Protocol

Title: Pilot Study of Appropriate Dosing for Reducing and Removing Hyaluronic Acid Filler with Hyaluronidase: A Randomized Control Clinical Trial

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<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AE</td>
<td>Adverse Event</td>
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<td>FDA</td>
<td>Food and Drug Administration</td>
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<td>IRB</td>
<td>Institutional Review Board</td>
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<td>SAE</td>
<td>Serious Adverse Event</td>
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<td>WHO</td>
<td>World Health Organization</td>
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PROTOCOL SYNOPSIS

STUDY TITLE: Pilot Study of Appropriate Dosing for Reducing and Removing Hyaluronic Acid Filler with Hyaluronidase: A Randomized Control Clinical Trial

1. STUDY OBJECTIVES:
Hyaluronic acid fillers are versatile, FDA-approved, soft-tissue augmentation materials, the popularity of which derives in part from their ability to be reversed quickly by injection of hyaluronidase. Despite the standard availability of hyaluronidase for injection, there is an absence of systematic guidance on the appropriate dosage and usage of hyaluronidase for reduction or removal of hyaluronic acid implants.

The study objective is to ascertain the smallest dose of hyaluronidase sufficient to eliminate subcutaneous nodules of hyaluronic acid fillers.

2. STUDY DESIGN
This study is a pilot randomized control trial of 5 subjects, each with 6 treatment sites and 2 control sites. Subjects will be injected with injectable hyaluronic acid filler material (Juvederm Ultra XC, Allergan Inc, Riverside, CA; and Restylane-L, Medicis Corp., Scottsdale, AZ). Juvederm and Restylane are distributed in 0.8cc and 1.0cc vials respectively with plans to use about 2 vials of each per subject during the course of the study. Each of 5 subjects will have 8 sites marked along their bilateral upper inner (medial) arms (4 on each arm). Four 0.5cm marks will be placed using a surgical marker and spaced 5 cm apart along the midline of each upper inner arm. Injections will begin (i.e., be no closer than) 1.5 cm medial to each marked site. Each injection will deliver 0.4cc of the filler (Juvederm or Restylane) into the high subcutaneous tissue, at the dermal-subcutaneous junction, using the standard injection apparatus with 30-gauge needle. A fresh needle will be used for each injection. Injected sites in each patient will be approximately round (button-shaped). Study agent will be delivered from a single point of insertion. From this point on sites that received hyaluronic acid injections will be referred to as “buttons”. Doses of both Juvederm Ultra XC and Restylane-L used in this study are in the approved range as indicated on their respective package inserts.

For all subjects, each arm will be randomized during the baseline visit to receive either of the two formulations of hyaluronic acid (Juvederm Ultra XC or Restylane-L). Subsequently, during the week 1 visit, each arm will be separately block randomized to receive all of the following treatments randomly across the 4 buttons previously injected with filler:

1.5 units hyaluronidase (volume 0.1cc)
3 units hyaluronidase (volume 0.1cc)
9 units hyaluronidase (volume 0.1cc)
Normal saline treatment control (volume 0.1cc)

The above concentrations will be achieved using normal saline to dilute the stock hyaluronidase (200 units per 1.2cc) as necessary to achieve the desired number of units in 0.1cc of fluid. The same block randomization process will be utilized for the 4 sites on the opposite arm that received the other formulation of hyaluronic acid as well. The control sites will be placed in
order to make sure that the resolution of buttons can be attributed to hyaluronidase and not to the body’s own natural process of breaking down the substance (to help prove that hyaluronidase hastens this process and improves reabsorption compared to no treatment, i.e. control normal saline).

During week 2 and 3 visits, the same treatments will be applied to each button exactly as was applied during week 1 but will be discontinued once the individual button is no longer detectable by all of 3 detectability measures (described later in protocol) as determined by a blinded rater (dermatologist). There will be two follow-up visits after the last scheduled injection session. At the end of the study, should the control or any other buttons still be detectable using our detectability measures, we will inject these with hyaluronidase to decrease any residual cosmetic defects to the subject. Vitrase, and other brands of hyaluronidase, are used regularly by physicians to reverse any inadvertent effects caused by cosmetic filler. Currently there are no established dosages for this indication. This study will attempt to determine the best dosing for this indication starting with dosages already in use regularly.

The primary outcome measure will be the number of units injected per site until the individual button is no longer detectable by a blinded dermatologist rater by various detectability thresholds. Digital photographs will be taken before and after each injection. Additional photographs will be taken at 1 month and 4 months from the initial visit.

