aim was not met due to the low recruitment numbers, caused by the Covid-19 pandemic however, there was a large proportion of responders in each group (13 or 11 responders out of 16) when using a Hylase dose of 50 I.U or 75 I.U.

This result was confirmed by the data derived from the explorative analysis: With each filler used the response was considerably better compared to the saline control with a response rate of 75.0%, 81.3% and 56.3% vs 6.3%, 0% and 18.8%, respectively.

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A similar response rate per dose - independently of the filler – could be achieved applying a sensitivity analysis.

There were only a few AEs related to the test product that were of mild intensity so that safety profile of Hylase “Dessau” can be considered as being very good.

Overall, from the results presented it can be concluded that a dose of 50 I.U. of Hylase® "Dessau" as well as a dose of 75 I.U. was effective in the treatment of overcorrections, irrespective of the HA filler used. This statement is true despite the fact that no significance could be established, partlydue to the low recruitment numbers.

Date of report: October 2020

2.1 Clinical Study Protocol / Amendments

The clinical study protocol was approved on May 16, 2019. There was one amendment, dated November 18, 2019 to the protocol that was approved on Jan 03, 2020.

2.2 Study Centers

The study was conducted at 2 centers in Germany:

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3. Table of Contents

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4. List of Abbreviations and Definition Terms

ADR	Adverse Drug Reaction
AE	Adverse Event
BMI	Body Mass Index
BP	Blood Pressue
CI	Confidence Interval
CRF	Case Report/Record Form
CRO	Contract Research Organization
CSR	Clinical Study Report
DMP	Data Management Plan
EMA	European Medicines Agency
FAS	Full Analysis Set
GCP	Good Clinical Practice
HA	Hyaluronic acid
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IRB	Independent Review Board
ITT	Intention to treat
IU	International Unit
MedDRA	Medical Dictionary for Regulatory Activities
PI	Principal Investigator
PP	Per protocol
RMV	Regular monitoring visits
SAE	Serious Adverse Event
SAF	Safety Analysis Set
SAP	Statistical Analysis Plan
s.c.	subcutaneously
SD	Standard Deviation
SDV	Source Data Verification
SIV	Site initiation visit
SmPC	Summary of Product Characteristics
SOC	System Organ Class

TEAE	Treatment emergent adverse event

5. Ethics

5.1 Independent Ethics Committee (EC) or Institutional Review Board (IRB)

The clinical study report (CSR) is based on the study protocol final version 2.0 dated November 18, 2019. The study protocol as well as the patient information and the Informed Consent Form (ICF) were reviewed and approved by the EC of Cottbus as leading EC, as well as by the ECs of Hamburg, Germany.

The trial has the EudraCT Nr 2019-001151-40.

5.2 Ethical Conduct of the Study

This study was designed and conducted, recorded and reported according to the principles of GCP, the World Medical Associate Declaration of Helsinki (1989) and International Conference on Harmonization guidelines. The investigator conducted all aspects of this study in accordance with all national, state and local laws of the pertinent regulatory authorities.

Before study initiation, the Investigator obtained approval or favorable opinion of the research protocol and informed consent form (ICF) from a competent IRB/IEC. The Investigator assured IRB/IEC compliance with applicable regulations.

5.3 Patient Information and Consent

Informed consent was obtained from each subject before the performance of any study-related activity. The investigator explained the nature of the study and the treatment in such a manner that the subject was aware of his/her rights and responsibilities, as well as potential benefits and risks. The investigator was also responsible for answering any questions the subject had throughout the study and for sharing any new information, in a timely manner, that could have been relevant to the subject's willingness to continue his/her participation in the study. The ICF also included indications about the insurance. Subjects were also informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice to their current or future care. Patients were also informed about data protection regulations. Documentation of the discussion and the date of informed consent were recorded in the subject's medical record.

The informed consent form can be found in appendix 17.1.3.

6. Investigators and Study Administrative Structure

This clinical study was sponsored by RIEMSER Pharma GmbH, Greifswald-Berlin (Germany).

The responsible contract research organization (CRO) was Convidia Clinical Research, Münster, Germany.

The clinical part was conducted at two sites in Germany, according point 2.2 study sites.

The Leading Investigator and Scientific Expert of this study was Dr. med. Tanja Fischer, Haut- & Lasercentrum Potsdam, Kurfürstenstraße 40, 14467 Potsdam, Germany

Monitoring was conducted by Convidia Clinical Research.

The statistician in charge was Dr Daniela Adolf.

The author of the report was Dr Sigrun Niemitz.

7. Introduction

Hyaluronic acids (HA) are hydrophilic mucopolysaccharides with a high water-binding capacity (up to 6 litres of water per gram) and they are an ubiquitous component of the extracellular matrix of organisms (Becker et al, 2009). Hyaluronic acid combined with water forms a gel-like material, which for example is also a main component of the synovia. Hyaluronic acid is subject to a dynamic equilibrium between continuous build-up and breakdown (turnover) by HA synthases and hyaluronidases. Depending on the tissue, the half-life is about 4 minutes in blood, 1 – 2 days in skin, and 1 – 3 weeks in cartilage.

Hyaluronic acid is used in the cosmetic industry as a freeze-dried powder or as an aqueous solution, furthermore in the form of its potassium or sodium salts. Hyaluronic acid is mainly produced by bacterial fermentation (Necas et al, 2008). Since a decrease in the HA content of the skin is a major characteristic of skin ageing, physicians in aesthetic dermatology use the ability of HA to build up volume due to its high water-binding capacity and use HA injections as dermal fillers - a treatment that is becoming increasingly popular (Buhren et al, 2018; Cohen et al, 2015).

However, treatment with dermal fillers can also lead to adverse drug reactions (ADRs) such as overcorrection. Since the injections usually aim at a long-lasting effect, the iatrogenic deformities resulting from overcorrection can lead to patient dissatisfaction with the treatment (Valerian et al, 2005). Other potential complications of dermal filler treatment include the Tyndall effect or edema of the lower eyelid, granulomas, infections up to tissue necrosis, or even blindness due to vascular occlusion (Buhren et al, 2016; Cohen et al, 2015).

Hyaluronidases have been on the market in the USA since 1948. Approval for certain indications is based on the ability of hyaluronidases to enhance the distribution and dispersion of other injected substances. However, there is no consensus on the indications and treatment protocols for the use of hyaluronidases, and there are no standardized guidelines in place (Cohen et al, 2015).

Hylase® "Dessau" with the active ingredient hyaluronidase is an enzyme preparation that splits up both endogenous and synthetically produced HA by breaking down glycoside bridges but also other mucopolysaccharides that occur in connective tissue. In the field of dermatology/aesthetic medicine, the use of hylases as an antidote in off-label use is considered the gold standard in the treatment of overcorrection during dermal injection with HA (Buhren et al, 2018). Here the enzyme dissolves HA applied into the skin within the scope of a dermatological treatment.

The activity of different formulations of hyaluronidase is determined *in vitro* by the ability of hyaluronidase to depolymerize HA. The assay is referenced in the United States Pharmacopeia as a standard assay. The activity of hyaluronidase is expressed in units. One unit is defined as the amount of enzyme that releases 1 µmol of N-acetylglucosamine from HA per minute at 37°C and at a pH of 4.0. The activity of hyaluronidase is pH dependent. Hyaluronidase from bull testicles has an activity of 70% at a pH of 4, and retains this activity even at a pH of 7.5, i.e. it acts in a much wider pH range than other hyaluronidases (Dunn et al, 2010).

The availability of a specific antidote, hyaluronidase, for the treatment of overcorrection and complications in filler treatments is a major reason for the preferred use of hyaluronic acid-based fillers over other injectable fillers such as calcium hydroxylapatite or poly-1-lactic acid.

The degradation of a HA filler by hyaluronidase has already been investigated and confirmed in several *in vitro* tests (Sall et al. 2007, Jones et al. 2010, Buhren et al 2018).

Background information on Hylase® "Dessau

The information is based on the current summary of product characteristics (SmPC) of Hylase® "Dessau" 150 I.U. (dated from Nov 2018). Hyaluronidase (hyaluron glucosaminidase) cleaves components (the glucosamine bridges between C1 of the N-acetylglucosamine unit and C4 of glucoronic acid) of the extracellular matrix, hydrolyzing HA-type mucopolysaccharides. Substrates for testicular hyaluronidase are HA, chondroitin, chondroitin 4-sulfate and chondroitin 6-sulfate. The enzyme causes the extracellular matrix to become more permeable, the viscosity decreases and the permeability of the connective tissue is increased. This mechanism leads to accelerated absorption of fluids after subcutaneous (sc) and intramuscular injections. Therefore, Hyaluronidase is also known as diffusion factor.

Hylase® "Dessau" is obtained by extraction from bovine testes and purified via several stages of precipitation and adsorption. Hylase® “Dessau” is inactivated at a pH > 7.5 or < 4.0 and temperatures > 50 °C.

After an intravenous administration of 500 International Units (I.U.) of hyaluronidase per kg body weight, the elimination half-life in humans is 2.4 - 4.1 min. One min after injection serum levels are 8 - 10.7 I.U./mL serum. No enzyme activity in the plasma can be detected after s.c. administration.

Hyaluronidase is mainly distributed in the liver, kidney, and the gastrointestinal tract. The blood-brain barrier prevents accumulation in the central nervous system. After only 2 minutes 60% of the radioactivity can be found in the liver and it drops to 20% within 4 hours. The corresponding values in the kidney are 4.8% and 10% of the applied dose. These concentrations can not be found in any other organ.

In the tissue, hyaluronidase is split up by trypsin and pepsin and in the bloodstream it is rapidly inactivated by serum inhibitors. In a second phase, the enzyme moves from the intravascular to the extravascular space within 2 hours. In a 3rd phase lasting 1 – 3 hours the hyaluronidase is inactivated and eliminated.

The hyaluronidase effect or activity in the tissue lasts at least 12 hours despite a short half-life. The original condition of the tissue is restored after 2 – 4 days.

Data based on pre-clinical studies on acute and chronic toxicity do not give any indication of specific hazards for humans.

After application of Hylase® “Dessau” 150 I.U. allergic reactions are rarely experienced, these can rarely be accompanied by a shock symptom. The frequency of allergies, to which anaphylaxis-like reactions belong, is less than 0.1%, with possible occurrence of the following symptoms: chest pain or chest tightness, dizziness, tachycardia or racing heartbeat, shortness of breath or dyspnoea, skin flush, urticaria or itching, swelling of the face, lips, neck, ears, arms or legs, throat thightness, slight inflammations, redness or pain, nausea or vomiting.

In addition, existing infections can be increased by hyaluronidase application. In rare cases, an increase in temperature, loosening in teeth or intensified menstrual bleeding may occur. Furthermore, unpleasant injection pains upon application have been reported in single cases.

This ADR profile is consistent with ADR reports from studies with other hyaluronidase preparations (Cohen et al, 2015).

8. Study Objectives

Primary objective:
The primary objective of the study was the determination of a safe and effective dose of Hylase® "Dessau" (bovine hyaluronidase) as antidote for the treatment of overcorrection (blue lines, persistent swelling, vascular involvement) on the back of the hands caused by HA fillers.

Secondary objectives:
Secondary endpoints were the assessment of the safety and tolerability of Hylase® “Dessau” 150 I.U. in clinical use as an antidote to HA filler overcorrection (number of AEs per dose and HA filler, number of unexpected AEs per dose and filler) and the assessment of the volume reduction with 3D camera.

9. Investigational Plan

9.1 Overall Study Design and Plan-Description

The aim of this prospective, multicenter, randomized, single-blind, five-arm phase II pilot study was to find an effective and safe dose of Hylase® "Dessau" (hyaluronidase) as an antidote in the treatment of overcorrection caused by HA (so-called 'filler'). For this purpose, HA fillers were injected s.c. artificially on the back of the hands of healthy volunteers.

On the back of both hands, two 0.5 mL aliquots out of the three HA fillers used in this study were injected according to the scheme presented in Figure 1 (artificial overinjection). The injection of the HA filler was done per injection site after a single puncture of the skin into the dermis (injection depth according to the manufacturer's instructions) in one pass, so that the complete 0.5 mL were injected at one site. After 1-2 days, the control (saline solution) and four different concentrations of Hylase were injected randomly into the over-injected skin areas (following the 4-eye principle). The evaluation of the study was performed by volume measurement using imaging techniques (ultrasound and photography) and palpation by examining the subjects at different time points (Table 1) for the presence of nodules at the injection site and defining a response for each site.

The response at the injection sites was evaluated by a blinded investigator using volume determination by imaging techniques and palpation using a 5-part palpation rating scale (Table 2). A response was achieved if the palpation rating scale is 0 or 1, and if a complete volume reduction of more than 90% occurred. Furthermore, the occurrence of AEs and ADRs after treatment with Hylase® "Dessau" was examined, taking into account also AEs that occurred after administration of the HA filler. Adverse events were recorded up to and including day 14.

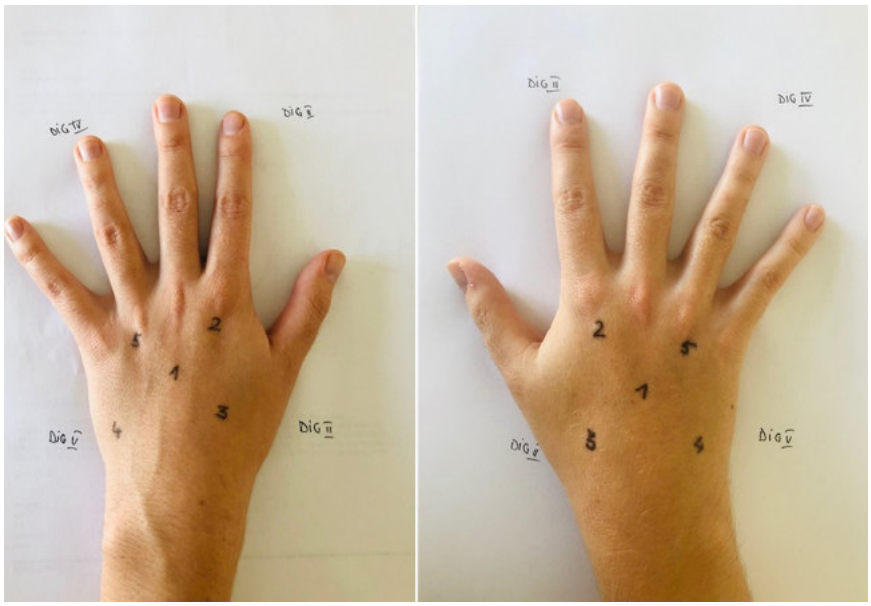

Figure 1: Examples of injection sites at the back of both hands

9.2 Discussion of Study Design, including the Choice of Control Groups

A five-arm study design was considered suitable and sufficient to find an appropriate dose and to investigate the efficacy and safety of Hylase® "Dessau" as antidote in this clinical trial. Although there are more than 3 HA fillers on the market, the used fillers were selected according to the degree of cross-linking (all 3 used fillers have a high degree of crosslinking).

Despite the fact that in the field of dermatology/aesthetic medicine the use of hyaluronidase as antidote in the treatment of AEs for example vascular occlusion, blindness, Tyndall effect, and overcorrections in dermal injection with HA is considered the gold standard, currently hardly any controlled studies exist investigating efficacy. In addition, the most effective and safe dose has not been determined in a controlled trial to date. Therefore, in this clinical trial different doses of Hylase® "Dessau" administered after an artificial overdose of an HA filler were tested for their efficacy and tolerability and examined by means of objective imaging techniques (ultrasound and photographic documentation with 3D camera). In addition, palpation was always performed by the same experienced, blinded dermatologist per patient, who classified the findings according to the criteria listed in Table 2.

To determine the efficacy of Hylase® "Dessau" study participants were observed for 1-2 days after Hylase injection (evaluation of the primary endpoint 24 h after the addition of Hylase. Based on the half-life of hyaluronidase, this timeframe appeared to be sufficient. Hyaluronidase is known to have a plasma half-life of two minutes, but it has a duration of action of 24-48 hours in the tissues.

To assess safety and tolerability, AEs were documented at all visits. On day 14 (V7), a final examination was performed at the study sites. If at V7 a follow-up Hylase® “Dessau” injection was required, an additional contact was realized 14 days later to document the safety information.

The current SmPC of Hylase® “Dessau” 150 I.U. (dated from Nov 2018) was used to assess the expectedness.

9.3 Selection of Study Population

1. Male and female adults, age ≥ 18 - ≤65 years at the time of enrollment
2. Signed informed consent and ability to understand and follow the study instructions
3. Study participants are healthy and in good physical condition
4. Heart frequency of > 50 - < 90 beats per minute and systolic blood pressure (BP) > 90 - < 140; diastolic BP > 50 - < 90 mmHg

9.3.1 Inclusion and exclusion Criteria

Inclusion criteria:

1. Male and female adults, age ≥ 18 - ≤65 years at the time of enrollment
2. Signed informed consent and ability to understand and follow the study instructions
3. Study participants were healthy and are in good physical condition
4. Heart frequency of > 50 - < 90 beats per minute and systolic blood pressure (BP) > 90 - < 140; diastolic BP > 50 - < 90 mmHg

Participants were not eligible for inclusion into the study, if one of the following criteria was met:

Exclusion criteria:

1. Known hypersensitivity against hyaluronidase, bovine protein and/or gelatin
2. Pregnancy or breast-feeding during the whole study
3. Women of childbearing potential who did not use contraceptive methods with a Pearl Index ≤ 1, e.g. "pill", implant, depot injection, sterilization (exception: menopause since ≥1 year)
4. Subjects with congenital heart defects, venous congestion or shock symptoms
5. Known allergy against inset poison
6. Subjects with infections, wounds and skin diseases and/or swelling caused by stitches or bites in the treatment area
7. Alcohol and/or drug dependency
8. Subjects who were simultaneously taking drugs containing antihistamines, heparin, morphine, chondroitin sulfate B, cholic acid, dicumarol, vitamin C, flavonoids, sulfonate detergents, salicylic acid derivatives and heavy metal ions (Fe, Mn, Cu, Zn, Hg), salicylic acid derivatives, histamine, calcium or phosphates These substances can inhibit or enhance the effect of hylase.
9. Subjects with cancer and autoimmune diseases (e.g. psoriasis)
10. Subjects who have received or had received a plasma infusion, in case the serum protein levels of the subject were < 55 g/L
11. Subjects with known immunodeficiency
12. Tendency to keloid formation and/or hypertrophic scarring
13. Participation in another clinical trial within the last 30 days
14. Subjects unwilling or unable to follow the requirements of the study protocol
15. Employees/relatives of the sponsor or employees/relatives of the principal investigator (PI)

9.3.1.1 *Concomitant therapy restrictions*

Not applicable

9.3.2 Removal of Patients from Therapy or Assessment

Subjects could withdraw from the trial at any time and without giving reasons, either at their own request, or at the request of the investigator or the sponsor.

Reasons for the exclusion of a subject from the trial as per protocol are defined in chapter 9.3.2.1.

9.3.2.1 *Criteria for study withdrawal:*

- The subject did not comply with the requirements and rules for the clinical trial;
- The subject wished to leave the trial at his own request (each subject had the right to leave the trial at any time and without giving reasons, without being prejudiced)
- The subject developed an AE that made withdrawal necessary, or any concomitant medication had to be administered during the course of the study that was not permitted;
- Continuation of the clinical trial led to a violation of the inclusion or exclusion criteria;
- In the opinion of the investigator, discontinuation of the trial was in the best interest of the subject;
- The clinical trial was terminated by the sponsor.

If a subject no longer appears to the visits and cannot be reached by phone, it should be attempted by other means to establish contact in order to terminate the clinical trial for this subject.

If a subject terminates the trial prematurely, the subject was asked to be available for a phone interview. The reasons, circumstances and findings of a premature termination were to be documented in the CRF. All rights of the subjects were to be respected.

If the subject withdraws consent, no further investigations had been carried out and no more data were collected.

Subjects who were excluded before the administration of the trial medication were replaced.

Subjects who were excluded after administration of the trial medication were not replaced.

9.3.2.2 *Criteria for stopping the whole study*

The sponsor reserved the right to terminate the clinical trial at any time (e.g. due to safety concerns, new data, ethical or administrative reasons, insufficient recruitment rates, or a high number of withdrawals). The sponsor informed the investigator of the termination of the trial and the reasons for it. The sponsor instructed the investigator whether investigations were necessary beyond the procedures specified in the protocol. In addition, the investigator had the right to terminate the trial prematurely for safety

reasons; in this case, an immediate notification to the sponsor, stating the reasons, was required.

9.4 Treatments

9.4.1 Treatments administered

9.4.2 Hylase® „Dessau“ 150 I.U. subcutaneous injection Identity of Investigational Product(s)

Hylase® „Dessau“ 150 I.U is a white to slightly yellow powder for preparation of a solution for injection containing hyaluronidase as active ingredient. Its Anatomic Therapeutic Chemical (ATC) Code is B06AA03. The Marketing Authorization Holder is RIEMSER Pharma GmbH, An der Wiek 7, 17493 Greifswald – Insel Riems, Germany.

9.4.3 Method of assigning Patients to Treatment Groups

Subjects were randomized to the 3 strata (two-fold combination of the three HA fillers) in a ratio of 1:1:1 and randomly assigned to the injection sites on the backs of the hands (compare to Figure 1), and to the sequence of injection sites for the different dosages. Randomization was realized using the SAS 9.4 programme (SAS Institute Inc., Cary, NC, USA). For each subject a randomization envelope was made available to the center to be opened immediately before treatment. A four-digit randomization number was assigned to the subject and was filed together with the subject ID. If in case of an emergency a need of unblinding had occurred, this information would have been available immediately.

Due to the necessity of various random assignments and the relatively small subject number, randomization was realized in three steps in order to ensure a strong balance:

1. Assignment of subjects to the HA fillers (combination of two fillers out of three possible ones) was carried out via block randomization with random block length.

2Assignment of the two fillers to the site of the back of the hand was also done within the three strata (combination of two fillers out of three possible ones) from step 1 by means of a block randomization with random block length.

3. Allocation of the five doses

1. Hylase® "Dessau" 15 I.U.
2. Hylase® "Dessau" 25 I.U.
3. Hylase® "Dessau" 50 I.U.
4. Hylase® "Dessau" 75 I.U.
5. Saline solution,

to the 5 predefined puncture sites per back of the hand was performed within the three strata created after step 2 by simulating 5 random numbers from an equal distribution over [0.1]. Dosages were then assigned to the defined injection sites in analogy to the ascending size sorting of the random numbers (e.g. if the fourth random number was the smallest, Hylase® "Dessau" 15 I.U. was used at the fourth injection site).

9.4.4 Selection of Doses in the Study

Single dose of 15, 25, 50 and 75 I.U. / 0,5 mL. If deemed necessary by the investigator a consecutive injection with Hylase® "Dessau" was performed at visit 6 and 7. The required dose was chosen by the investigator and documented

9.4.5 Selection and Timing of Dose for each Patient

An aliquot of 0.5 mL of the HA filler to be tested was injected on the back of both hands according to Figure 1 (artificial overinjection). A 1 mL disposable syringe was used. The syringe type and needle size were the same for all injections. The following dosages were given to achieve an artificial overdose:

- Allergan HA Filler Voluma® 10 mg/0,5 mL, s.c. injection
- Merz HA Filler, Volume® 13 mg/0.5 mL, s.c. injection
- Galderma HA Filler Restylane Volyme® 10 mg/0.5 mL, s.c. injection

The injection of the HA fillers was done per injection site after a single puncture of the skin into the dermis (injection depth according to the manufacturer's instructions) in one pass, so that the complete volume was injected at one site. Special attention was paid to the standardization of the injection depth.

After 1 – 2 days, 4 different concentrations of Hylase (15, 25, 50, and 75 I.U. /0.5 mL) and 0.5 mL 0.9% saline solution as a control were injected into the pre-treated skin according to the randomization scheme. The injection sites were circled with a marker and were numbered 1-5. The randomization, meaning the Hylase concentration used in an assigned area and injection of the control, was filed in the subject’s record. The investigator in charge of the evaluation (palpation assessment) had no access to this part of the patient file.

Since the subjects' hands needed to be in their original condition at study end, any findings (swelling visible and/or palpable at the injection sites) were treated with additional Hylase injections.

9.4.5.1 *Dose Delays/Dose Modifications*

Not applicable

9.4.6 Blinding

This clinical trial was conducted blind for the purpose of palpation assessment by the investigator (). The subjects were randomized in a 1:1:1 ratio to the 3 strata (two-fold combination of the three HA fillers). The distribution of the 5 injection sites on the backs of the hands (injection of different Hylase® "Dessau" concentrations as well as saline solution) was also randomized. The randomization (related to the allocation of the fillers, the back of the hand sites for each filler and the sequence of injection sites for the different dosages) was programmed beforehand, transmitted sealed to the center and opened for the respective subject when the HA filler was injected. The investigator who performed the injections was informed about the stratum and the different concentrations of Hylase® "Dessau" (treatment arms). The investigator responsible for the evaluation (palpation assessment) had no access to this part of the subject file. The subjects were trained by the study personnel not to divulge any information about the injections to the investigator (choice of fillers and concentrations of Hylase or control).

9.4.7 Prior and concomitant Therapy

All drugs taken or used within 7 days before the first visit (whether or not they were still taken at the start of the study) were defined as previous medication.

All drugs (previous or new medication) subjects received from the time of inclusion in the trial in addition to the investigational medicinal products were defined as concomitant medication and had to be included in the subject's treatment records.

9.4.7.1 *Prohibited Medications*

Use of hand crème was not permitted during the course of the study.

9.4.7.2 *Ancillary Treatment*

Not applicable

9.4.8 Treatment Compliance

Not applicable since only one dose was administered by the investigator. It was documented that the subjects were treated with the dosages as stated in the protocol.

9.5 Efficacy and Safety Variables

9.5.1 Efficacy and Safety Measurement assessed and Flow Chart

An overview of the assessments performed during the study and the corresponding time points is provided in the study flow chart in **Table 1** below.

Table 1: Schedule of assessments

	V1: Day 0 (T0) **HA Injection**	**V2: Day 1 (+1) (T1.1), Hylase Injection**	**V3: Day 1(+1) (T1.2), (15 min post Hylase Injection)**	**V4: Day 1(+1) (T1.3), (60 min post Hylase Injection)**	**V5: Day 2+(1)** **(24 h post Hylase Injection)**	**V6: Day 7 or 8 (post Hylase Injection)**	**V7: Day 14 (+/-1), Final examination**
Informed consent	X						
In- and exclusion criteria	X	X					
Medical history, anamnesis	X						
Physical examination, vital signs[1]	X						X
Pregnancy test in urine[2]	X						
Injection of HA-filler[3]	X						
Injection of Hylase® „Dessau“[4]		X				X[7]	X[8]
Injection of saline solution 0,9%[4]		X					
Ultrasound of skin areas		X[6]	X	X	X	X	X[9]

	V1: Day 0 (T0) HA Injection	V2: Day 1 (+1) (T1.1), Hylase Injection	V3: Day 1(+1) (T1.2), (15 min post Hylase Injection)	V4: Day 1(+1) (T1.3), (60 min post Hylase Injection)	V5: Day 2+(1) (24 h post Hylase Injection)	V6: Day 7 or 8 (post Hylase Injection)	V7: Day 14 (+/-1), Final examination
Photographs of skin areas	X[10]	X[6]	X	X	X	X	X
Palpation[5]		X[6]	X	X	X	X	X
Documentation of AEs	X	X	X	X	X	X	X
Documentation of concomitant medication	X	X	X	X	X	X	X

AE=Adverse Event; HA=Hyaluronic acid

1) Measurement of body temperatue, blood pressure and heart frequency
2) Pregnancy test was performed in urine (women of childbearing potential only)
3) 0.5 mL aliquots of HA filler were injected into the back of both hands. The HA filler was injected per injection site after single puncture of the skin into the dermis in one pass, so that the complete 0.5 mL was injected at one site.
4) 0.5 mL saline solution as control and four different concentrations of Hylase (0.5 mL each) were injected into the over-injected skin areas. Reconstitution, dilution and injection had always to be performed following the four eye principle
5) Evaluation of the injection sites by the blinded investigator using a 5-part palpation rating scale (**Fehler! Verweisquelle konnte nicht gefunden werden.**)
6) At this visit measurement before injection of Hylase® „Dessau“ were performed. Recorded values represented the baseline values (100%) before administration of the investigational drug
7) Hylase injection on an as needed basis, if swelling at the injection sites could still be seen or palpated. The dose required was chosen by the investigator.
8) Hylase injection on an as needed basis, if swelling at the injection sites could still be seen or palpated. The dose required was chosen by the investigator and documented. The next contact by the study site was done after 14 days.
9) This ultrasound examination was only necessary if residual findings were tob e treated with Hylase at visit 6.
10) Before injection of the HA-Filler the baseline condition oft he hands was documented photographically.

9.5.1.1 Efficacy Assessment

24 h after Hylase injection the response of the Hylase dosages was determined. A response is defined as a volume reduction of 90%, which was detected by ultrasound, and a nodule which is hardly or no longer tactile by palpation. The evaluation of the effectiveness of the Hylase dose was based on the following examinations:

- Ultrasound examination: volume decrease of the injection site by more than 90%.
- Palpation: A score of 0 or 1 based on the palpation rating scale (Table 2).

Using these procedures, the volume of artificially applied HA at the injection site was examined at 6 different timepoints (V2 - V7) (Table 1) by palpation and by ultrasound at 0, 15 and 60 minutes, at 24 h, 7 and 14 days after Hylase injection. The measured volume was then expressed as a percentage of the baseline value (Hylase injection = T1.1 = 100%).

- Palpation: Evaluation of the injection site by a blinded investigator using the following palpation evaluation scale (Table 2). The same investigator should always perform all evaluations for one subject. .

Table 2: Palpation-Rating-Scale (according to Vartanian et al., 2005)

Grade	Palpable Finding
0	Node at the injection site not palpable anymore
1	Node hardly palpable
2	Slightly elevated palpable node
3	Moderately elevated node
4	Maximum elevation directly after injection of the HA filler and Hylase

9.5.1.2 Safety and Tolerability Assessments

Definitions

An **adverse event (AE)** is any untoward medical occurrence in a subject to whom an investigational medicinal product has been administered and which does not necessarily have a causal relationship with this treatment.

Therefore, an AE can be any unfavourable and unintended sign (e.g. an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Any deterioration (severity or frequency) of an existing condition or any newly diagnosed condition in a trial had to be documented as an AE.

An **adverse drug reaction (ADR)** is a response to a medicinal product which is noxious and unintended. Response in this context means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility.

A **serious adverse event (SAE)** or **serious adverse drug reaction (SADR)** is any untoward medical occurrence that at any dose

- results in death
- is life-threatening[1)]
- requires hospitalization or prolongation of existing hospitalisation
- results in persistent or significant disability/ incapacity
- consists of a congenital anomaly or birth defect

[1)] Life-threatening in the definition of a serious adverse event or serious adverse drug reaction referred to an event in which the subject was at risk of death at the time of event; it did not refer to an event which hypothetically might have caused death if it were more severe.

Important medical events that were not fatal, life-threatening, or did not require hospitalization may be considered serious if the investigator assessed that there was a risk to the patient and medical intervention was necessary to prevent an outcome as listed above.

If the nature or severity of an adverse drug reaction was not consistent with the applicable product information (e.g. Investigator's Brochure for an unapproved investigational product or package insert / summary of product characteristics for an approved product) then it was called **unexpected**.

A suspected unexpected serious adverse reaction (SUSAR) means a serious adverse reaction, the nature, severity and outcome of which is not consistent with the reference safety information.

Assessment of an AE

Irrespective of whether the AE was classified as serious, non-serious and/or unexpected, the **severity** of the event was classified as follows:

- Mild: Temporary symptoms, which did not interfere with routine activities
- Moderate: Clear symptoms, disturbances of the normal daily routine of the subject occured
- Severe: Considerable disturbance of the normal daily routine of the subject

A severe adverse event needed not to be serious in nature and a serious adverse event needed not, by definition, to be severe. Regardless of severity all serious adverse events had to be reported as described below.

The investigator's assessment of the **causal relationship** of an AE to the investigational product was based on the following classification:

- There was a relationship: There was clear evidence of a causal link and other factors were not considered. The clinical event (including, for example, a laboratory result that deviated from the norm) occurred within a plausible time window after administration of the investigational product and could not be explained by a concomitant disease or by the use of another drug or substance.
- There was no correlation: the AE was completely independent of the investigational product and/or there was clear evidence of another origin. An alternative explanation should be documented by the investigator.

The following outcome of an AE was possible: recovered/resolved, recovering/resolving, not recovered/not resolved, recovered/resolved with sequelae, fatal, unknown.

The sponsor assessed the expectedness of an AE using the current SmPC (dated Nov 2018).

Documentation and reporting of an AE

All concomitant diseases had to be recorded at study start. The worsening of the concomitant disease within the study had to be documented as AE in any case.

All AEs that occurred after the subject had signed the informed consent form until the end of the trial had to be documented by the investigator.

All AEs that occurred during the trial were electronically documented in the electronic case report form (eCRF) section of the subject's records and include a description, start and end dates, severity, causality and outcome, and any medical procedures (e.g., medication, other treatments, hospitalization). The contact persons for drug safety in the clinical trial had access to the electronic database in order to use necessary data of the subject for the evaluation of an AE.

The investigator reported all non-serious AEs via an AE form with assessment of causality within 72 h by fax or e-mail to the sponsor's address given below.

Serious adverse events: The investigator notified the sponsor immediately, but no later than 24 h after becoming aware of the occurrence of an SAE. An SAE Form was completed for all SAEs that come to the attention of the investigator, both during and after the end of the trial. The investigator assessed the causal relationship to the investigational product, completed the form in full, and sent it by fax or e-mail to the Pharmacovigilance Department of RIEMSER Pharma GmbH:

Fax number: +49 38351 76 778; e-mail: drugsafety@riemser.com

For details regarding timepoints for documentation of safety and tolerability of Hylase® „Dessau“ refer to Table 3.

Table 3: Documentation of adverse events

Timepoint	Documentation
V1 – (T0)	Immediately after the injection with HA-Filler subjects were questioned about AEs and those were documented in the subject file.
V2-V6 (T1 – T7)	Recording of AEs after administration of Hylase® „Dessau“ by the investigator at the respective visit
V7 (T14)	Recording of AEs by the investigator at final examination at the study site

The documentation of AEs also included the extent to which reversible and/or irreversible furrows and dents occurred in the tissue after sc injection of Hylase® "Dessau".

Adverse events were recorded for all subjects up to and including day 14 (final visit).

If another injection with Hylase was necessary due to residual findings at visit 7 (day 14), it was documented accordingly in the subject's file.

In this case, the study site contacted the subject again on day 28 (14 days after visit 7) to follow up on AEs and residual findings, and the results were documented in the subject’s file. Adverse events were also documented accordingly in the eCRF.

The sponsor documented all AEs reported by the investigator. The documentation can be made available to the BfArM on request.

The sponsor reported any SUSAR within 15 days to the EudraVigilance Clinical Trial Modul, to the responsible ethics committee, and participating investigators. In the case of a fatal or life-threatening SUSAR, the sponsor reported as soon as possible and in any case no later than seven days.

The sponsor has to submit an annual report to the BfArM and responsible ethics committee on the safety of the investigational medicinal product for the duration of the clinical study.

Follow up of adverse events

All AEs that had been assessed by the investigator as 'investigational product-related' were tracked by the investigator until the subject was either restored, recovered with consequences, or deceased, whichever occurred first. All other AEs were followed by the investigator until recovery or until the end of the follow-up period (up to 30 days after the last visit), whichever came first, and until all questions regarding the AE were resolved. The investigator took all medically appropriate and necessary steps to treat the AE, if possible. All efforts to obtain follow-up information were documented in the source data.

Follow-up information were documented on the AE Form and/or SAE Form. The investigator submitted follow-up information on SAEs to the sponsor's Pharmacovigilance Division by fax or e-mail within 24 hours of notification.

Pregnancy

In order to guarantee the safety of the subjects, any pregnancy occurring during the use of the investigational product was to be reported to the sponsor's pharmacovigilance department within 24 h of becoming aware of the pregnancy. The pregnancy was to be followed through to its end to ascertain its outcome, including voluntary or spontaneous termination, details of delivery, and the presence or absence of birth defects, congenital abnormalities, or complications in the mother or newborn.

Pregnancy had to be documented on an appropriate form and the investigator had to inform the Medical Monitor. Pregnancy follow-up was to be documented on the same form and the outcome had to include an assessment of a possible causal relationship with the investigational product. Any SAE occurring during pregnancy was to be documented on the SAE Form.

9.5.2 Appropriateness of Measurements

The measures of efficacy (palpation and ultrasound) and safety in this study are standard measurements, widely used and generally recognized as reliable, accurate, and relevant. The safety measurements recorded in this clinical study are those employed in most clinical studies, including the recording of AEs.

9.5.3 Primary Efficacy Variable(s)

24 hours after injection of the four Hylase doses or saline solution into the HA nodules, the complete volume reduction was determined by palpation and ultrasound. Evaluation of the effectiveness of the Hylase dose was based on the following examinations:

- Ultrasound examination: volume decrease of the nodules by more than 90%.
- Palpation: A score of 0 or 1 based on the palpation rating scale (Table 2)

Using these procedures, the volume of artificially applied HA at the injection site was examined at 6 different timepoints V2 - V7 (Table 1) by palpation and by ultrasound at 0, 15 and 60 minutes, as well as at 24 h, 7 and 14 days after Hylase injection. The

measured volume was then expressed as a percentage of baseline (Hylase injection = T1.1 = 100%).

Ultrasound

The measurements were contucted with the device SonidoSmart Plus (Zimmer MedizinSysteme GmbH). The used sonde with a width of 38 mm (linear sonde L8LN) had a frequency of 5,0 – 12 MHz.

Palpation

Evaluation of the injection site by a blinded investigator using the following palpation assessment scale (Table 2). The same investigator always performed the evaluation for each subject.

9.5.4 Secondary Efficacy Variable

- Evaluation of the volume reduction by means of a 3D camera

9.5.5 Secondary Study Variable(s)

9.5.5.1 *Safety and tolerability*

- Number of adverse events (AE) per dose and HA filler
- Number of expected adverse drug reactions (ADR) per dose and HA filler
- Number of unexpected ADRs per dose and HA filler
- Detection of AEs and ADRs after treatment with HA fillers

Physical examination, vital signs

A short physical examination was performed including the following: general appearance, lungs and thorax, ears, cardiovascular system, abdomen, nasopharynx, muscular and skeletal system, skin, extremities and lymph nodes.

Vital signs: Body temperature, blood pressure and heart rate according to the inclusion criteria.

Systolic and diastolic blood pressure and heart rate were measured on the right or left arm in supine or seated position after 15 minutes at rest. Body temperature was measured in the ear.

9.5.6 Demographics and Baseline Characteristics

For main demographic and baseline characteristics following variables were summarized:

- Age and gender
- Menopausal status

9.5.7 Drug Concentration Measurements

No drug concentration measurements had been performed in this study.

9.6 Data Quality Assurance

9.6.1 Data confidentiality

The protocol, the documentation, all data and all other documents and information derived from the clinical trial were strictly confidential. No information or data derived from the trial were disclosed to unauthorized third parties without the prior written consent of the sponsor.

Subject-related data were kept confidential in accordance with the European Data Protection Directive (2016/679 (General Data Protection Regulation) and local regulations.

The investigator ensured that the pseudonymization of participants' data was maintained and that their identity was protected from access by unauthorized persons. On the CRF and all other documents submitted to the sponsor or its agents, subjects were identified only coded. The use of name, address, telephone number, or similar information was not permitted. The investigator had a list for decoding (containing the full name and date of birth of each subject) in his or her records. This document was strictly confidential.

9.6.2 Personal training

The study staff was trained at the initiation visit: The eCRF to be used was presented, randomization and blinding procedures were discussed, and the performance of the ultrasound examination was trained.

9.6.3 Monitoring

The intensity of monitoring and source data verification was specified in a monitoring plan.

Before starting the trial, a site initiation visit (SIV) was performed by the monitor who discussed the protocol and eCRF with the investigators and their study staff. No subjects were included before this visit. During the conduct of the trial, regular monitoring visits (RMV, two visits in Potsdam and one visit in Hamburg) as defined in the monitoring plan were conducted in accordance with ICH GCP, the sponsor's requirements, and the local guidelines. The data transferred into CRFs were checked against source data to ensure compliance with the protocol and ICH GCP. The completeness, accuracy and consistency of the data was also checked. In addition, the monitor reviewed the progress of inclusions and ensured that the trial drug was stored, dispensed and accounted for according to the requirements. Staff were available during these visits to support the monitor.

The investigators allowed the monitor access to the subjects' data. During the conduct of the clinical trial, all data were identified by a subject number only.

At the end of the study the monitor performed a site closed out visit to formally close the study site.

9.6.4 Data management

In this clinical trial, an eCRF (ACTide by Nubilaria s.r., Novara, Italy) was used for documentation of the test person data. Nubilaria performed a study specific validation of the eCRF including layout, user interface, user experience and custom functionalities testing. All data collected after signing the ICF were entered into this eCRF. The investigator ensured that all data were entered promptly, completely and correctly,

according to the instructions in the eCRF (eCRF manual), and in accordance with the source data.

Only the investigators and authorized staff were permitted to make entries in the eCRF. Fully completed eCRFs (per visit) were electronically signed and dated by the investigator. Any changes or additions made thereafter required a new signature. The sponsor's staff responsible for drug safety in the clinical trial had access to the subject database.

In addition, the investigator ensured that all important data were collected in the trial subject's file, such as anamnesis, concomitant diseases, date of inclusion in the clinical trial, dates of visits, results of investigations, administration of drugs and AEs.

All data management activities were performed by a representative of the sponsor according to standardized guidelines including the development of the database.

Statconsult, Magdeburg, Germany was responsible for data cleansing, including plausibility checks, for checking the medical data, and for monitoring of the query process. Computer-aided validation checks were used for completeness, correctness and plausibility of data. All discrepancies generated a query using forms for cleansing the data and were forwarded to the investigator.