3. **BACKGROUND AND RATIONALE**
Hyaluronic acid fillers are versatile soft-tissue augmentation materials, the popularity of which derives in part from their ability to be reversed quickly by injection of hyaluronidase. Despite the safety and availability of hyaluronidase for injection, there is an absence of systematic guidance on the appropriate dosage and usage of hyaluronidase for reduction or removal of hyaluronic acid implants. The purpose of this study is to develop guidelines for injection of hyaluronidase by assessing the clinical effect of injections of different concentrations. Based on the study results, it may be possible to predict the minimal concentration and total unit dose of hyaluronidase to dissolve a standard amount of injected hyaluronic acid.

4. **STUDY POPULATION**

4.1 **Inclusion Criteria**
- Age 18 and over
- The subjects are in good health
- The subject has the willingness and the ability to understand and provide informed consent for the use of their tissue and communicate with the investigator

4.2 **Exclusion Criteria**
- Under 18 years of age
- Pregnancy or Lactation
- Subjects who are unable to understand the protocol or to give informed consent
- Subjects with mental illness
- Recent Accutane use in the past 6 months
- Subjects prone to hypertrophic and keloidal scarring
- Subjects with tattoos and/or scars on upper medial arms (the treatment area)
-Subjects with known hypersensitivity to hyaluronic acid

5. STUDY PROCEDURES
5.1 Patient Recruitment:
Subjects for the study will include normal volunteers. All or some of the following may be used after going through IRB approval for recruitment of volunteers: Written announcements posted in the Northwestern University Feinberg School of Medicine and Northwestern Memorial Hospital, mailed to patients of the Northwestern dermatology clinic and/or posted on the internet. Patients will be recruited via in-network Northwestern referrals and local doctors in the downtown Chicago area.

5.2 Screening, Randomization, and Hyaluronic Acid Injection Visit:
- Screening: inclusion/exclusion criteria along with medical history will be discussed and if subject is eligible and willing, an informed consent form will be signed.
- Photographs: After consent form signed, subject will have upper, inner arms photographed as a pre-treatment baseline.
- Marking: Each subject will have 8 sites marked along their bilateral upper inner (medial) arms (4 on each arm). Four 0.5cm marks will be placed using a surgical marker and spaced 5cm apart along the midline of each upper inner arm.
- Button placement (Hyaluronic acid injections): For each subject, each arm will be block randomized to receive one of two brands of hyaluronic acid filler (Juvederm Ultra XC, Allergan Inc, Riverside, CA; and Restylane-L, Medicis Corp., Scottsdale, AZ). Once the formulation for each arm is determined via randomization, each arm will receive four buttons of the assigned formulation of filler (volume of 0.4cc each). The injections will be placed no closer than 1.5cm medial to each marked site. Each injection will consist or 0.4cc of the appropriate formulation of filler into the high subcutaneous tissue, at the dermal-subcutaneous junction, using the standard injection apparatus with 30-gauge needle. A fresh needle will be used for each injection. Injected sites in each patient will be approximately round, button-shaped, with the study agent delivered from a single point of insertion.
- Photographs will be obtained again after injections are complete.

5.3 Hyaluronidase (Vitrase) Injections (Weeks 1, 2, 3):
- Before the hyaluronidase injections at each visit, a blinded dermatologist will rate the detectability of each button of hyaluronic acid on a 5-point scale for each of the following detectability measures: “Visual detection”, “light palpability” and “firm palpability” (see outcome measures).
- Patients will self-report detectability of buttons verbally and this information will be recorded
- Photographs will be obtained of the injected sites before and after the injections.
- During the week 1 visit, each arm will then be separately block randomized to receive all of the following treatments randomly across the 4 buttons previously injected with filler:

   1.5 units hyaluronidase (volume 0.1cc)
   3 units hyaluronidase (volume 0.1cc)
   9 units hyaluronidase (volume 0.1cc)
   Normal saline treatment control (volume 0.1cc)
The above concentrations will be achieved using normal saline to dilute the stock hyaluronidase (200 units per 1.2cc) as necessary to achieve the desired number of units in 0.1cc of fluid. The same block randomization process will be utilized for the 4 sites on the opposite arm that received the other formulation of hyaluronic acid as well. During week 2 and 3 visits, the same treatments will be applied to each button exactly as was applied during week 1 but will be discontinued once the individual button is no longer detectable by all of 3 detectability measures (described later in protocol) as determined by a blinded rater (dermatologist). A luer lock 1cc syringe with 30-gauge needle will be used to inject the treatment sites. To avoid imprecise injection due to needle hub deadspace, 0.1cc injections will be performed such that total syringe volume is greater than the amount required and decreases by 0.1cc with each injection, and the remainder will be discarded.