Self-explanatory corrections (e.g. typing errors) were done by the data manager in agreement with the investigator and can be traced by an audit trail.

The database was provisionally closed as soon as it was complete and correct (i.e. all CRF entries were included and the data cleansing process had been performed). The database was locked in agreement with the sponsor after all changes were discussed in the data review meeting and the database was complete and accurate. All changes were traceable (audit trail).

9.6.5 Coding conventions

Not applicable

9.6.6 Audits

No audits were performed during the study.

9.7 Statistical Methods Planned in the Protocol and Determination of Sample Size

9.7.1 Statistical and Analytical Plans

All data from the CRF were included in the evaluation. The statistical evaluation of population related data (inclusion and exclusion criteria, visit dates and times, protocol conformity of the study conduct, demographic data and vital signs as well as measurement of skin areas before Hylase® "Dessau" injection, premature termination and membership of the analysis population) was done descriptively.,

Categorical variables were presented in form of frequency tables using absolute values and percentages. Percentages are calculated using one decimal place.
Continuous variables were presented on the basis of the number of subjects, the number of subjects without missing examinations (N), mean, standard deviation (SD), median,

minimum (min), maximum (max), and of the 1st and 3rd quartiles. As baseline, all measurements at visit 2 (day 1) 10 minutes (±5 minutes) before injection of Hylase® "Dessau" were used.

All efficacy analyses were performed for the ITT and PP set and separately for HA filler. The four different doses of Hylase® "Dessau" were tested within a hierarchical analysis. The saline solution was tested as a fifth dose (placebo control) as part of the randomisation. The analysis was based on the assumption that a higher Hylase concentration causes a higher response rate. Thus, there was no loss of power due to parallel testing of the doses. The following null hypothesis was tested:
Null hypothesis: π_Response=80%
Alternative hypothesis: π_Response>80%, i.e. if the lower limit of the 95% confidence interval (CI) of the response estimate was above 80%, the null hypothesis could be rejected.
If the null hypothesis for the highest dose could be rejected, the next highest dose at the same significance level could be tested. This was done until the smallest dose was tested. If, however, no significance could be achieved at one of these hierarchical levels, the test stopped and all subsequent levels were also assessed as not significant. This strict hierarchy ensured strict compliance with the first type of error in terms of multiple testing.

The analytical methods are described in detail in a separate document (SAP - Statistical Analysis Plan) dated 07.05.2020. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

9.7.1.1 *Analysis populations:*

The following analyses groups were defined:

Full Analysis Set (FAS): Included participants: All participants that had signed the informed consent and were eligible, based on the results of the screening examination.

Safety Analysis Set (SAS): For determination of safety: All included participants who were randomized and received at least one dose of the HA filler or the investigational product Hylase® “Dessau”.

Intent to treat Set (ITT): Complete analysis: All participants of the SAS that attended at least one visit after receiving the investigational product Hylase® “Dessau”.

Per Protocol Set (PP): All participants of the ITT without any major protocol deviations, which could also apply to single injection sites. Major protocol deviations were defined in a data review meeting after reviewing all data collected. For example, these were violations of the inclusion and exclusion criteria, wrong dosage of the investigational product or the injection of the wrong HA filler.

9.7.2 Determination of Sample Size

Assuming a response rate of 90%, a lower limit of the 95% confidence interval above 80% is achieved with at least 36 subjects. Since the statement had to be valid for each of the three fillers, and each respondent received two fillers, at least 54 test persons were required. With an assumed drop-out rate of 5%, 57 subjects were therefore to be included.

9.7.3 Analysis

The disposition of subjects and analysis groups (including protocol violations, which lead to exclusion from the PP population) were reported. In and exclusion criteria as well as

the status of the termination of the clinical trial was shown in table form. In case of a premature departure of a subject, the reason for termination was documented. The number of subjects per visit was shown.

All variables from screening and the baseline characteristics were compiled to describe the population of the clinical trial. Details are presented in the statistical analysis plan (SAP). Data were presented for all subjects in the analysis population (if required).

Analysis of endpoints was done in the ITT as well as in the PP population.

9.7.3.1 *Primary endpoint*

The primary endpoint was thedetermination of the Hylase dosage with which a complete volume reduction of the HA nodules, proven by palpation and ultrasound, could be determined 24 hours after injection..

The definition of the response was based on volume measurements using imaging procedures (ultrasound) and palpation by the blinded investigator (see Table 2). The investigator assigned a score of 0 - 4 for the respective palpated site, whereby the '4' corresponded to a maximum skin elevation, and the '0' to 'no longer palpable'. Details are provided in the SAP.

9.7.3.2 *Analysis of safety*

All safety relevant data were analyzed in SAF.

The occurrence of AEs (both expected and unexpected), as documented by the investigator and coded and evaluated by the sponsor, was investigated.

AEs were coded according to the Medical Dictionary for Regulatory Activities (MedDRA) coded. The MedDRA version 22.1 was used for coding of AEs. AEs were combined and presented in tables, listed by primary system organ class (SOC) and preferred term (PT). The analysis focussed on treatment-emergent adverse events (TEAEs).

For the analyses, an assessment of the severity, the seriousness, or the relationship with the investigational product was performed. AEs were evaluated for both the investigational product Hylase® "Dessau" (and placebo) as well as for the hyaluronic acid fillers.

A list of all subjects experiencing an AE was compiled for both, all reported AEs as well as for all included subjects. All AEs were reported in absolute numbers as well as in percentages.

More details on the analysis of the AEs are described in the SAP.

Results of the laboratory examinations (pregnancy test) were listed.

Results of the physical examination as well as of vital signs were presented descriptively.

Previous and concomitant medication was listed.

9.7.4 Sensitivity analysis related to HA filler

Sensitivity analysis related to HA filler
The primary analysis of the efficacy of Hylase was carried out exclusively with regard to the HA filler, as these can be split by hyaluronidase with varying effectiveness. As a general effect of the Hylase "Dessau", however, a sensitivity analysis was performed to determine whether the response rate per dose was also estimated similarly independent of the filler.

Since each subject was treated with two HA fillers, dependent data were available for each dose, so that a generalized mixed model was used for estimating the response rate 24 hours after Hylase injection, in which the subject identification number was also estimated as a random effect, and thus subject-related dependencies could be taken into account. In the same way, the center affiliation was modelled as a random effect. The hierarchical testing was performed as already described.
The sensitivity analysis based on imputed data (if more than 5% of the test persons were no-responders), was not done due to premature termination of recruitment.

9.7.5 Interim Analysis

There was no interim analysis.

9.8 Changes in the Conduct of the Study or Planned Analyzes

Due to the corona epidemic and the associated lack of opportunities for recruiting volunteers, the study was terminated prematurely in March 2020. The database was locked and the data export for analysis took place after finalization of the SAP. Thus, the target number of participants could not be reached, so that even if the assumed response rate of 90% was reached, the lower limit of 80% could not be exceeded significantly. In order to discuss the results of all doses, the response rates for all HA fillers and doses including their 95% CI (probability of error not adjusted) were given exploratively for all HA fillers and doses.

In the course of the study, the evaluation and volume determination using the 3D camera has shown as being very time-consuming, which is why this could not be carried out onsite by the study staff and an external service provider had to be called in. It turned out that the complete evaluation of all images is very time-consuming and costly. To get a first impression of the images and measurement results, the sponsor had individual test persons evaluated. Compared to the evaluation with ultrasound or palpation, it turned out that the 3D camera is an unsuitable method to evaluate the volume change of the nodules

Moreover an additional statistical analysis was carried out. Further information are described under 11.4.4.

10. Study Patients

10.1 Disposition of Patients

From August 12, 2019 to March 31, 2020 28 study participants were screened and included into the study. All 28 subjects completed the study, however, only 24 subjects attended all visits. Four subjects missed visit 5 (Statistical output, Table 1.1.3)

10.2 Protocol Deviations

There were the following protocol deviations during the course of the study:

- The inclusion or exclusion criteria were violated in one subject – major deviation.
- Hylase Dessau was not injected correctly at all 5 injection sites in one subject – major deviation.
- Not all values related to the primary endpoint were available in 4 subjects – major deviation.
- V5 was not performed 1 day after injection of Hylase Dessau in 4 subjects – major deviation.
- There was an AE before injection of Hylase (after injection of the hyaluronic acid filler) in 2 subjects – minor deviation.
- Palpation as well as volume measurement were done at V6 (day 3 or 4) according to protocol version 1 instead of day 7 or 8 in 4 subjects – minor deviation.
- The time window for V6 and V7 was longer than ±1 day in 9 subjects – minor deviation. (Statistical output Table 1.1.5)

All injection sites of patients with major protocol deviations were not included in the PP population.

11. Efficacy Evaluation

11.1 Data Sets Analyzed

All enrolled patients were included in the ITT population for efficacy evaluation (n=28).

All patients that received at least one dose of study drug were included in the SAF population for safety evaluation (n=28, 280 injection sites).

All (injection sites of) patients without major protocol deviations were included in the PP population (n=23, 208 injection sites).

All analyses were performed per Hylase® Dessau dose, since the study aimed to determine an efficient and tolerable dose for the treatment of hyaluronic acid filler-induced overinjections.

In addition, most of the analyses was done according to the hyaluronic acid filler used:

- Allergan HA-Filler Voluma® (10 mg/0,5 mL Hyaluronsäure)
- Merz HA-Filler Volume® (13 mg/0,5 mL Hyaluronsäure)
- Galderma HA-Filler Restylane Volyme® (10 mg/0,5 mL Hyaluronsäure)

An analysis not separated by the filler used was done as a sensitivity analysis for the primary endpoint only.

11.2 Demographic and Other Baseline Characteristics

Twenty-two females and 6 males were included in the study. The gender of subjects per analysis population is shown in Table 4.

Table 4 : Gender of subjects per analysis population

Gender						
Analysis Population	**Female**		**Male**		**Total**	
	N	**%**	**N**	**%**	**N**	**%**
FAS	22	78.6	6	21.4	28	100
SAF	22	78.6	6	21.4	28	100
ITT	22	78.6	6	21.4	28	100
PP	18	78.3	5	21.7	23	100

(Source: Statistical output Table 1.3.1)

The mean age of the subjects of the ITT population was 49.5 years, range 20.0 – 65.0 years. The mean age in females was 50.5 years, range 21.0 – 65.0 years, and mean age in males was 46.0 years, range 20.0 – 64.0 years.

The mean age of the subjects of the PP population was 50.0 years, range 20.0 – 65.0 years. The mean age in females was 52.1 years, range 21.0 – 65.0 years, and mean age in males was 42.4 years, range 20.0 – 65.0 years. (Statistical output 1.3.2)

11.3 Measurements of Treatment Compliance

According to section **Fehler! Verweisquelle konnte nicht gefunden werden.** no data are available.

11.4 Efficacy Results and Tabulations of Individual Patient Data

11.4.1 Analysis of Efficacy

11.4.1.1 *Primary endpoint:*

Determination of the Hylase® dose that achieved complete volume reduction, defined by palpation and ultrasound after 24 hours. Hylase® was administeredone or 2 day(s) after treatment with a HA filler. Response was defined by a palpation result of 0 or 1 and a volume reduction ≥90% determined by ultrasound. Results are shown for the ITT as well as for the PP population.

A descriptive evaluation of the response after 24 hours is shown in Table 5.

Table 5: Response after 24 hours - ITT population

Hyaluronic acid filler	Dose	Response after 24 h (%)	N (Responder / N	N (missing)
Allergan HA Filler Voluma	Hylase Dessau 75 I.U.	75.0	12 / 16	2
	Hylase Dessau 50 I.U.	81.3	13 / 16	2
	Hylase Dessau 25 I.U.	56.3	9 / 16	2
	Hylase Dessau 15 I.U.	37.5	6 / 16	2
	Saline solution	6.3	1 / 16	2
Merz HA-Filler Volume	Hylase Dessau 75 I.U.	81.3	13 / 16	3
	Hylase Dessau 50 I.U.	62.5	10 / 16	3
	Hylase Dessau 25 I.U.	62.5	10 / 16	3
	Hylase Dessau 15 I.U.	62.5	10 / 16	3
	Saline solution	0	0 / 16	3
Galderma HA-Filler Restylane Volyme	Hylase Dessau 75 I.U.	56.3	9 / 16	3
	Hylase Dessau 50 I.U.	68.8	11 / 16	3
	Hylase Dessau 25 I.U.	62.5	10 / 16	3
	Hylase Dessau 15 I.U.	56.3	9 / 16	3

Hyaluronic acid filler	Dose	Response after 24 h (%)	N (Responder / N	N (missing)
	Saline solution	18.8	3 / 16	3

HA: Hyaluronic acid; I.U.: International Units
(Source: Statistical output Table 2.1.1.1)

Regarding the Allergan HA Filler there were most responders (n=13 out of 16) when using a Hylase dose of 50 I.U. Using the Merz HA Filler there were 13 responders out of 16 with a Hylase dose of 75 I.U. and with the Galderma HA filler there were most responders (n=11 out of 16) with a Hylase dose of 50 I.U.

Figure 2 is corresponding to these results. White fields mean that there was no response (0%, e.g.Merz HA filler and saline solution). A darker colour points towards a higher response rate (e.g. 81.3% Allergan HA filler and Hylase 50 I.U.)

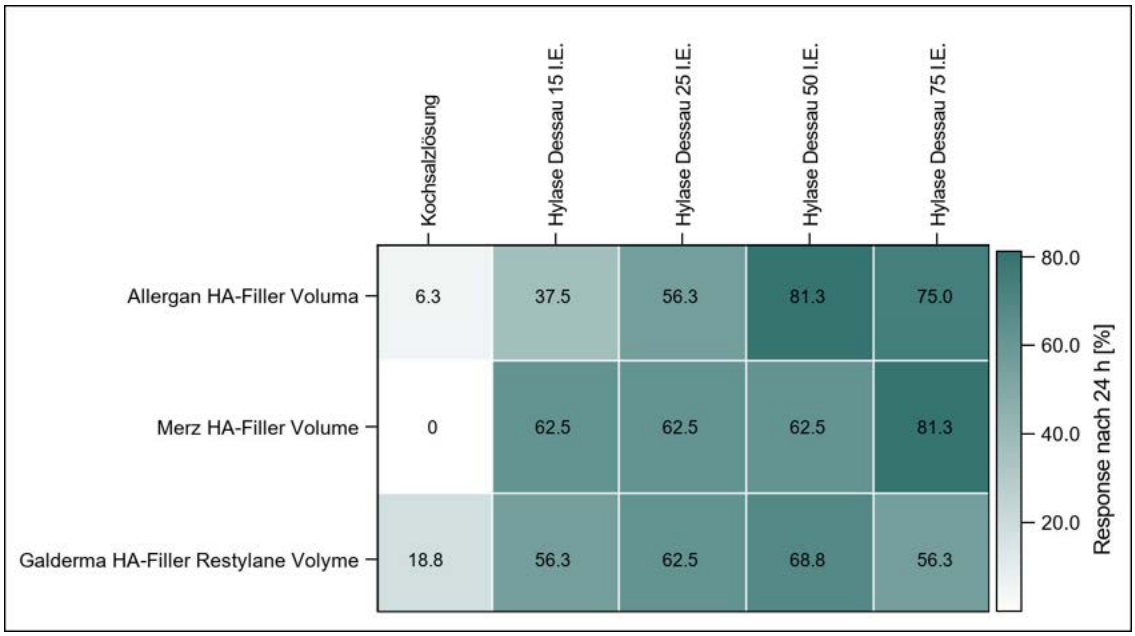

Figure 2: Responder rates in relation to Hylase dose and filler used

(Source: Statistical output Figure 2.1.1.1)

The next table provides all responses after 24 hours per dose and HA filler for the PP population.

Table 6: Response after 24 hours per dose and HA filler (PP population)

Hyaluronic acid filler	Dose	Response after 24 h (%)	N (Responder / N	N (missing)
Allergan HA Filler Voluma	Hylase Dessau 75 I.U.	71.4	10 / 14	0
	Hylase Dessau 50 I.U.	90.9	10 / 11	0
	Hylase Dessau 25 I.U.	53.3	8 / 15	0
	Hylase Dessau 15 I.U.	28.6	4 / 14	0
	Saline solution	0	0 / 13	0
Merz HA-Filler Volume	Hylase Dessau 75 I.U.	78.6	11 / 14	0
	Hylase Dessau 50 I.U.	71.4	10 / 14	0
	Hylase Dessau 25 I.U.	60.0	9 / 15	0
	Hylase Dessau 15 I.U.	64.3	9 / 14	0
	Saline solution	0	0 / 14	0
Galderma HA-Filler Restylane Volyme	Hylase Dessau 75 I.U.	57.1	8 / 14	0
	Hylase Dessau 50 I.U.	73.3	11 / 15	0
	Hylase Dessau 25 I.U.	66.7	8 / 12	0
	Hylase Dessau 15 I.U.	57.1	8 / 14	0
	Saline solution	13.3	2 / 15	0

HA: Hyaluronic acid; I.U.: International Units
(Source: Statistical output Table 2.2.1.1)

The results in the PP population confirm the results from the ITT population: Regarding the Allergan HA Filler there were most responders (n=10 out of 11) when using a Hylase dose of 50 I.U. Using the Merz HA Filler there were 11 responders out of 14 with a Hylase dose of 75 I.U. and with the Galderma HA filler there were most responders (n=11 out of 15) with a Hylase dose of 50 I.U.

These results are graphically displayed in Figure 3.

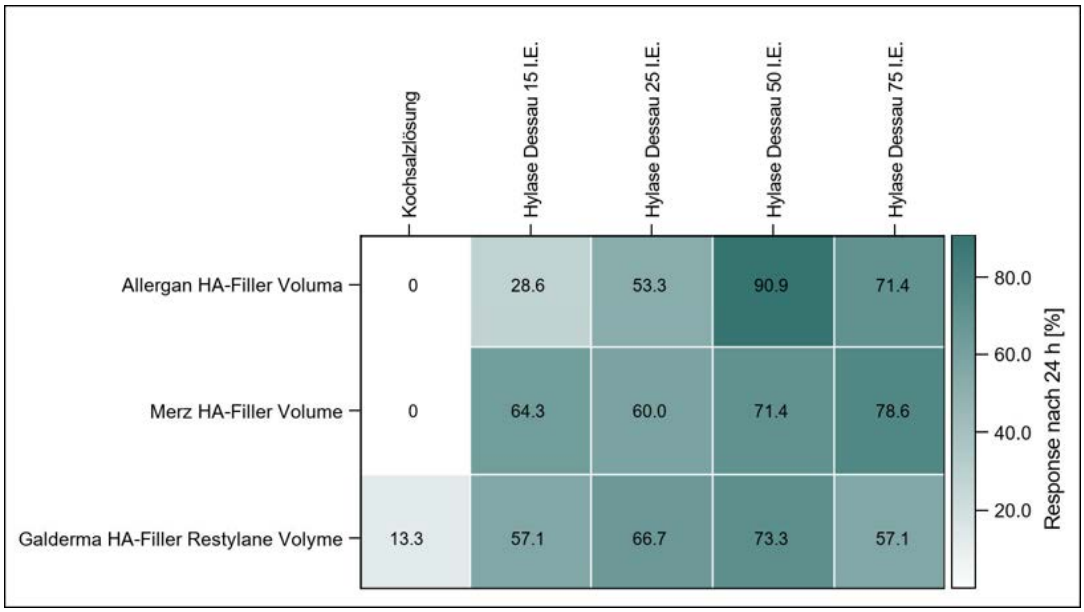

Figure 3: Response after 24 hours per dose and HA filler (PP population)

HA: Hyaluronic acid; I.U.: International Units
(Source: Statistical output Figure 2.2.1.1)

Figure 3 is corresponding to the above mentioned results. White fields mean that there was no response (0%, e.g.Merz HA filler and saline solution). A darker colour points towards a higher response rate (e.g. 90.9% Allergan HA filler and Hylase 50 I.U.)

Primary efficacy analysis was realized as hierarchical analysis to show the response after 24 hours per HA filler (Table 7).

Table 7: Hierarchical analysis: Response after 24 hours per HA filler (ITT population)

				Confidence interval		
HA filler	**Dose**	**N (Responder)/ N**	**Response after 24 hours (%)**	**Lower CI**	**Upper CI**	**Result**
Allergan HA-Filler Voluma	Hylase 75 I.U.	12/16	75.0	53.8	96.2	Not significant – all other doses were not statistically significant, too
	Saline solution	1/16	6.3	0	18.1	
Merz HA-Filler Volume	Hylase 75 I.U.	13/16	81.3	62.1	100.0	Not significant – all other doses were not statistically significant, too
	Saline solution	0/16	0	0	0	

				Confidence interval		
HA filler	**Dose**	**N (Responder)/ N**	**Response after 24 hours (%)**	**Lower CI**	**Upper CI**	**Result**
Galderma HA-Filler Restylane Volyme	Hylase 75 I.U.	9/16	56.3	31.9	80.6	Not significant – all other doses were not statistically significant, too
	Saline solution	3/16	18.8	0	37.9	

CI: Confidence interval; HA: Hyaluronic acid; I.U.: International Unit
Source: Statistical output Table 2.1.1.2

In this hierarchical analysis it is shown that no dose of Hylase „Dessau“ achieved a statistically significant result. Their lower limit of the respective 95% CI was in no case above 80%, so that the hierarchical test procedure was terminated after this first dose and all other Hylase® "Dessau" doses were also not considered significant.

However, it could be demonstrated clearly, that with each filler used the response was considerably better compared to the saline control with a response rate of 75.0%, 81.3% and 56.3% vs 6.3%, 0% and 18.8%, respectively.

In Table 8 the hierarchical analysis is presented for the PP population.

Table 8: Hierarchical analysis Response after 24 hours per HA filler (PP population)

				Confidence interval		
HA filler	**Dose**	**N (Responder)/N**	**Response after 24 hours (%)**	**Lower CI**	**Upper CI**	**Result**
Allergan HA-Filler Voluma	Hylase 75 I.U.	10/14	71.4	47.8	95.1	Not significant – all other doses were not statistically significant, too
	Saline solution	0/13	0	0	0	
Merz HA-Filler Volume	Hylase 75 I.U.	11/14	78.6	57.1	100.0	Not significant – all other doses were not statistically significant, too
	Saline solution	0/14	0	0	0	

				Confidence interval		
HA filler	**Dose**	**N (Responder)/N**	**Response after 24 hours (%)**	**Lower CI**	**Upper CI**	**Result**
Galderma HA-Filler Restylane Volyme	Hylase 75 I.U.	8/14	57.1	31.2	83.1	Not significant – all other doses were not statistically significant, too
	Saline solution	2/15	13.3	0	30.5	

CI: Confidence interval; HA: Hyaluronic acid; I.U.: International Unit
Source: Statistical output Table 2.1.1.2

Again, no statistical significance could be shown but it could be demonstrated, that with each filler used the response was considerably better compared to the saline control with a response rate of 71.4%, 78.6% and 57.1% vs 0%, 0% and 13.3%, respectively.

In the following tables the response after 24 hours, each for palpation and volume reduction, per dose and HA filler, is presented (Table 9 - Table 11) for the ITT population. This was done to find out if a missing response could be caused by the method used (systematic error).

The meaning of the table is explained using the Allergan HA filler as an example: At a Hylase 'Dessau' dose of 75 I.U. a response assessed by palpation and ultrasound after 24 h is seen in 12 participants (66.7%). Considering palpation alone, 14 participants (77%) had a response. Two of these participants (11.1%, <90%) showed no response in ultrasound. Data were missing in 2 out of 18 participants (11.1%). In contrast to Table 5 missing data were included into the calculation of response.

Table 9: Response after 24 hours, each for palpation and volume reduction, per dose and HA filler (Allergan HA Filler) – ITT population

		Volume reduction (response after 24 hours					
Dose	**Palpation (response after 24 hours)**	**>90%**		**≤90%**		**missing**	
		N	**%**	**N**	**%**	**N**	**%**
Hylase Dessau 75 I.U.	**0,1**	12	66.7	2	11.1	0	0
	2,3,4	0	0	2	11.1	0	0
	missing	0	0	0	0	2	11.1
Hylase Dessau 50 I.U.	**0,1**	13	72.2	1	5.6	0	0
	2,3,4	0	0	2	11.1	0	0
	missing	0	0	0	0	2	11.1

		Volume reduction (response after 24 hours					
Dose	**Palpation (response after 24 hours)**	**>90%**		**≤90%**		**missing**	
Hylase Dessau 25 I.U.	**0,1**	9	50.0	4	22.2	0	0
	2,3,4	0	0	3	16.7	0	0
	missing	0	0	0	0	2	11.1
Hylase Dessau 15 I.U.	**0,1**	6	33.3	8	44.4	0	0
	2,3,4	0	0	2	11.1	0	0
	missing	0	0	0	0	2	11.1
Saline solution	**0,1**	1	5.6	8	44.4	0	0
	2,3,4	0	0	5	27.8	2	11.1
	missing	0	0	0	0	2	11.1

I.U.: International Units
(Source: Statistical output Table 2.1.1.5)

Table 10: Response after 24 hours, each for palpation and volume reduction, per dose and HA filler (Merz HA Filler) – ITT population

		Volume reduction (response after 24 hours					
Dose	**Palpation (response after 24 hours)**	**>90%**		**≤90%**		**missing**	
		N	**%**	**N**	**%**	**N**	**%**
Hylase Dessau 75 I.U.	**0,1**	13	68.4	2	10.5	0	0
	2,3,4	1	5.3	0	0	0	0
	missing	0	0	0	0	3	15.8
Hylase Dessau 50 I.U.	**0,1**	10	52.6	4	21.1	0	0
	2,3,4	1	5.3	1	5.3	0	0
	missing	0	0	0	0	3	15.8
Hylase Dessau 25 I.U.	**0,1**	10	52.6	4	21.1	0	0
	2,3,4	0	0	2	10.5	0	0
	missing	0	0	0	0	3	15.8
	0,1	10	52.6	5	26.3	0	0

		Volume reduction (response after 24 hours					
Dose	**Palpation (response after 24 hours)**	**>90%**		**≤90%**		**missing**	
Hylase Dessau 15 I.U.	**2,3,4**	0	0	1	5.3	0	0
	missing	0	0	0	0	3	15.8
Saline solution	**0,1**	0	0	6	31.6	0	0
	2,3,4	0	0	9	47.4	1	5.3
	missing	0	0	0	0	3	15.8

I.U.: International Units
(Source: Statistical output Table 2.1.1.5)

Table 11: Response after 24 hours, each for palpation and volume reduction, per dose and HA filler (Galderma HA Filler) – ITT population

		Volume reduction (response after 24 hours					
Dose	**Palpation (response after 24 hours)**	**>90%**		**≤90%**		**missing**	
		N	**%**	**N**	**%**	**N**	**%**
Hylase Dessau 75 I.U.	**0,1**	9	47.4	6	31.6	0	0
	2,3,4	1	5.3	0	0	0	0
	missing	0	0	0	0	3	15.8
Hylase Dessau 50 I.U.	**0,1**	11	57.9	3	15.8	0	0
	2,3,4	1	5.3	1	5.3	0	0
	missing	0	0	0	0	3	15.8
Hylase Dessau 25 I.U.	**0,1**	10	52.6	5	26.3	0	0
	2,3,4	0	0	1	5.3	0	0
	missing	0	0	0	0	3	15.8
Hylase Dessau 15 I.U.	**0,1**	9	47.4	7	36.8	0	0
	2,3,4	0	0	0	0	0	0
	missing	0	0	0	0	3	15.8
Saline solution	**0,1**	3	15.8	4	21.1	0	0
	2,3,4	0	0	8	42.1	1	5.3

		Volume reduction (response after 24 hours					
Dose	**Palpation (response after 24 hours)**	**>90%**		**≤90%**		**missing**	
	missing	9	47.4	6	31.6	0	0

I.U.: International Units
(Source: Statistical output Table 2.1.1.5)

A graphical presentation of palpation and volume reduction after 24 hours, per dose and HA filler is shown in Figure 4.

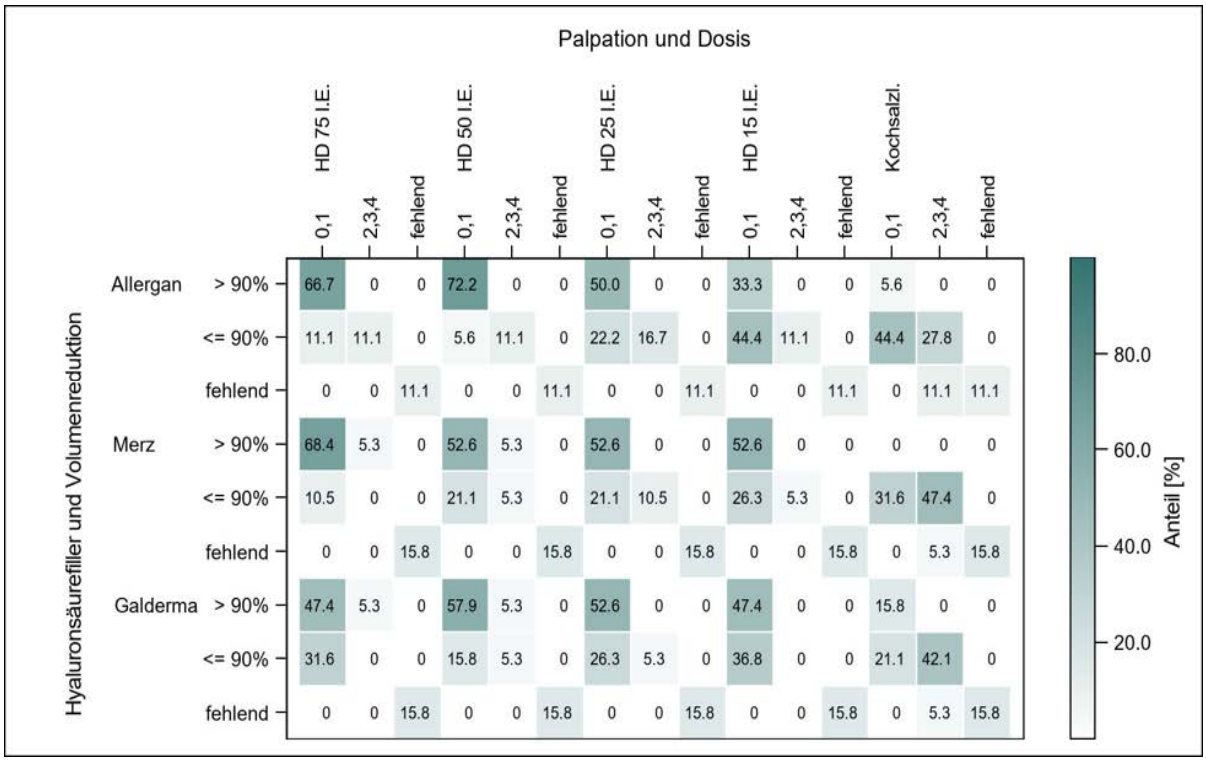

Figure 4: Palpation and volume reduction after 24 hours, per dose and HA filler

(Source: Statistical output Figure 2.1.1.2)

The corresponding relative frequencies per HA filler and Hylase dose are illustrated in this figure (Figure 4). In each case, missing values were taken into account. For example, in 12 cases there was a response to the Allergan HA-Filler Voluma and the Hylase® "Dessau" 75 I.U.; in two cases there was no response (the volume reduction was too small), although a value of 0 or 1 was achieved on palpation; in two cases no response could be achieved for both methods used, and in a further two cases both results were missing. The latter were included in the relative frequencies, so that the response in 12 cases was 12/18

In the following tables (Table 12 -

Table 14) results of palpation and volume reduction per dose and HA filler are provided for the PP population:

Table 12: Response after 24 hours each for palpation and volume reduction per dose and per HA-filler (Allergan HA filler) - PP population

		Volume reduction (response after 24 hours					
Dose	**Palpation (response after 24 hours)**	**>90%**		**≤90%**		**missing**	
		N	**%**	**N**	**%**	**N**	**%**
Hylase Dessau 75 I.U.	**0,1**	10	71.4	2	14.3	0	0
	2,3,4	0	0	2	14.3	0	0
	missing	0	0	0	0	0	0
Hylase Dessau 50 I.U.	**0,1**	10	90.9	0	0	0	0
	2,3,4	0	0	1	9.1	0	0
	missing	0	0	0	0	0	0
Hylase Dessau 25 I.U.	**0,1**	8	53.3	4	26.7	0	0
	2,3,4	0	0	3	20.0	0	0
	missing	0	0	0	0	0	0
Hylase Dessau 15 I.U.	**0,1**	4	28.6	8	57.1	0	0
	2,3,4	0	0	2	14.3	0	0
	missing	0	0	0	0	0	0
Saline solution	**0,1**	0	0	6	46.2	0	0
	2,3,4	0	0	5	38.5	2	15.4
	missing	0	0	0	0	0	0

I.U.: International Units
(Source: Statistical output Table 2.1.1.5)

Table 13: Response after 24 hours each for palpation and volume reduction per dose and per HA-filler (Merz HA filler) - PP population

		Volume reduction (response after 24 hours					
Dose	**Palpation (response after 24 hours)**	**>90%**		**≤90%**		**missing**	
		N	**%**	**N**	**%**	**N**	**%**
Hylase Dessau 75 I.U.	**0,1**	11	78.6	2	14.3	0	0
	2,3,4	1	7.1	0	0	0	0
	missing	0	0	0	0	0	0
Hylase Dessau 50 I.U.	**0,1**	10	71.4	3	21.4	0	0
	2,3,4	0	0	1	7.1	0	0
	missing	0	0	0	0	0	0
Hylase Dessau 25 I.U.	**0,1**	9	60.0	4	26.7	0	0
	2,3,4	0	0	2	13.3	0	0
	missing	0	0	0	0	0	0
Hylase Dessau 15 I.U.	**0,1**	9	64.3	4	28.6	0	0
	2,3,4	0	0	1	7.1	0	0
	missing	0	0	0	0	0	0
Saline solution	**0,1**	0	0	4	28.6	0	0
	2,3,4	0	0	9	64.3	1	7.1
	missing	0	0	0	0	0	0

I.U.: International Units
(Source: Statistical output Table 2.1.1.5)

Table 14: Response after 24 hours each for palpation and volume reduction per dose and per HA-filler (Galderma HA filler) - PP population

		Volume reduction (response after 24 hours					
Dose	**Palpation (response after 24 hours)**	**>90%**		**≤90%**		**missing**	
		N	**%**	**N**	**%**	**N**	**%**
Hylase Dessau 75 I.U.	**0,1**	11	78.6	2	14.3	0	0
	2,3,4	8	57.1	5	35.7	0	0
	missing	1	7.1	0	0	0	0
Hylase Dessau 50 I.U.	**0,1**	0	0	0	0	0	0
	2,3,4	11	73.3	3	20.0	0	0
	missing	1	6.7	0	0	0	0
Hylase Dessau 25 I.U.	**0,1**	0	0	0	0	0	0
	2,3,4	8	66.7	3	25.0	0	0
	missing	0	0	1	8.3	0	0
Hylase Dessau 15 I.U.	**0,1**	0	0	0	0	0	0
	2,3,4	8	57.1	6	42.9	0	0
	missing	0	0	0	0	0	0
Saline solution	**0,1**	0	0	0	0	0	0
	2,3,4	2	13.3	4	26.7	0	0
	missing	0	0	8	53.3	1	6.7

I.U.: International Units
(Source: Statistical output Table 2.1.1.5)

11.4.1.2 *Sensitivity Analysis related to Hyaluronic Acid Fillers*

A sensitivity analysis was performed to determine whether a similar response rate per dose - independently of the filler – could be achieved and thus a better power was possible. As each subject was treated with two HA fillers, dependent data are available for each dose. Therefore, a generalised mixed model was used for the filler-independent estimation of the response rate 24 hours after Hylase injection:

The following response rates were found:

- Hylase® „Dessau“ 75 I.U.: 71.3% [95% CI: 36.7%; 91.4%]
- Hylase® „Dessau“ 50 I.U.: 73.0% [95% CI: 37.7%; 92.4%]
- Hylase® „Dessau“ 25 I.U.: 60.1% [95% CI: 40.4%; 76.9%]

- Hylase® „Dessau“ 15 I.U.: 51.5% [95% CI: 34.9%; 67.8%]

Also with this analysis no response rate >80% (lower limit of the 95% confidence interval) could be achieved.
Additionally, the chance of a response was quantified for one HA filler compared to a second. If the odds ratio including the corresponding 95% confidence interval is above 1, the chance of a response with the first HA-filler is significantly higher; if it is below 1, the chance of a response with the second HA-filler is higher.

A comparison of the HA fillers is shown in Table 15, revealing no significant differences between HA fillers.

Table 15: Comparison of HA fillers (ITT population)

			95% Confidence interval	
Dose	**Category**	**Odds ratio**	**Lower CI**	**Upper CI**
Hylase Dessau 75 I.U.	Allergan HA-Filler Voluma vs Merz HA-Filler Volume	0.623	0.098	3.961
	Galderma HA-Filler Restylane Volyme vs Merz HA-Filler Volume	0.270	0.047	1.569
Hylase Dessau 50 I.U.	Allergan HA-Filler Voluma vs Merz HA-Filler Volume	3.270	0.468	22.862
	Galderma HA-Filler Restylane Volyme vs Merz HA-Filler Volume	1.219	0.202	7.368
Hylase Dessau 25 I.U.	Allergan HA-Filler Voluma vs Merz HA-Filler Volume	0.720	0.157	3.310
	Galderma HA-Filler Restylane Volyme vs Merz HA-Filler Volume	0.944	0.202	4.419
Hylase Dessau 15 I.U.	Allergan HA-Filler Voluma vs Merz HA-Filler Volume	0.358	0.075	1.712
	Galderma HA-Filler Restylane Volyme vs Merz HA-Filler Volume	0.761	0.163	3.563

CI: Confidence interval; HA: Hyaluronic acid filler; I.U.: International Units
(Source: Statistical output Table 2.1.1.3)

The same generalised mixed model was used for the filler-independent estimation of the response rate 24 hours after Hylase injection related to the PP population:

The following response rates were found:

- Hylase® „Dessau“ 75 I.U.: 68.0% [95% CI: 21.1%; 94.4%]
- Hylase® „Dessau“ 50 I.U.: 80.1% [95% CI: 41.2%; 95.9%]
- Hylase® „Dessau“ 25 I.U.: 59.2% [95% CI: 41.8%; 74.6%]
- Hylase® „Dessau“ 15 I.U.: 50.3% [95% CI: 32.8%; 67.6%]

Also with the PP populario analysis no response rate >80% (lower limit of the 95% confidence interval) could be achieved.Thus, the results related tot he ITT population

were confirmed in the PP population. The following table (Table 16) presents the results corresponding to the PP population:

Table 16: Comparison of HA fillers (PP population)

			95% Confidence interval	
Dose	**Category**	**Odds ratio**	**Lower CI**	**Upper CI**
Hylase Dessau 75 I.U.	Allergan HA-Filler Voluma vs Merz HA-Filler Volume	0.645	0.089	4.669
	Galderma HA-Filler Restylane Volume vs Merz HA-Filler Volume	0.354	0.052	2.405
Hylase Dessau 50 I.U.	Allergan HA-Filler Voluma vs Merz HA-Filler Volume	4.345	0.294	64.238
	Galderma HA-Filler Restylane Volume vs Merz HA-Filler Volume	1.148	0.166	7.956
Hylase Dessau 25 I.U.	Allergan HA-Filler Voluma vs Merz HA-Filler Volume	0.751	0.154	3.655
	Galderma HA-Filler Restylane Volume vs Merz HA-Filler Volume	1.275	0.226	7.182
Hylase Dessau 15 I.U.	Allergan HA-Filler Voluma vs Merz HA-Filler Volume	0.233	0.041	1.331
	Galderma HA-Filler Restylane Volume vs Merz HA-Filler Volume	0.754	0.142	4.013

CI: Confidence interval; HA: Hyaluronic acid filler; I.U.: International Units
(Source: Statistical output Table 2.1.1.3)

11.4.1.3 *Sensitivity analysis due to premature termination of recruitment*

The lower limit of response of 80%, as defined in the primary analysis, was also used as the basis for planning the sample size. However, due to the premature end of recruitment, the planned number of subjects could not be reached. Therefore, even if the assumed response rate of 90% had been achieved, no significant exceeding of the above-mentioned 80% was possible. Within the hierarchical test procedure the lower dosages of Hylase® "Dessau" could not be tested under these assumptions.

However, in order to be able to discuss the results of all dosages, the response rates including their 95% confidence intervals (not adjusted) were given exploratively for all HA fillers and dosages. This was done for the time crucial for the primary endpoint (V5) as well as for V6. The highest response rate after 24 h was achieved with Allergan HA-Filler Volume using Hylase® "Dessau" 50 I.U., and with Merz HA-Filler Volume using Hylase® "Dessau" 75 I.U. (81.3% [62.1%; 100%]). At visit 6, Galderma HA-Filler Restylane Volyme achieved a response in all subjects with Hylase® "Dessau" 75 I.U. as well as with Hylase® "Dessau" 15 I.U. Detailed results are shown in Table 17 for the ITT population and in

Table 18 for the PP population.