5.4 Follow-up office visit #1 (Week 4):
-Subjects will return for follow-up 1 week after the 3rd and last hyaluronidase injection session.
-Injection sites will be photographed.
-Detectability on the three measures for each of the 8 injection sites will be measured by the blinded rater, and reported by the patient.

5.5 Follow-up office visit #2 (4 Months):
-Subjects will return for a follow-up 3 months after the last follow-up visit.
-Injection sites will be photographed.
-Detectability on the three measures for each of the 8 injection sites will be measured by the blinded dermatologist raters and reported by the subject using a survey (Appendix I).
-At this visit, if any previous injection sites are still detectable (using the 3 measures described previously) then we will inject hyaluronidase again to ensure a good cosmetic outcome for the subject at the end of the study. This includes control buttons.

5.6 Data Collection and Reporting:
Primary Outcome Measures
The primary outcome measure will be the number of units injected per site until the individual button is no longer detectable by a blinded dermatologist rater by each of three detectability measures. Detectability measures include “visual detection,” “light palpability” and “firm palpability.”

Visual detection: Defined in this study as deviation from smoothness or visible bulge when examining the button from the side, both with and without side lighting and with and without 3-5x magnification with a hand-held magnifier.

Light Palpability: Defined in this study as detection of the button upon gentle movement of the dominant index finger fingertip tip across the button.

Firm palpability: Defined in this study as detection of a papule, nodule, or other skin mass (not related to normal skin or underlying skin pathology) with increasing pressure from light touch to firm pressure while moving the dominant index finger fingertip tip across the button.
Detectability (each measure separately) will be measured on a 5-point scale as follows (0-4):
   0 = Undetectable
   1 = Faintly perceptible
   2 = Mildly perceptible
   3 = Moderately perceptible
   4 = Very perceptible

For each site, and for each experimental condition, the mean number of units of hyaluronidase required from the time of button placement to loss of detectability by each of the three measures will be computed. Additionally, the number of units per 1-unit change in detectability measures will be computed.

Secondary Outcome Measures
Secondary outcome measures will be the same statistics computed based on patient self-report of detectability.

The occurrence and extent of any adverse events (AE) during the entire duration of the study will be noted as well. This includes if the subject contacts the investigators on days that are not visit days.

6. DATA DISCLOSURE AND SUBJECT CONFIDENTIALITY
Subject medical information obtained as a result of this study is considered confidential and disclosure to third parties other than the principal investigator and the co-investigators is prohibited. All reports and communications relating to subjects in this study will identify each subject only by their initials and study identification number. Data generated as a result of this study are available to inspection on request by Food and Drug Administration or other government regulatory agency auditors, and the Northwestern University Institutional Review Board (IRB).

Subject identity will be protected through use of a coded list of identifiers which will be maintained separately from the data set. Source documents and CRFs are kept in a secured area (in a locked cabinet in a locked room) and all electronic data is password protected so that only authorized personnel can have access. Photographs will be labeled only by subject initials and identification number. All photos will be stored on a password protected server accessed by a password protected computer.

7. EFFICACY ASSESSMENT
Efficacy Assessment will be done based on the primary and secondary outcome measures mentioned above.

8. RISKS AND BENEFITS
8.1 Risks
Hyaluronidase (Vitrase) injections: Possible risks include lumpy surface after injection, temporary skin discoloration, infection, bleeding or bruising, and swelling at the injection site. Pruritis or erythema at injection site can also occur. Allergic reactions (urticaria, angioedema) have been reported in <0.1% of patients receiving hyaluronidase in higher dosages and for separate indications.
Hyaluronic acid injections: Possible risks include lumpy surface after injection, temporary skin discoloration, infection, bleeding or bruising, and swelling at the injection site.

Photographs: There is a possibility that you will be able to be identified from the photographs that will be taken for the study. This risk is minimal given the area to be photographed and the fact that people with large tattoos, scars or other identifying marks in the area being photographed will be excluded from this study.

8.2 Benefits
This study may produce guidelines on the dosage of hyaluronidase necessary to reduce and remove hyaluronic acid from the skin.