Table 17: Explorative analysis due to premature termination of recruitment (ITT population)

					95% Confidence interval	
HA filler	**Dose**	**Visit**	**N (Responder / N)**	**Response (%)**	**Lower limit**	**Upper limit**
Allergan HA-Filler Voluma	Hylase Dessau 75 I.U.	V5	12 / 16	75.0	53.8	96.2
		V6	16 / 18	88.9	74.4	100
	Hylase Dessau 50 I.U.	V5	13 / 16	81.3	62.1	100
		V6	15 / 18	83.3	66.1	100
	Hylase Dessau 25 I.U.	V5	9 / 16	56.3	31.9	80.6
		V6	16 / 18	88.9	74.4	100
	Hylase Dessau 15 I.U.	V5	6 / 16	37.5	13.8	61.2
		V6	15 / 18	83.3	66.1	100
	Saline solution	V5	1 / 16	6.3	0	18.1
		V6	9 / 17	52.9	29.2	76.7
Merz HA-Filler Volume	Hylase Dessau 75 I.U.	V5	13 / 16	81.3	62.1	100
		V6	18 / 19	94.7	84.7	100
	Hylase Dessau 50 I.U.	V5	10 / 16	62.5	38.8	86.2
		V6	16 / 18	88.9	74.4	100
	Hylase Dessau 25 I.U.	V5	10 / 16	62.5	38.8	86.2
		V6	16 / 19	84.2	67.8	100
	Hylase Dessau 15 I.U.	V5	10 / 16	62.5	38.8	86.2
		V6	16 / 17	94.1	82.9	100
	Saline solution	V5	0 / 16	0	0	0
		V6	6 / 19	31.6	10.7	52.5
	Hylase Dessau 75 I.U.	V5	9 / 16	56.3	31.9	80.6
		V6	19 / 19	100	100	100
	Hylase Dessau 50 I.U.	V5	11 / 16	68.8	46.0	91.5
		V6	18 / 19	94.7	84.7	100
		V5	10 / 16	62.5	38.8	86.2

					95% Confidence interval	
HA filler	**Dose**	**Visit**	**N (Responder / N)**	**Response (%)**	**Lower limit**	**Upper limit**
Galderma HA-Filler Restylane Volyme	Hylase Dessau 25 I.U.	V6	18 / 19	94.7	84.7	100
	Hylase Dessau 15 I.U.	V5	9 / 16	56.3	31.9	80.6
		V6	18 / 18	100	100	100
	Saline solution	V5	3 / 16	18.8	0	37.9
		V6	7 / 19	36.8	15.2	58.5

CI: Confidence interval; HA: Hyaluronic acid filler; I.U.: International Units

(Source: Statistical output Table 2.1.1.4)

Table 18: Explorative analysis due to premature termination of recruitment (PP population)

					95% Confidence interval	
HA filler	**Dose**	**Visit**	**N (Responder / N)**	**Response (%)**	**Lower limit**	**Upper limit**
Allergan HA-Filler Voluma	Hylase Dessau 75 I.U.	V5	10 / 14	71.4	47.8	95.1
		V6	11 / 11	100	100	100
	Hylase Dessau 50 I.U.	V5	10 / 11	90.9	73.9	100
		V6	8 / 8	100	100	100
	Hylase Dessau 25 I.U.	V5	8 / 15	53.3	28.1	78.6
		V6	11 / 12	91.7	76.0	100
	Hylase Dessau 15 I.U.	V5	4 / 14	28.6	4.9	52.2
		V6	10 / 11	90.9	73.9	100
	Saline solution	V5	0 / 13	0	0	0
		V6	6 / 10	60.0	29.6	90.4
Merz HA-Filler Volume	Hylase Dessau 75 I.U.	V5	11 / 14	78.6	57.1	100
		V6	10 / 10	100	100	100
	Hylase Dessau 50 I.U.	V5	10 / 14	71.4	47.8	95.1
		V6	10 / 10	100	100	100
	Hylase Dessau 25 I.U.	V5	9 / 15	60.0	35.2	84.8
		V6	10 / 11	90.9	73.9	100
	Hylase Dessau 15 I.U.	V5	9 / 14	64.3	39.2	89.4
		V6	11 / 11	100	100	100
	Saline solution	V5	0 / 14	0	0	0
		V6	5 / 10	50.0	19.0	81.0
Galderma HA-Filler Restylane Volyme	Hylase Dessau 75 I.U.	V5	9 / 16	56.3	31.9	80.6
		V6	19 / 19	100	100	100
	Hylase Dessau 50 I.U.	V5	11 / 16	68.8	46.0	91.5
		V6	8 / 14	57.1	31.2	83.1
		V5	13 / 13	100	100	100

					95% Confidence interval	
HA filler	**Dose**	**Visit**	**N (Responder / N)**	**Response (%)**	**Lower limit**	**Upper limit**
	Hylase Dessau 25 I.U.	V6	11 / 15	73.3	51.0	95.7
	Hylase Dessau 15 I.U.	V5	14 / 14	100	100	100
		V6	8 / 12	66.7	40.0	93.3
	Saline solution	V5	10 / 11	90.9	73.9	100
		V6	8 / 14	57.1	31.2	83.1

CI: Confidence interval; HA: Hyaluronic acid filler; I.U.: International Units

(Source: Statistical Output Table 2.1.1.4)

11.4.2 Secondary Endpoint – Volume reduction measured with a 3D camera

No data for this endpoint are available (see section 9.8).

During the course of the study it turned out that the complete evaluation of all images is very time-consuming and costly. In addition, assessments of individual test persons showed that the 3D camera is an unsuitable method to evaluate the volume change of the nodules. This became apparent when comparing the evaluation with ultrasound or palpation versus evaluation with 3-D-camera.

11.4.3 Exploratory Endpoints

As explorative endpoints, all measurements of skin areas (ultrasound, palpation) after Hylase injection were analysed for the respective visits (V3, V4, V5, V6 and V7) per dose and HA filler. Changes in the ultrasound measurement values compared to the initial values of visit 2 were also displayed as box plots.

Ultrasound results of the skin per dose and HA-filler are presented in the following figures (boxplots, Figure 5 - Figure 8).

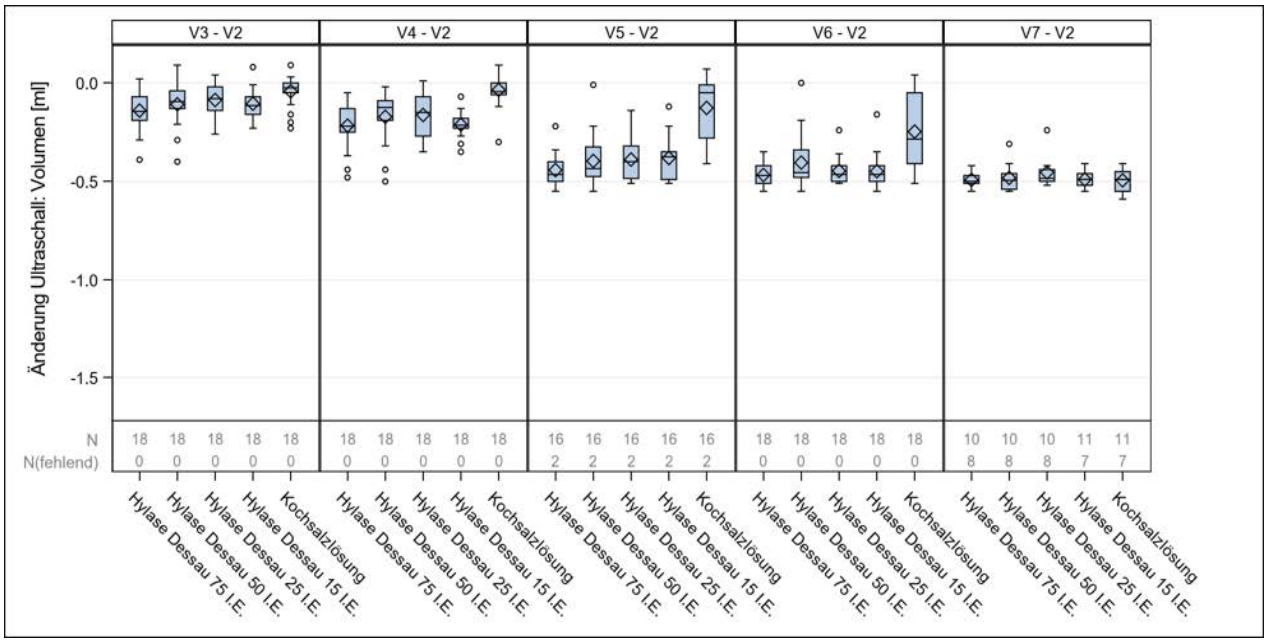

Figure 5: Ultrasound results of the skin per dose – Allergan HA filler (ITT population)

(Source: Stats output Figure 2.1.3.2)

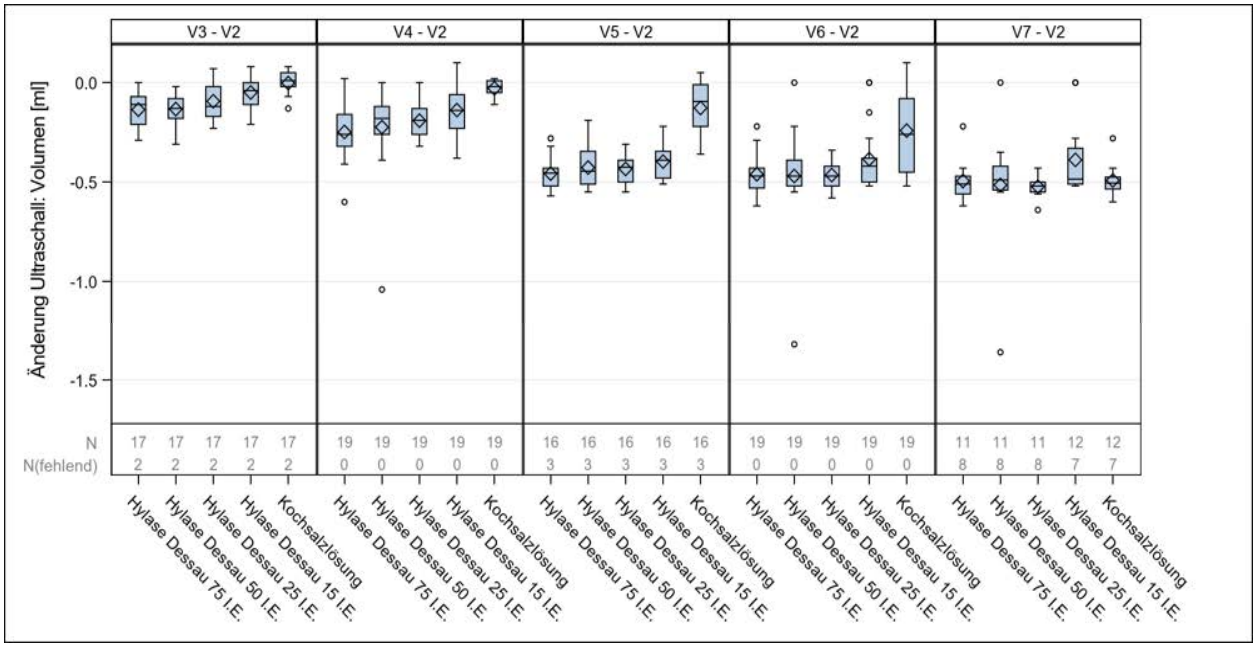

Figure 6: Ultrasound results of the skin per dose – Merz HA filler (ITT population)

(Source: Stats output Figure 2.1.3.2)

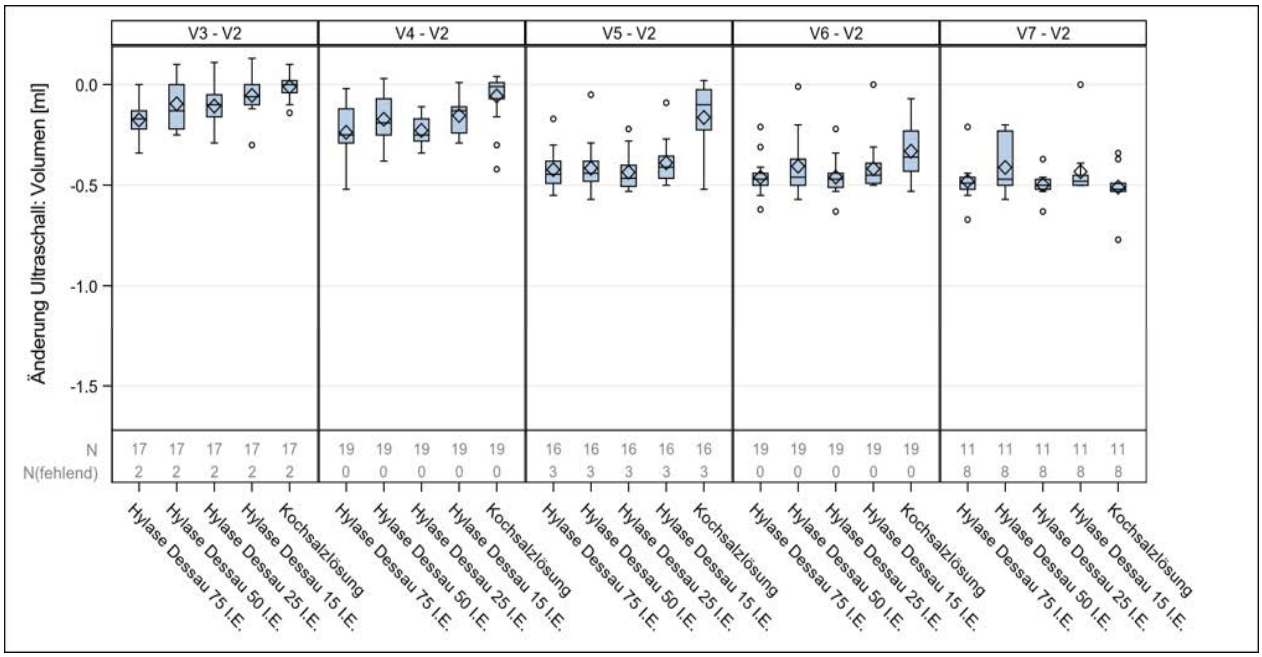

Figure 7: Ultrasound results of the skin per dose – Galderma HA filler (ITT population)

(Source: Stats output Figure 2.1.3.2)

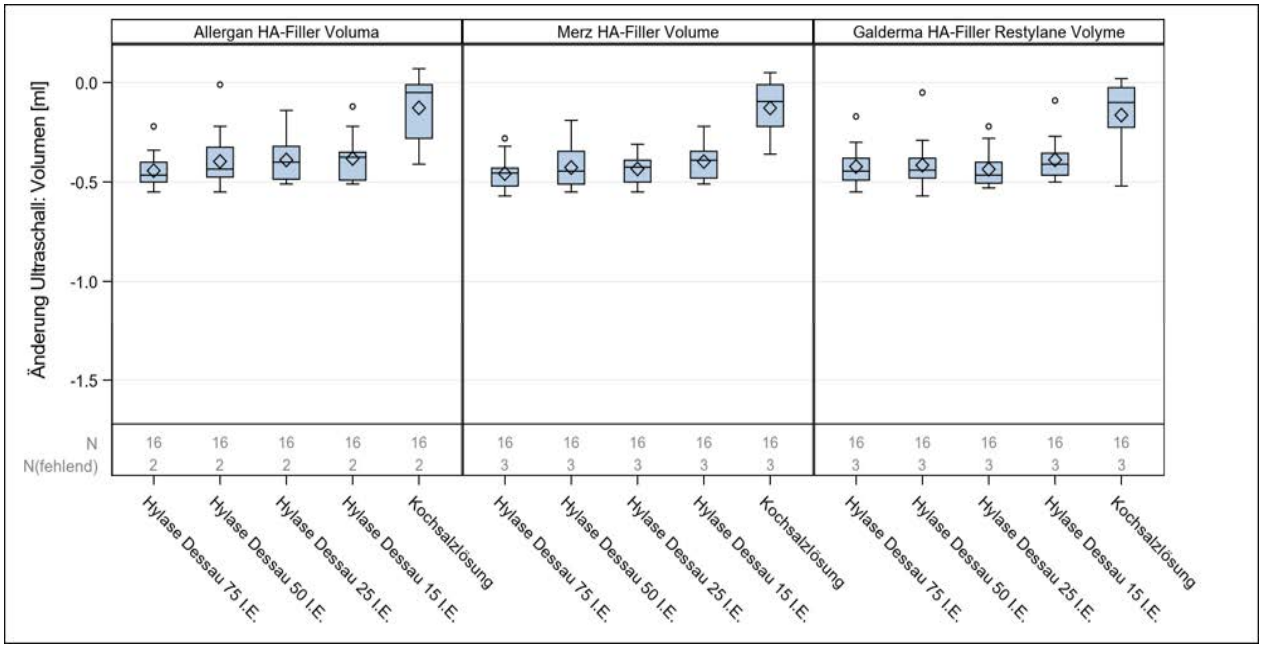

Figure 8: Ultrasound results of the skin per dose and HA filler; V5 – V2 (ITT population)

(Source: Stats output Figure 2.1.3.2)

In the following figures results of the palpation per dose and HA filler from V3 to V7 are presented (Figure 9 - Figure 13).

Changes in the palpation categories were visualized using heat maps. The color intensity of these maps correlates to the relative proportion of the subjects. There is one heatmap for each combination of HA filler and Hylase dose.

It can be said that white fields characterize missing response (0%). The darker the green of the fields, the higher the response rate of the test persons. The lowest response is thus shown for the areas treated with saline solution, while higher response rates are generally shown with increasing doses of Hylase® "Dessau".

For example, the results for Hylase® "Dessau" 75 I.U. with Allergan HA-Filler Voluma are explained herewith: Of the six volunteers who were injected with the Allergan filler and had a palpation value of 4 at V2, after treatment with Hylase® "Dessau" 75 I.U. one subject already had a value of 1 at V3; at V5 three subjects had a value of 0 (1 missing value), which increased to five subjects at V6. Finally, at V7 all six volunteers treated with Hylase® "Dessau" 75 I.U. after Allergan HA filler had a palpation value of 0. This can be seen in Figure 9.

Allergan HA filler:

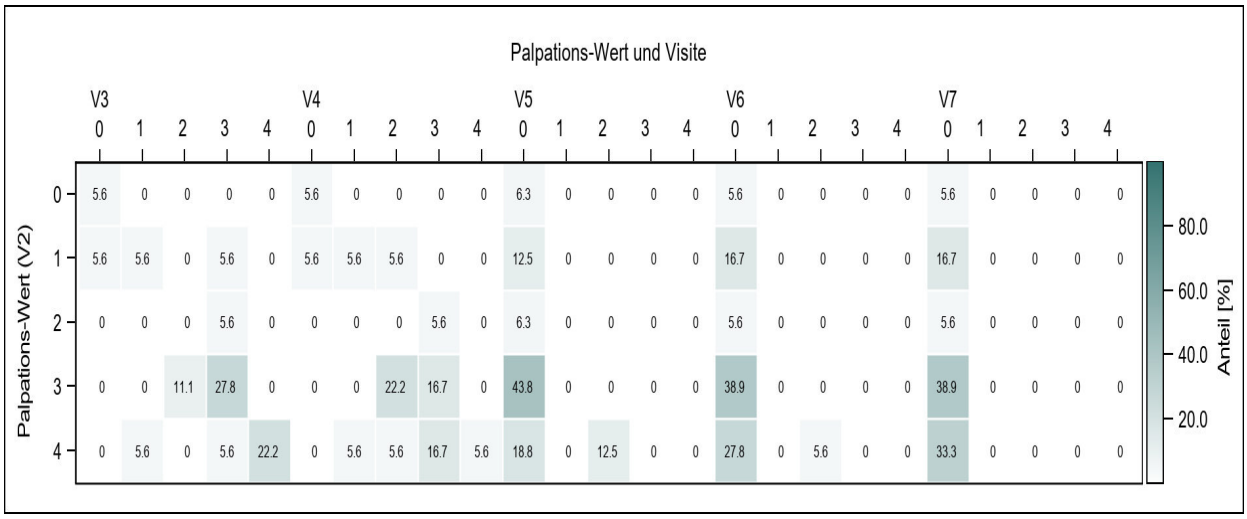

Figure 9: Palpation results per dose (75 I.U.) and HA filler - Allergan HA filler (ITT population)

(Source: Statistical output Figure 2.1.3.3)

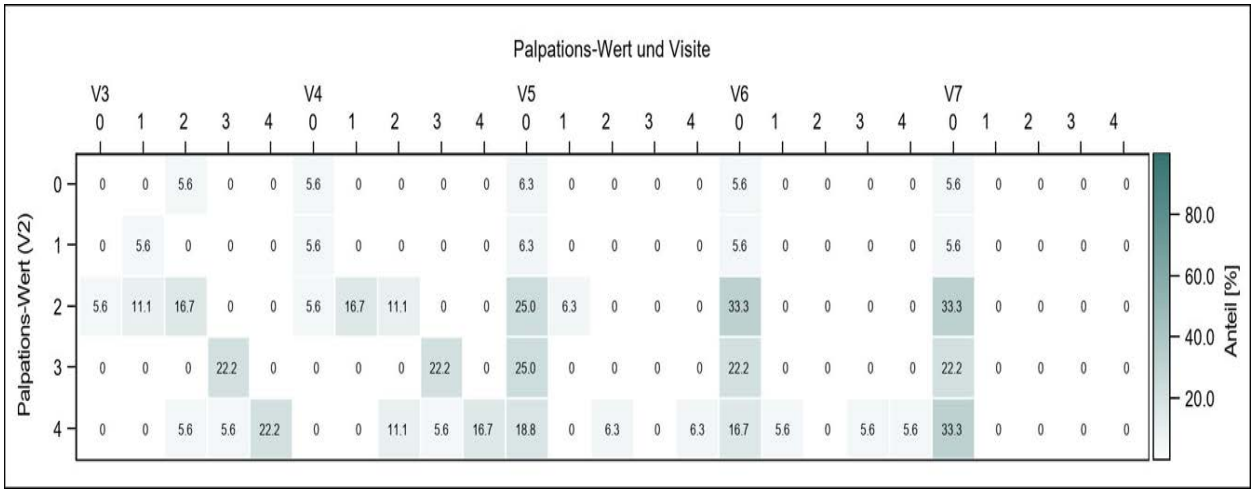

Figure 10: Palpation results per dose (50 I.U.) and HA filler - Allergan HA filler (ITT population)

(Source: Statistical output Figure 2.1.3.3)

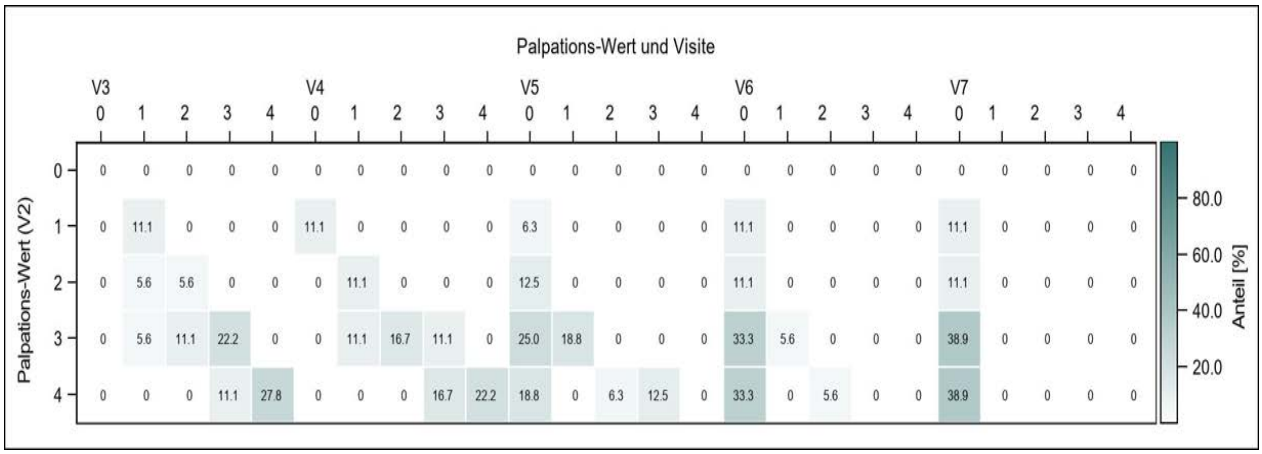

Figure 11: Palpation results per dose (25 I.U.) and HA filler - Allergan HA filler (ITT population)

(Source: Statistical output Figure 2.1.3.3)

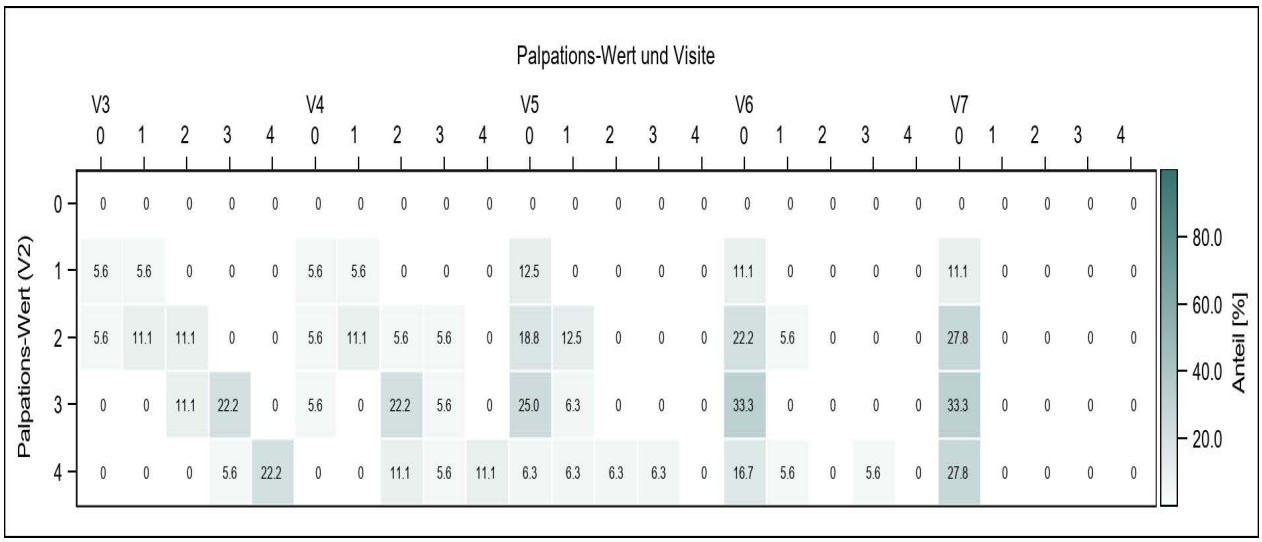

Figure 12: Palpation results per dose (15 I.U.) and HA filler - Allergan HA filler (ITT population)

(Source: Stats output Figure 2.1.3.3)

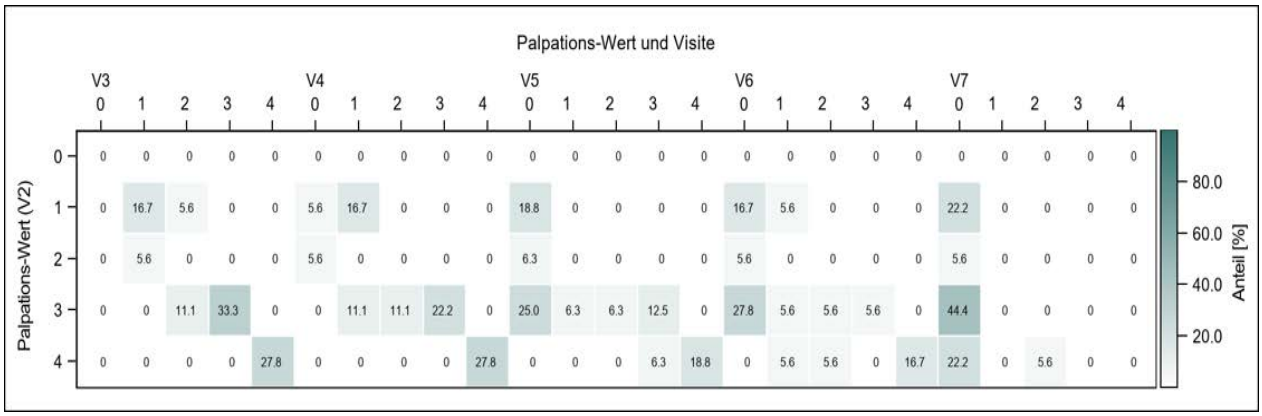

Figure 13: Palpation results per dose (Saline solution) and HA filler - Allergan HA filler (ITT population)

(Source: Statistical output Figure 2.1.3.3)

Merz HA filler:

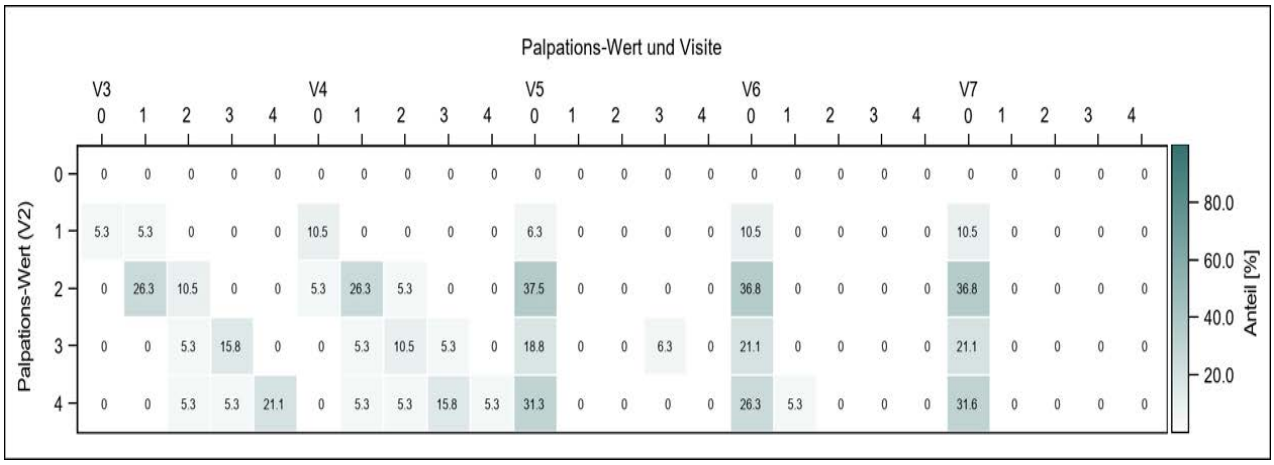

Figure 14: Palpation results per dose (75 I.U.) and HA filler - Merz HA filler (ITT population)

(Source: Statistical output Figure 2.1.3

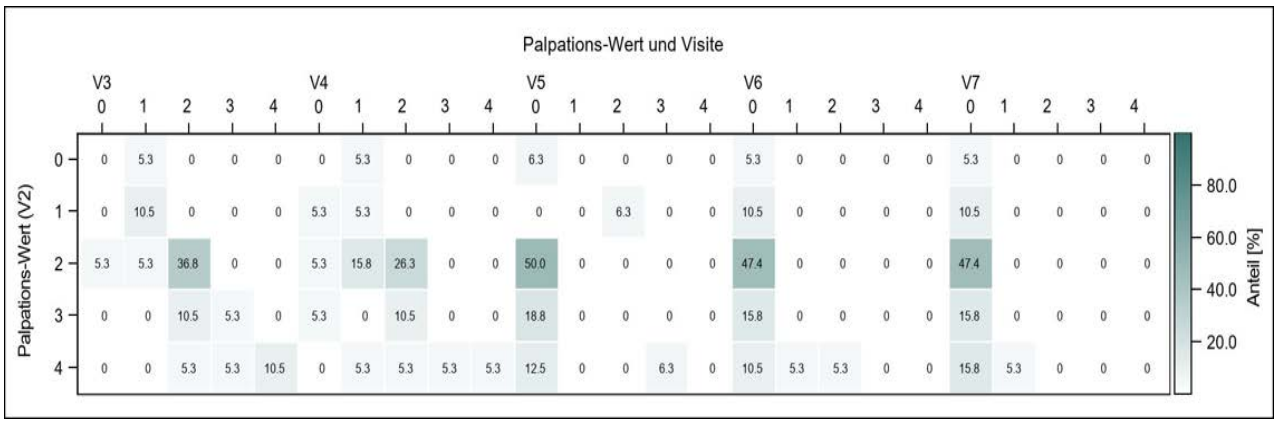

Figure 15: Palpation results per dose (50 I.U.) and HA filler - Merz HA filler (ITT population)

(Source: Statistical output Figure 2.1.3.3)

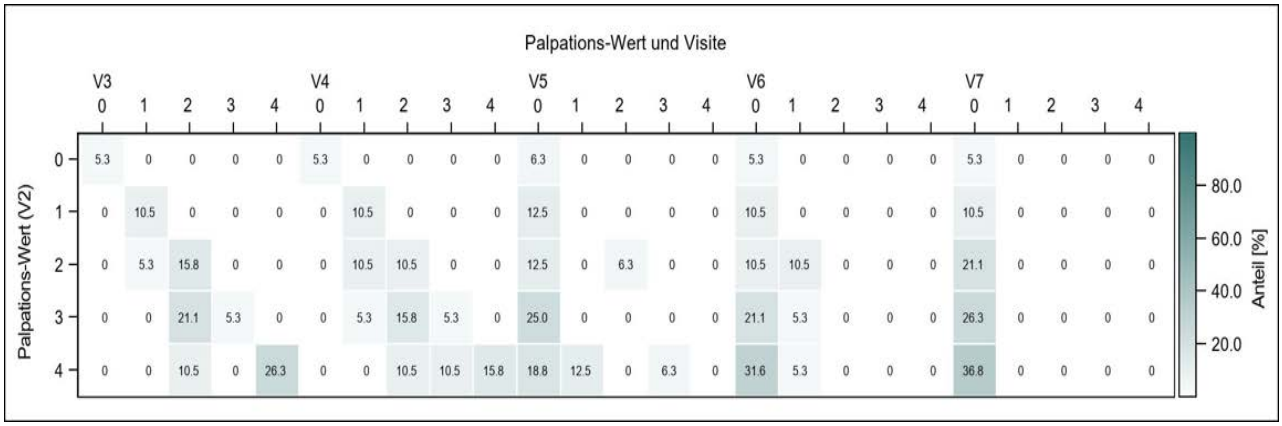

Figure 16: Palpation results per dose (25 I.U.) and HA filler - Merz HA filler (ITT population)

(Source: Stats output Figure 2.1.3.3)

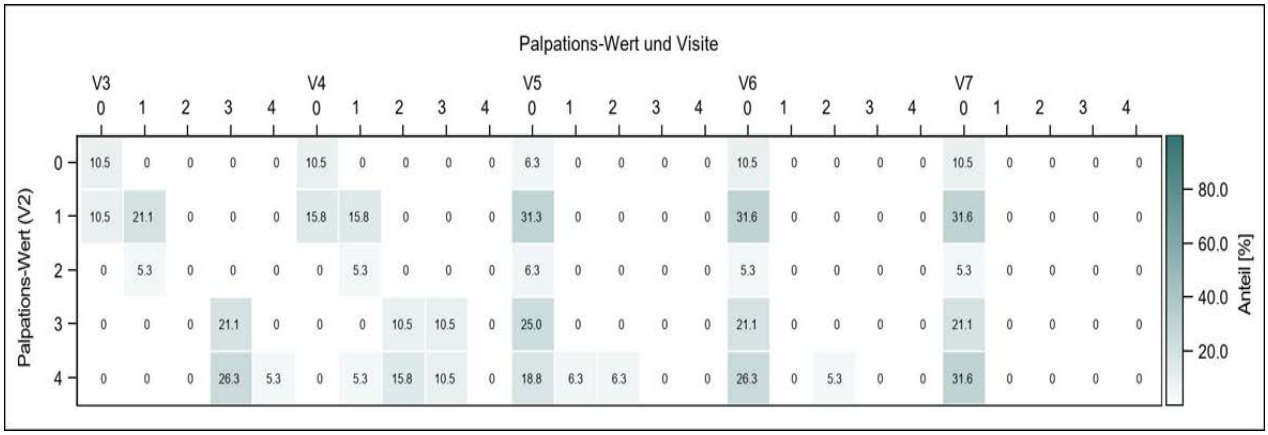

Figure 17: Palpation results per dose (15 I.U.) and HA filler - Merz HA filler (ITT population)

(Source: Statistical output Figure 2.1.3.3)

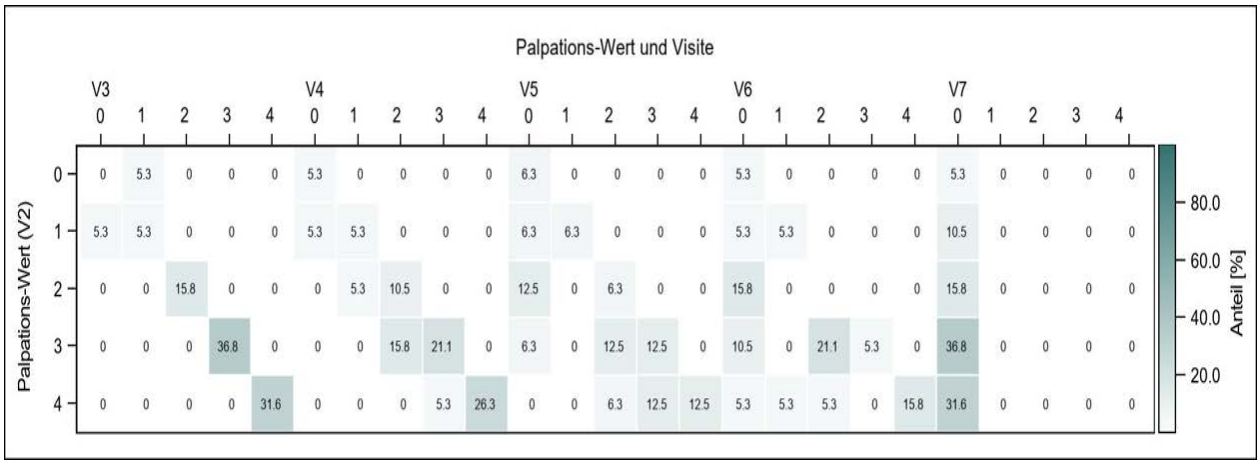

Figure 18: Palpation results per dose (Saline solution) and HA filler - Merz HA filler (ITT population)

(Source: Statistical output Figure 2.1.3.3)

Galderma HA Filler:

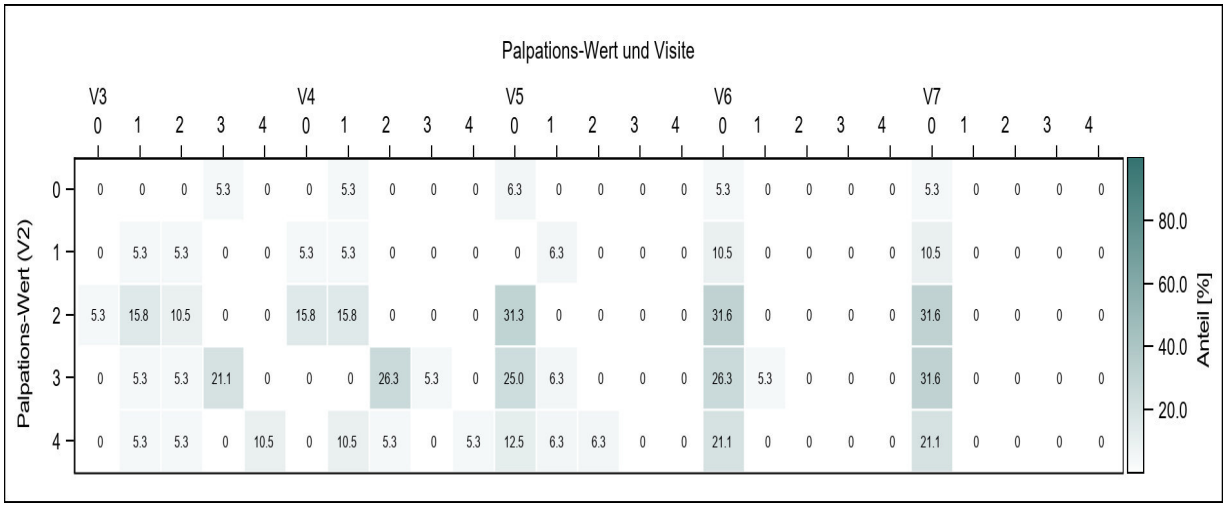

Figure 19: Palpation results per dose (75 I.U.) and HA filler - Galderma HA filler (ITT population)

(Source: Statistical output Figure 2.1.3.3)

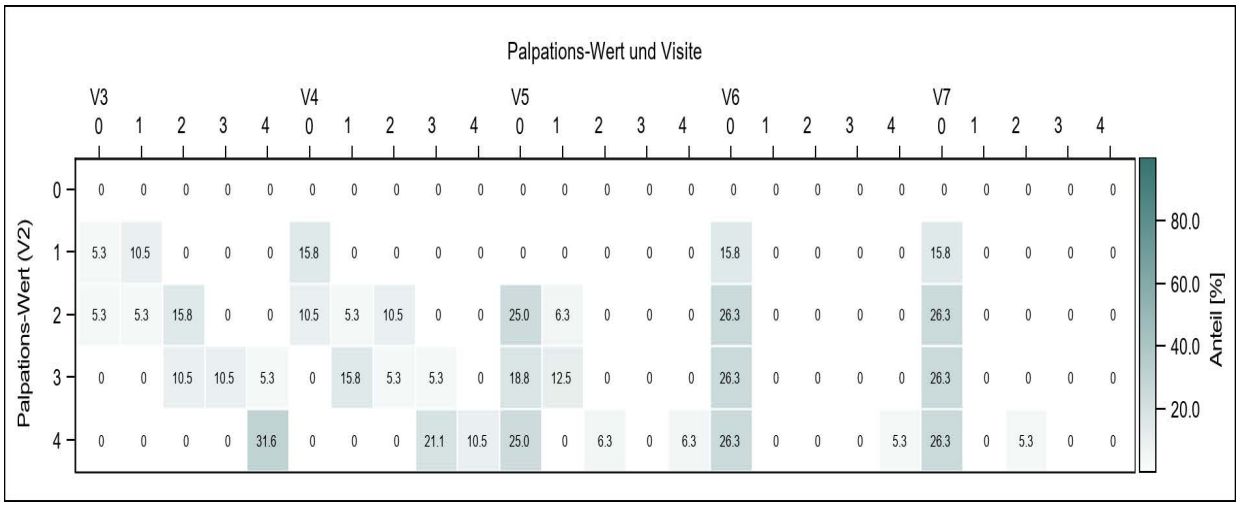

Figure 20: Palpation results per dose (50 I.U.) and HA filler - Galderma HA filler (ITT population)