8.3 Adverse Event Reporting
Any adverse events will be noted and recorded.

9. STATISTICAL CONSIDERATIONS
Statistical analysis will be performed after the completion of the study. Analysis will be done on de-identified data by Emir Veledar, PhD. The units required for elimination of detectability and the units required for a decrease in detectability by one unit will be compared for the three concentrations of hyaluronidase used. Subgroup analysis will be used to investigate any differences associated with the type of hyaluronic acid preparation. For 10 buttons injected with 9 units hyaluronidase each we can expect average detectability to drop from 4 (Very perceptible) to 0 to 0.5; for buttons injected with 3 units, average detectability after first injection can be expected to be 1 to 1.5 and for buttons injected with 1.5 units average detectability is expected to be 2 to 2.5. With 10 buttons per each treatment and placebo and alpha level of 0.05 we will have 99%, 98% and 88% power to find expected differences between levels of treatment and placebo, and 98% and 88% power to find significant dose effect.

10. STUDY SITE
Northwestern Medical Facility Foundation Dermatology clinic
676 North St. Clair Street, 16th Floor
Northwestern University Feinberg School of Medicine, Chicago, Illinois 60611

11. ETHICAL CONSIDERATIONS
11.1 Human Subjects Protection
A periodic review must be submitted to the IRB at least once a year. The IRB must be notified of completion of the study. After study completion or termination, a final report must be provided to close the study. The investigator must maintain an accurate and complete record of all submissions made to the IRB, including a list of all reports and documents submitted. Adverse events that are reported to the FDA and IND Safety Reports must be submitted promptly to the IRB per IRB guidelines. At least once per year, the IRB must review and give written approval in order to continue the study. This trial will be conducted in accordance with Good Clinical Practices and the Declaration of Helsinki.
11.2 Consent Form
Prior to study entry, a signed informed consent must be obtained from the subject (age 18 and over). A copy of the subject’s signed consent form must be retained in the study file as well as given to the patient.

11.3 Protocol Amendments
All changes must be submitted to the IRB. Protocol modifications that impact subject safety or the validity of the study must be approved by the IRB and submitted to the FDA before initiation.

11.4 Retention of Records
Food and Drug Administration and Good Clinical Practice guidelines require that an Investigator retain subject identification codes, subject files, and source data for the maximum period of time permitted by the hospital, institution, or private practice, but not less than 15 years after the completion or discontinuation of the trial.

11.5 Use of Information and Publication
The Principal Investigator, sub-investigators may publish the results of this study in conjunction with appropriate scientific and medical personnel.
12. REFERENCES


Appendix I: Subject Questionnaire

<table>
<thead>
<tr>
<th>Subject#</th>
<th>Date</th>
<th>Visit #</th>
<th>Investigator</th>
<th>Initials</th>
</tr>
</thead>
</table>

1. Please rate each of the injection “buttons” (numbered 1-8 on the stick-person diagram on the last page of this questionnaire) on a scale of 0 to 4 as follows in terms of detecting any bumpiness in these areas by _looking_ at the areas only.

<table>
<thead>
<tr>
<th>Button</th>
<th>0 = Undetectable</th>
<th>1 = Faintly Perceptible</th>
<th>2 = Mildly Perceptible</th>
<th>3 = Moderately Perceptible</th>
<th>4 = Very Perceptible</th>
</tr>
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<tbody>
<tr>
<td>1 (R)</td>
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<td>2 (R)</td>
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<td>8 (L)</td>
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2. Please rate each of the injection “buttons” (numbered 1-8 on the stick-person on the last page of this questionnaire) on a scale of 0 to 4 as follows in terms of detecting any bumpiness at these areas by running the finger-tip of your dominant (the hand you write with) index finger _lightly_ and _firmly_ over the areas.

<table>
<thead>
<tr>
<th>Button</th>
<th>0 = Undetectable</th>
<th>1 = Faintly Perceptible</th>
<th>2 = Mildly Perceptible</th>
<th>3 = Moderately Perceptible</th>
<th>4 = Very Perceptible</th>
</tr>
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<tbody>
<tr>
<td>1 (R)</td>
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<td>2 (R)</td>
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<td>3 (R)</td>
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<td>4 (R)</td>
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<td>6 (L)</td>
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<td>7 (L)</td>
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<td>8 (L)</td>
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Figure 1: Diagram of Button Numbering (front of arms)