(Source: Statistical output Figure 2.1.3.3)

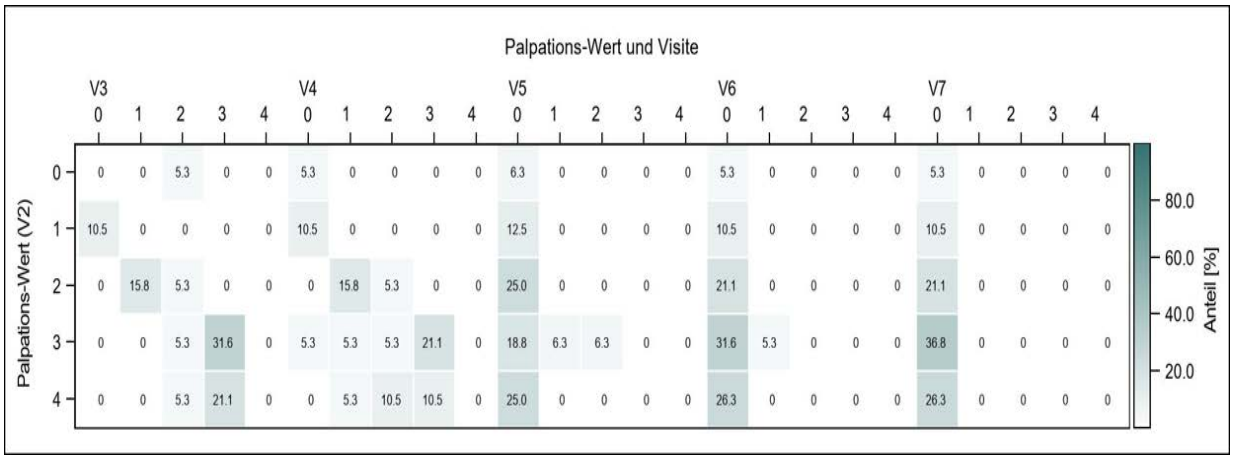

Figure 21: Palpation results per dose (25 I.U.) and HA filler - Galderma HA filler (ITT population)

(Source: Stats output Figure 2.1.3.3)

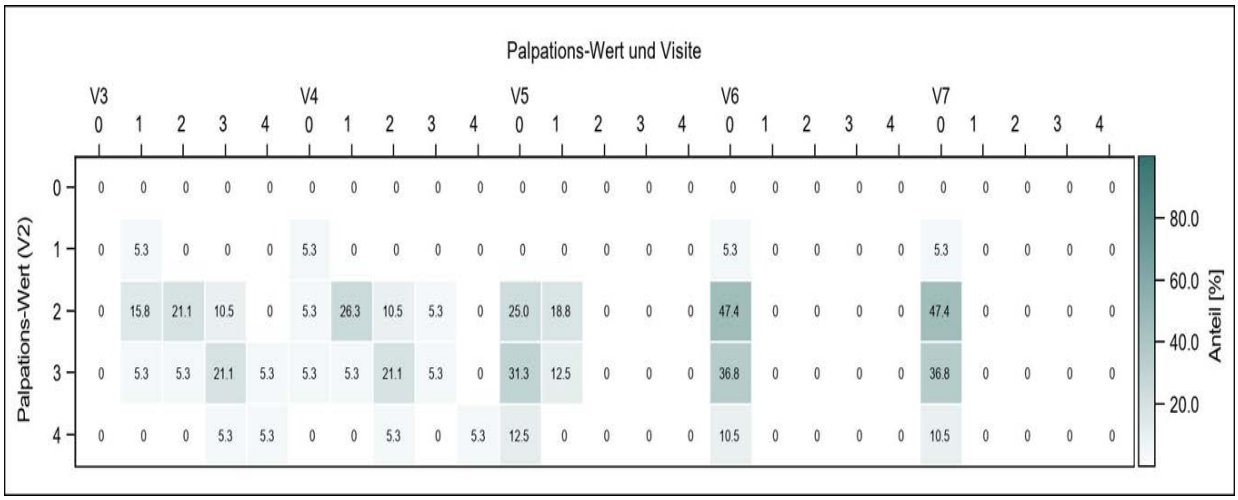

Figure 22: Palpation results per dose (15 I.U.) and HA filler - Galderma HA filler (ITT population)

(Source: Stats output Figure 2.1.3.3)

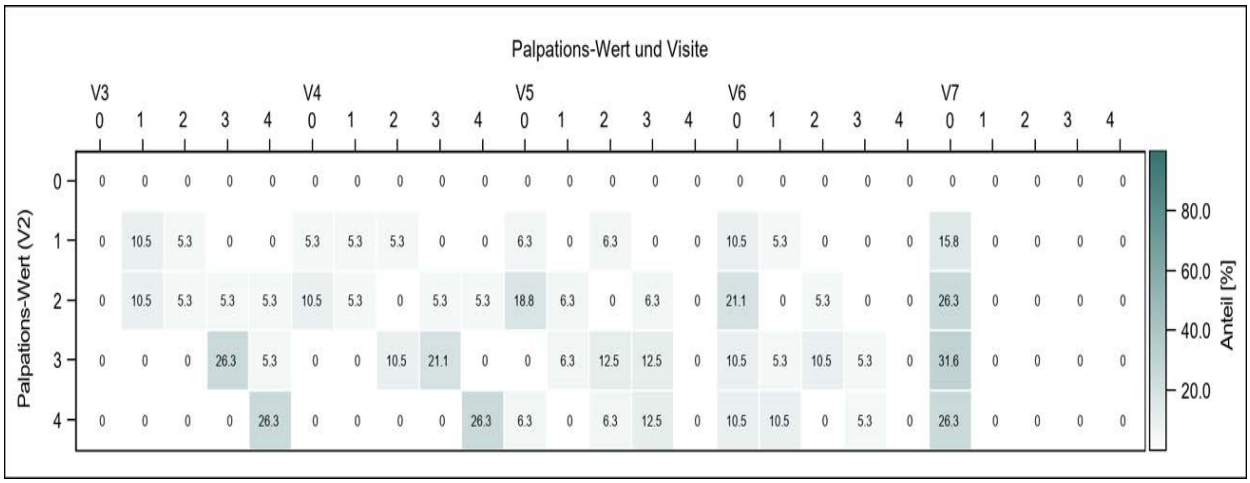

Figure 23: Palpation results per dose (Saline solution) and HA filler - Galderma HA filler (ITT population)

(Source: Statistical output Figure 2.1.3.3)

11.4.4 Statistical/Analytical Issues

After finalization of the study, an extended statistical analysis was performed, focussing on three issues: First of all, this included the decoupled analysis of the response rates for the ultrasound and palpation measurement methods.

The isolated consideration of sonography and palpation showed good response rates independently from the method used.

Moreover, response rates were calculated at three further cut-off values (80%, 65% and 50%) for volume reduction. Here, a cut-off value of 80% already showed significantly better response rates than a target value of 90% volume reduction.

Finally, a repeated analysis of the (original) response rate from palpation and 90% volume reduction was carried out, taking into account the location of the injection sites, differentiating between the central injection site and the four lateral sites.

The response rates for differentiating the localisation (one central injection site versus four lateral injection sites) were hardly comparable descriptively, as only few data were available for the central injection site in particular.

The extended statistical analysis was not part of the study plan. All analyses were performed using the SAS 9.4 software (SAS Institute Inc., Cary, NC, USA).

The statistical methodology is outlined in Section **Fehler! Verweisquelle konnte nicht gefunden werden.** of this CSR.

11.4.4.1 *Adjustments for Covariates*

Not applicable

11.4.4.2 *Handling of Drop-outs or Missing Data*

Not applicable

11.4.4.3 *Interim Analyzes and Data Monitoring*

Not applicable.

11.4.4.4 *Multi center Studies*

During the sensitivity analysis related to the HA filler the patient code as well as the site were estimated as a random effect to take into account subject and site-related dependencies. These resulted in the following response rates:

- Hylase® „Dessau“ 75 I.U.: 71.3% [36.7%; 91.4%]
- Hylase® „Dessau“ 50 I.U.: 73.0% [37.7%; 92.4%]
- Hylase® „Dessau“ 25 I.U.: 60.1% [40.4%; 76.9%]
- Hylase® „Dessau“ 15 I.U.: 51.5% [34.9%; 67.8%]

Thus, even when considering the HA fillers in summary, neither the highest dose nor the lower dosages of Hylase® "Dessau" achieved a response rate above 80% (lower limit of the 95% confidence interval).

As an additional result of this model, the influence of fixed and random effects can be assessed: For the random effect related to the site, no random effect is detectable for any of the Hylase doses; all p-values are not significant. This applies to the HA filler, too:

- Hylase® „Dessau“ 75 I.U.: p(site) = 0.445; p(HA-Filler) = 0.294
- Hylase® „Dessau“ 50 I.U.: p(site) = 0.511; p(HA-Filler) = 0.432
- Hylase® „Dessau“ 25 I.U.: p(site) = 0.667; p(HA-Filler) = 0.892
- Hylase® „Dessau“ 15 I.U.: p(site) = 0.956; p(HA-Filler) = 0.386

This was true for the ITT population as well as for the PP population.

11.4.4.5 *Multiple Comparisons/Multiplicity*

Not applicable

11.4.4.6 *Use of an ‘Efficacy Subset’ of Patients*

The efficacy analyses were performed in the ITT as well as in the PP population.

11.4.4.7 *Active-control Studies intended to show Equivalence*

Not applicable.

11.4.4.8 *Examination of Subgroups*

All analyses were performed per dose of Hylase® "Dessau", as the planned aim was to determine the lowest effective dose to treat overinjections caused by HA fillers.

In addition, the majority of the analyses were performed per HA filler:

Allergan HA-Filler Voluma® (10 mg/0.5 mL HA)

Merz HA-Filler Volume® (13 mg/0.5 mL HA)

In addition, the dosage of Hylase® "Dessau" was taken into account as well as the respective visit (partly). Interactions were not considered.

11.4.5 Tabulation of Individual Response Data

Not applicable

11.4.6 Drug Dose, Drug Concentration and Relationship to Response

Not applicable

11.4.7 Drug-Drug and Drug-Disease Interactions

Not applicable

11.4.8 By-Patient Displays

Graphical presentations of individual patient data are not available.

11.4.9 Efficacy Conclusions

It was the primary endpoint of this study to determine the Hylase® dose that achieved complete volume reduction, defined by palpation and ultrasound after 24 hours of Hylase injection. This was done one or 2 day(s) after treatment (overcorrection) with a HA filler. Response was defined by a palpation result of 0 or 1 and a volume reduction >90% determined by ultrasound.

ITT population: Regarding the Allergan HA Filler there were most responders (n=13 out of 16) when using a Hylase dose of 50 I.U. Using the Merz HA Filler there were 13 responders out of 16 with a Hylase dose of 75 I.U. and with the Galderma HA filler there were most responders (n=11 out of 16) with a Hylase dose of 50 I.U.

PP population: Regarding the Allergan HA Filler there were most responders (n=10 out of 11) when using a Hylase dose of 50 I.U. Using the Merz HA Filler there were 11 responders out of 14 with a Hylase dose of 75 I.U. and with the Galderma HA filler there were most responders (n=11 out of 15) with a Hylase dose of 50 I.U.

No dose effect could be observed but all doses showed the desired effect achieving a volume reduction of at least 80.0% whereas after placebo injection a reduction of approximately 20.0% was noted. It has to be taken into account that the low number of participants biased the results, so that further investigation including more subjects are warranted.

Response rates of the hierarchical analysis in the ITT population were as follows:

- 75.0% [53.8%; 96.2%] - Allergan HA-Filler Voluma
- 81.3% [62.1%; 100%] - Merz HA-Filler Volume und
- 56.3% [31.9%; 80.6%] - Galderma HA-Filler Restylane Volyme.

Their lower limit of the respective 95% CI was in no case above 80%, so that the hierarchical test procedure was terminated after this first dose and all other Hylase® "Dessau" doses were also not considered significant. Thus, no (lowest) dose of Hylase could be determined at which a suitable response after 24 hours, i.e. a complete volume reduction by palpation and ultrasound, was observed after 1 or 2 day(s) after treatment with a HA filler.

Within the sensitivity analysis the response rates related to the concentration of Hylase “Dessau” as well as to the HA fillers were as follows:

- Hylase® „Dessau“ 75 I.U.: 71.3% [36.7%; 91.4%]

- Hylase® „Dessau“ 50 I.U.: 73.0% [37.7%; 92.4%]
- Hylase® „Dessau“ 25 I.U.: 60.1% [40.4%; 76.9%]
- Hylase® „Dessau“ 15 I.U.: 51.5% [34.9%; 67.8%]

Thus, even when considering the hyaluronic acid fillers in summary, neither the highest dose nor the lower dosages of Hylase® "Dessau" achieved a response rate above 80%.

When viewing the response rates of the sensitivity analysis caused by the premature stop of recruitment the highest response rate after 24 hours was achieved with Allergan HA-Filler Volume using Hylase® "Dessau" 50 I.U. and with Merz HA-Filler Volume using Hylase® "Dessau" 75 I.U. (81.3% [62.1%; 100%] each). At V6, Galderma HA-Filler Restylane Volyme achieved a response in all subjects with Hylase® "Dessau" 75 I.U. and Hylase® "Dessau" 15 I.U.

The results of the analysis of the primary endpoint based on the PP population differ only slightly from those of the ITT population. Again, the hierarchical analysis for each of the three hyaluronic acid fillers was not significant for Hylase® "Dessau" 75 I.U.

Again, the results of the sensitivity analysis related to the HA filler confirm the results of the ITT population. With the exception of the dose of Hylase® "Dessau" 50 I.U., for which a response rate of 80.1% [41.2%; 95.9%] was estimated by modelling the mixed model, the estimates of the response rate at V5 are slightly below the results for the ITT population. Neither the center effect nor the HA filler are significant.

Looking at the response rates related to the sensitivity analysis due to the premature stop of recruitment the highest response rate of 90.9% [73.9%; 100%] after 24 hours was achieved with Allergan HA-Filler Voluma using Hylase® "Dessau" 50 I.U. At visit 6 a response was achieved in all subjects for all three HA fillers using Hylase® "Dessau" 75 I.U. and Hylase® "Dessau" 50 I.U. This was also achieved with Hylase® "Dessau" 15 I.U. for Merz HA-Filler Volume and Galderma HA-Filler Restylane Volyme.

From the results presented it can be concluded that a dose of 50 I.U. of Hylase® "Dessau" as well as a dose of 75 I.U. was effective in the treatment of overcorrections, independently from the HA filler used. This statement is true despite the fact that no significance could be established as the effectiveness was not as high as originally estimated. To establish statistical significance further investigations involving more subjects are needed.

12. Safety Evaluation

Safety evaluation was performed in all subjects of the SAF population (n=28).

12.1 Extent of Exposure

On the back of both hands, 0.5 mL aliquots of HA fillers were injected into each hand; the following fillers were injected:

- Allergan HA filler Voluma: n=18, 32.1%
- Merz HA filler Volume: n=19, 33.9%
- Galderma HA filler Restylane Volyme: n=19, 33.9%

All subjects received their injections according to the protocol but in 2 subjects the randomization scheme was not correctly applied (1 subject in the Allergan HA filler group and 1 subject in the Galderma HA filler group).

The injection points 1 and 3 were mixed up in one subject (Source Statistical output Table 1.2.1.2, 1.2.2.1, 1.2.3.1, 1.2.3.2). The following combinations of HA fillers were applied:

Table 19: Combinations of HA fillers

Hyaluronic acid filler				
Allergan HA filler	Merz HA filler	Galderma HA filler	N	%
	X	X	10	35.7
X	X		9	32.1
X		X	9	32.1

(Source: Statistical output Table 1.2.1.1)

12.1.1 Follow-up Injections of Hylase® „Dessau“

In the event that the dose was not sufficient to treat the overcorrection, additional Hylase® “Dessau” injections were necessary. This was the case for 17 subjects who received a total of 48 additional injections. Relating the additional Hylase® “Dessau” injections to the administered dose in 4 nodules out of 56 nodules a second injection was necessary using a dose of 75 IU. Using 50 IU or 25 IU an additional dose was necessary in 6 out of 56 nodules each, and with a dose of 15 IU another injection was required to treat 3 nodules out ofr 56 nodules. In the control group (using NaCl) a follow-up injection was applied into 29 out of 56 nodules. Furthermore, it has to be noted that the lowest number of follow-up injections was necessary in the group who received the Galderma HA filler Restylane Volume (n=3 in total) (Stats output Table 2.1.4.2).

In the PP population, there was a swelling in eight subjects at V6, and the follow-up injections were made exclusively at the injection sites that had been treated with saline solution.

12.2 Adverse Events (AEs)

12.2.1 Brief Summary of Adverse Events

Twenty-seven AEs were developed by 5 out of 28 subjects (17.9%); the remaining subjects had no AE. There was one subject (01-001) that experienced 16 AEs, one subject had 7 AEs (01-008), one subject had 2 AEs (01-016), and 2 subjects reported one AE each (01-007, 01-015).

The following AEs occurred during the course of the study and are listed as reported and as MedDRA PT:

- Injection site tenderness (PT Injection site pain), n=3
- Burning at injection sites (PT Injection site pain) (n=10)
- Dizziness (PT Dizziness) (n=1)
- Burning after further Hylase injection (15 I.U.) (PT Injection site pain), n=2
- Nerve injury (*N. ulnaris*) (PT Ulnar nerve injury), n=1
- Pain in the area of the carpal bones (PT Pain in extremity), n=1
- Swelling of both backs of the hands (PT Peripheral swelling), n=2
- Reddening of both backs of the hands (PT Erythema), n=4
- Hematoma at back of the left hand (PT Aplication site haematoma), n=2
- Hematoma at back of the right hand (PT Application site hematoma), n=1 (Statistical output Table 3.1.1.1, for an assignment of SOC and PT refer to Table 3.2.1.11 of the statistical output).

There was no serious adverse event (SAE) (Statistical output Table 3.1.1.2).

Five AEs occurred at V1 after injection of the HA filler, 20 AEs occurred at V2 after Hylase “Dessau” injection and 2 AEs occurred after the follow up injections at V6 or V7 (refer to Table 20) (Statistical output Table 3.1.1.3).

Table 20: Adverse events at the respective visits

	Total	
Time-based assignment	**n_e**	**n**
All subjects		28 (100%)
After HA filler injection V1	5	2 (7.1%)
After Hylase injection V2	20	5 (17.9%)
After further Hylase injection V6/V7	2	1 (3.6%)
Total	27	5 (17.9%)

(Source: Statistical output Table 3.1.1.3)

Twenty AEs were assessed as “mild”, no assessment of the severity grade was done in 7 AEs. All AEs were reported to be resolved at study end (Statistical output Table 3.2.1.3, Table 3.2.1.4).

Seventeen AEs out of the 20 AEs at V2 were assessed as being related to the injection of Hylase® “Dessau” (ADRs) (Table 21). Those were injection site pain (n=8), peripheral swelling (n=2), application site hematoma (n=1), erythema (n=4), Ulnar nerve injury (n=1), and pain in extremity (n=1). The event dizziness was assessed as not related to Hylase® “Dessau” and two AEs were assigned to injection of 0.9 % NaCl.

Both AEs after the follow up injection were also assessed as related to the study drug. These ADRs were “injection site pain” (n=2) and were developed by one patient who also suffered from these events after the first Hylase injection.Five AEs that occurred after injection of Hylase “Dessau” were treated pharmaceutically, 13 AEs required no intervention, 1 AE required protection of the affected hand and for 1 AE information related to treatment was missing (Statistical output Table 3.2.1.7).

All AEs were present in females, no male experienced an AE (Statistical output Table 3.2.1.9).

The relationship to the test drug classified according to SOC and PT is provided in the following table:

Table 21: Causal Relationship of AEs at V2 to the study drug classified according to SOC and PT

	Yes		**No**		**Total**	
System Organ Class (SOC) Preferred Term (PT)	**n_e**	**n**	**n_e**	**n**	**n_e**	**n**
Total subjects		28 (100%)		28 (100%)		28 (100%)
Total AEs (n_e) and subjects with at least 1 AE (n)	17	5 (17.9%)	1	1 (3.6%)	18	5 (17.9%)
General disorders and administration site conditions (10018065)	11	3 (10.7%)	-	-	11	3 (10.7%)
Injection site pain (10022086)	8	1 (3.6%)	-	-	8	1 (3.6%)
Peripheral swelling (10048959)	2	1 (3.6%)	-	-	2	1 (3.6%)
Application site haematoma (10068317)	1	1 (3.6%)	-	-	1	1 (3.6%)
Skin and subcutaneous tissue disorders (10040785)	4	2 (7.1%)	-	-	4	2 (7.1%)
Erythema (10015150)	4	2 (7.1%)	-	-	4	2 (7.1%)
Injury, poisoning and procedural complications (10022117)	1	1 (3.6%)	-	-	1	1 (3.6%)
Ulnar nerve injury (10045378)	1	1 (3.6%)	-	-	1	1 (3.6%)

	Yes		No		Total	
System Organ Class (SOC) Preferred Term (PT)	**n_e**	**n**	**n_e**	**n**	**n_e**	**n**
Musculoskeletal and connective tissue disorders (10028395)	1	1 (3.6%)	-	-	1	1 (3.6%)
Pain in extremity (10033425)	1	1 (3.6%)	-	-	1	1 (3.6%)
Nervous system disorder (10029205)	-	-	1	1 (3.6%)	1	1 (3.6%)
Dizziness (10013573)	-	-	1	1 (3.6%)	1	1 (3.6%)

Source: Statistical output Table 3.2.2.1.3
Two AEs that occurred after injection of saline solution were not included into this table.

Unexpected AEs according to SOC and PT are shown in Table 22.

Two ADRs (application site hematoma and ulnar nerve injury) out of the 17 ADRs were not expected (Statistical output Table 3.2.1.6).

Table 22: Unexpected ADRs at V2 according to SOC and PT

	Expected		Unexpected		Total	
System Organ Class (SOC) Preferred Term (PT)	**n_e**	**n**	**n_e**	**n**	**n_e**	**n**
Total subjects		28 (100%)		28 (100%)		28 (100%)
Total AEs (n_e) and subjects with at least 1 AE (n)	15	3 (10.7%)	2	2 (7.1%)	17	5 (17.9%)
General disorders and administration site conditions (10018065)	10	2 (7.1%)	1	1 (3.6%)	11	3 (10.7%)
Injection site pain (10022086)	8	1 (3.6%)	-	-	8	1 (3.6%)
Peripheral swelling (10048959)	2	1 (3.6%)	-	-	2	1 (3.6%)
Application site haematoma (10068317)	-	-	1	1 (3.6%)	1	1 (3.6%)
Skin and subcutaneous tissue disorders (10040785)	4	2 (7.1%)	-	-	4	2 (7.1%)
Erythema (10015150)	4	2 (7.1%)	-	-	4	2 (7.1%)
Injury, poisoning and procedural complications (10022117)	-	-	1	1 (3.6%)	1	1 (3.6%)
Ulnar nerve injury (10045378)	-	-	1	1 (3.6%)	1	1 (3.6%)
Musculoskeletal and connective tissue disorders (10028395)	1	1 (3.6%)	-	-	1	1 (3.6%)

	Expected		Unexpected		Total	
System Organ Class (SOC) Preferred Term (PT)	**n_e**	**n**	**n_e**	**n**	**n_e**	**n**
Pain in extremity (10033425)	1	1 (3.6%)	-	-	1	1 (3.6%)

Source: Statistical output Table 3.2.2.2.3

Adverse events per dose and filler used are displayed in Table 23**Fehler! Verweisquelle konnte nicht gefunden werden.** for the ITT population and in Table 24**Fehler! Verweisquelle konnte nicht gefunden werden.** for the PP population: Not all of the AEs observed could be assigned to a specific dose.

Table 23: Adverse events per dose and filler used (ITT population)

	Allergan HA filler		Merz HA filler		Galderma HA filler		No information about filler provided	
	n_e	**n**	**n_e**	**n**	**n_e**	**n**	**n_e**	**n**
Total subjects		18 (100%)		19 (100%)		19 (100%)		28 (100%)
Hylase Dessau 75 I.U.	1	1 (5.6%)	1	1 (5.3%)	1	1 (5.3%)	-	-
Hylase Dessau 50 I.U.	2	2 (11.1%)	-	-	1	1 (5.3%)	-	-
Hylase Dessau 25 I.U.	1	1 (5.6%)	-	-	1	1 (5.3%)	-	-
Hylase Dessau 15 I.U.	1	1 (5.6%)	-	-	1	1 (5.3%)	-	-
Saline solution	1	1 (5.6%)	-	-	1	1 (5.3%)	-	-
No dose provided	1	1 (5.6%)	2	1 (5.3%)	3	2 (10.5%)	2	2 (7.1%)
Total	7	3 (16.7%)	3	2 (10.5%)	8	3 (15.8%)	2	2 (7.1%)

HA: Hyaluronic acid filler; I.U.: International Unit
Source: Statistical output Table 3.4.1.1.1

Table 24: Adverse events per dose and filler used (PP population)

	Allergan HA filler		Merz HA filler		Galderma HA filler		No information about filler provided	
	n_e	n	n_e	n	n_e	n	n_e	n
Total subjects		15 (100%)		16 (100%)		15 (100%)		23 (100%)
Hylase Dessau 75 I.U.	-	-	1	1 (6.3%)	-	-	-	-
Hylase Dessau 50 I.U.	1	1 (6.7%)	-	-	-	-	-	-
Hylase Dessau 25 I.U.	-	-	-	-	-	-	-	-
Hylase Dessau 15 I.U.	-	-	-	-	-	-	-	-
Saline solution	-	-	-	-	-	-	-	-
No dose provided	1	1 (6.7%)	2	1 (6.3%)	3	2 (13.3%)	1	1 (4.3%)
Total	2	2 (13.3%)	3	2 (12.5%)	3	2 (13.3%)	1	1 (4.3%)

HA: Hyaluronic acid filler; I.U.: International Unit
Source: Statistical output Table 3.4.2.1.1

Expected and unexpected AEs per dose and HA filler are presented in Table 25 and

Table 26.

Table 25: Expected AEs per dose and HA filler

	Allergan HA filler		Merz HA filler		Galderma HA filler		No information about filler provided	
	n_e	n	n_e	n	n_e	n	n_e	n
Total subjects		18 (100%)		19 (100%)		19 (100%)		28 (100%)
Hylase Dessau 75 I.U.	1	1 (5.6%)	-	-	1	1 (5.3%)	-	-
Hylase Dessau 50 I.U.	1	1 (5.6%)	-	-	1	1 (5.3%)	-	-

Hylase Dessau 25 I.U.	1	1 (5.6%)	-	-	1	1 (5.3%)	-	-
Hylase Dessau 15 I.U.	1	1 (5.6%)	-	-	1	1 (5.3%)	-	-
No dose provided	1	1 (5.6%)	2	1 (5.3%)	3	2 (10.5%)	1	1 (3.6%)
Total	5	2 (11.1%)	2	1 (5.3%)	7	3 (15.8%)	1	1 (3.6%)

HA: Hyaluronic acid filler; I.U.: International Unit
Source: Statistical output Table 3.4.1.1.2

Table 26: Unexpected AEs per dose and HA filler

	Allergan HA filler		**Merz HA filler**		**Galderma HA filler**		**No information about filler provided**	
	n_e	**n**	**n_e**	**n**	**n_e**	**n**	**n_e**	**n**
Total subjects		18 (100%)		19 (100%)		19 (100%)		28 (100%)
Hylase Dessau 75 I.U.	-	-	1	1 (5.3%)	-	-	-	-
Hylase Dessau 50 I.U.	1	1 (5.6%)	-	-	-	-	-	-
Hylase Dessau 25 I.U.	-	-	-	-	-	-	-	-
Hylase Dessau 15 I.U.	-	-	-	-	-	-	-	-
No dose provided	-	-	-	-	-	-	-	-
Total	1	1 (5.6%)	1	1 (5.3%)	-	-	-	-

HA: Hyaluronic acid filler; I.U.: International Unit
Source: Statistical output Table 3.4.1.1.3

12.2.2 Display of Adverse Events

Refer to section 12.2.1. No further analysis of AEs was done.

12.2.3 Analysis of Adverse Events

Refer to section 12.2.1. No further analysis of AEs was done.

12.2.4 Listing of Adverse Events by Patient

All AEs are listed by patient in Appendix 17.2.

12.3 Deaths, other Serious Adverse Events, and other Significant Adverse Events

There were no deaths, other SAEs or significant AEs in this study.

12.3.1 Listing of Deaths, other Serious Adverse Events, and other Significant Adverse Events

12.3.1.1 *Deaths*

Not applicable

12.3.1.2 *Other Serious Adverse Events*

Not applicable

12.3.1.3 *Other Significant Adverse Events*

Not applicable

12.3.2 Narratives of Deaths, other Serious Adverse Events, and certain other Significant Adverse Events

Not applicable, narratives are not available.

12.3.3 Analysis and Discussion of Deaths, other Serious Adverse Events, and other Significant Adverse Events

Not applicable

12.4 Clinical Laboratory Evaluation

Not applicable.

12.5 Vital Signs, Physical Findings and other Observations related to Safety

12.5.1 Concomitant Medication

Concomitant medication used during the study is shown in Table 27.

Table 27: Concomitant medication

Concomitant medication	**n_e**	**n**
Total subjects		28 (100%)
ConMed in total (n_e) and total subjects with at least one ConMed (n)	9	7 (25.0%)
Asumate 20	1	1 (3.6%)

Candecor 16mg	1	1 (3.6%)
Clobetasolpropionat 0.05% + Octinidin 0.1% Creme	1	1 (3.6%)
Corvaton ret 8mg	1	1 (3.6%)
Jaydess Hormonspirale	1	1 (3.6%)
Jubrele Pille	1	1 (3.6%)
L-Thyroxin 100 µg	1	1 (3.6%)
Ramipril 10mg 2xtgl	1	1 (3.6%)
Venlafaxin Aristo Retard 150 mg	1	1 (3.6%)

12.6 Safety Conclusions

All subjects received their injections according to the protocol but in 2 subjects the randomization scheme was not correctly applied (1 subject in the Allergan HA filler group and 1 subject in the Galderma HA filler group).

In the event that the dose was not sufficient to treat the overcorrection, additional Hylase® "Dessau" injections were necessary. This was the case for 17 subjects who received a total of 48 additional injections.

In the PP population, there was a swelling in eight subjects at V6, and the follow-up injections were made exclusively at the injection sites that had been treated with saline solution.

There were 27 events in total in 5 subjects (17.9%); the remaining subjects had no AE. There was one subject that experienced 16 AEs, one subject had 7 AEs, one subject had 2 AEs, and 2 subjects reported one AE each.

All AEs were of mild intensity; there was no SAE during the study.

Seventeen AEs were assessed as being related to the injection of Hylase "Dessau". Those were injection site pain (n=8), peripheral swelling (n=2), application site hematoma (n=1), erythema (reddening, n=4), nerve injury (n=1), and pain in extremity (n=1). The remaining AEs occurred after injection of the HA fillers or no assessment of causality was done. Two AEs out of those17 AEs assessed as being related to the injection of Hylase "Dessau" were not suspected (Stats output Table 3.2.1.6).

Five AEs that occurred after injection of Hylase "Dessau" were treated pharmaceutically, in 13 AEs there was no intervention, in 1 AEs treatment was not specified, and in 1 AE information related to treatment was missing.

All AEs were present in females, no male experienced an AE.

Concomitant medication was used by 9 subjects.

Taken together, the safety profile of Hylase "Dessau" can be considered as very good.

13. Discussion and Overall Conclusions

13.1 Key results

It was the primary objective of this study to determine a tolerable and effective dose of Hylase® "Dessau" (bovine hyaluronidase) as an antidote for the treatment of overcorrections by HA fillers based on the treatment of artificially induced overinjections on the back of the hands.

One or 2 day(s) after nodule induction with a HA filler a Hylase dose should be determined, that achieved complete volume reduction, defined by a palpation result of 0 or 1 and ultrasound after 24 hours.

Due to the premature end of recruitment, the planned number of subjects could not be reached. The number of study participants that could be enrolled in the study was too low to significantly exceed the pre-defined 80% of responders in case of 90% response that was originally used to calculate the sample size. Therefore, the study had not sufficient power to meet its primary objective for the assumed results.

Twenty-eight subjects were injected with 0.5 mL aliquots of HA fillers (Allergan HA filler, Merz HA filler, and Galderma HA filler) into each hand. All injections were done according to the protocol as well as to the randomization scheme.

All analyses were done in the ITT population as well as in the PP population. In general, results in both populations were similar, with the exception of follow-up injections required. In the ITT population, follow-up injections were required in a total of 17 subjects at V6, with about half of the injection sites being those treated with saline solution.

In the PP population, follow-up injections were required in eight subjects at V6, and the follow-up injections were made exclusively at the injection sites that had been treated with saline solution. Therefore, in the PP population, no follow-up injection was necessary when injection sites had been treated with Hylase.

Therefore, even if the assumed response rate of 90% had been achieved, no significant exceeding of the above-mentioned 80% was possible. Within the hierarchical test procedure the lower dosages of Hylase® "Dessau" could not be tested under these assumptions.

13.1.1 Effectiveness:

According to the SmPC, Hylase “Dessau” is indicated in the field of ophthalmology in combination with local analgesics for surgical interventions. Approval for certain indications is based on the ability of hyaluronidases to enhance the distribution and dispersion of other injected substances. However, In the USA Hyaluronidases have been on the market since 1948, but there is no consensus on the indications and treatment protocols for the use of hyaluronidases, and there are no standardized guidelines in place (Cohen et al, 2015).

To address this the main objective of this study was to find a suitable dose of Hylase® "Dessau" to treat overcorrections caused by HA fillers. Response was defined by a palpation result of 0 or 1 and a volume reduction >90% determined by ultrasound.

As already mentioned the primary objective of this study could not be met due to the premature termination of recruitment. Thus, focus had further been on explorative results for all Hylase doses.

Treatment responses were analyzed descriptively as well as with hierarchical and sensitivity analyses. Furthermore, a sensitivity analysis due to the premature recruitment stop were made.

A higher responder rate was observed (13 or 11 responders out of 16) when using a Hylase dose of 50 I.U (Allergan HA filler or Galderma HA filler). Using the Merz HA Filler there were 13 responders out of 16 with a Hylase dose of 75 I.U.

In the hierarchical analysis the response after 24 hours per HA filler was assessed. No dose of Hylase „Dessau“ achieved a statistically significant result. Their lower limit of the respective 95% CI was in no case above 80%, so that the hierarchical test procedure was terminated after this first dose and all other Hylase® "Dessau" doses were also not considered significant.

However, it could be demonstrated clearly, that with each filler used the response was considerably better compared to the saline control with a response rate of 75.0%, 81.3% and 56.3% vs 6.3%, 0% and 18.8%, respectively.

A sensitivity analysis was performed to determine whether a similar response rate per dose - independently of the filler - could be achieved and thus a better power was possible. As each subject was treated with two HA fillers, dependent data are available for each dose. The generalised mixed model, used for the filler-independent estimation of the response rate 24 hours after Hylase injection resulted in response rates of 71.3%, 73.0%, 60.1% and 51.5% corresponding to Hylase® „Dessau“ concentrations of 75, 50, 25 and 15 I.U. Also with this analysis no response rate >80% could be achieved.

A response rate of >80 was also used as the basis for planning the sample size. Since the planned number of subjects could not be reached no significant exceeding of the above-mentioned 80% was possible, even if the assumed response rate of 90% had been achieved, Within the hierarchical test procedure the lower dosages of Hylase® "Dessau" could not be tested.

In order to be able to discuss the results of all dosages, the response rates including their 95% confidence intervals were given exploratively for all HA fillers and dosages. This was done for the time crucial for the primary endpoint (V5) as well as for V6. The highest response rate after 24 h was achieved with Allergan HA-Filler Volume using Hylase® "Dessau" 50 I.U., and with Merz HA-Filler Volume using Hylase® "Dessau" 75 I.U. (81.3% [62.1%; 100%]). At visit 6, Galderma HA-Filler Restylane Volyme achieved a response in all subjects with Hylase® "Dessau" 75 I.U. as well as with Hylase® "Dessau" 15 I.U.

13.1.2 Safety:

The safety analysis population for this analysis also consisted of 28 patients. There were 27 events in total in 5 subjects (17.9%); the remaining subjects had no AE. In almost all patients the AEs were assessed as ‘mild’ or no assessment of severity was performed (in 7 out of 27 events). There was no SAE in this study.

20 AEs were reported after the initial treatment with Hylase® “Dessau” of which 17 were assessed as related to the study drug (ADRs). The following ADRs were reported: injection site pain (n=8), peripheral swelling (n=2), application site haematoma (n=1), erythema (n=4), Ulnar nerve injury (n=1), and pain in extremity (n=1). The reported dizziness was assessed as not related to the study drug and two AEs were assigned to the injection of 0.9 % NaCl.

Most of the 17 ADRs were expected for Hylase® “Dessau” 150 I.U., only two (ulnar nerve injury and application site haematoma) were unexpected.

Moreover, five out of the 27 AEs occurred directly after injection of the HA fillers and two AEs (injection site pain) were reported after follow up injection which were also related to Hylase “Dessau”.

All AEs were present in females, no male experienced an AE, and all AEs were documented exclusively at study site 1.

Concomitant medication was used by 9 subjects.

Taken together, the injection of Hylase “Dessau” 150 I.U. was safe and well tolerated.

13.2 Limitations

There is limited generalizability due to the small sample size (n=28) caused by the premature stop of recruitment.

The results may have a small potential for bias because of missing data (n=4 subjects) and delayed visits.

Furthermore, injections were only performed in the back of the hands of healthy volunteers, therefore it has to be questioned whether the data collected in this study are completely transferable to other body regions or to patients.

13.3 Generalizability

During data collection and analyses, much attention and efforts were made to ensure inclusion and exclusion criteria were met and data quality was high.

Regular data checks by monitoring were performed (source data validation). The recorded parameters seemed to be sufficient and the assessments performed and the duration of the observation phase was considered appropriate to meet the objectives of this analysis.

The documentation of the Hylase “Dessau” treatment with 28 patients provides a valuable insight into the effectiveness and safety of the test drug.

14. Conclusion

This study was performed to find an effective and safe dose of Hylase “Dessau” to treat overinjections caused by HA fillers. The study aim was not met, in part due to the premature termination of recruitment, however, there was a large proportion of responders in each group (13 or 11 responders out of 16) when using a Hylase dose of 50 I.U or 75 I.U.

With each filler used, the response was considerably better compared to the saline control with a response rate of 75.0%, 81.3% and 56.3% vs 6.3%, 0% and 18.8%, respectively.

A similar response rate per dose - independently of the filler - could be achieved applying a sensitivity analysis.

During this clinical study (from randomization to database closure) 27 AEs (17 ADRs after initial treatment, 2 ADRs after follow up injection, no SAE) were developed by 5 out

of the 28 enrolled subjects. The ADRs were of mild intensity and only two ADRs were unexpected.

In summary, Hylase® “Dessau” was safe and well tolerated in the treatment of artificial HA filler overcorrection. Hylase® “Dessau” proved as a safe drug.

Overall, no dose effect could be observed but all doses showed the desired effect achieving a volume reduction of at least 80.0% whereas after placebo injection a reduction of approximately 20.0% only was noted. It has to be taken into account that the low number of participants biased the results, so that further investigation including more subjects are warranted to establish statistical significance.

15. Tables, Figures and Graphs Referred to but not Included in the Text

Not applicable

16. Reference List

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17. Appendices

17.1 Study Information

17.1.1 Protocol and protocol amendments

17.1.2 Sample Case Report Form (unique pages only)

17.1.3 List of ECs or IRBs (plus the name of the committee chair if required by the regulatory authority) - representative written information for patients and sample consent forms

17.1.4 List and description of investigators and other important participants in the study, including brief (1 page) CVs or equivalent summaries of training and experience relevant to the performance of the clinical study

17.1.5 Signatures of principal or coordinating investigator(s) or sponsor's responsible medical offer, depending on the regulatory authority´s requirement

17.1.6 Listing of patients receiving test drug(s)/investigational product(s) from specific batches, where more than one batch was used

17.1.7 Randomization scheme and codes (patient identification and treatment assigned)

17.1.8 Audit certificates (if available) and Monitoring plan

Monitoring plan Version 1.0

17.1.9 Documentation of statistical methods

Not applicable

17.1.10 Documentation of inter-laboratory standardization methods and quality assurance procedures if used

Not applicable

17.1.11 Publications based on the study

Not applicable

17.1.12 Important publications referenced in the report

Not applicable

17.2 Patient Data Listings

17.2.1 Discontinued patients

Not applicable

17.2.2 Protocol deviations

Statistical output listing 4.1.5

17.2.3 Patients excluded from the efficacy analysis

Not applicable

17.2.4 Demographic data

Statistical output listing 4.1.10

17.2.5 Compliance and/or drug concentration data (if available)

Not applicable

17.2.6 Individual efficacy response data

Not applicable

17.2.7 Adverse event listings (each patient)

Statistical output listing 4.3.1 and 4.3.2

17.2.8 Listing of individual laboratory measurements by patient, when required by regulatory authorities

Not applicable

17.3 Case Report Forms

17.3.1 CRFs of deaths, other serious adverse events and withdrawals for AE

Not applicable

17.3.2 Other CRFs submitted

Not applicable

Principal Investigator and Sponsor's Representative Signatures

Study Title: Phase II pilot study: Determination of an effective and tolerable dose of Hylase® "Dessau" (bovine hyaluronidase) in the treatment of hyaluronic acid filler-induced overinjections by injection into defined hyaluronic acid filler-injected skin areas of healthy volunteers

Study Author: Sigrun Niemitz

I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study.

Principal Investigator

Dr. med. Tanja Fischer		Haut- und Lasercentrum Potsdam
Name:		Affiliation:
Siganture:		
Date: 29.11.2020		

Sponsor's Representative

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Date 03.12.2020		

Biostatistician

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Date 08.12.2020		