CLINICAL TRIAL PROTOCOL

STUDY NAME:
Pilot Study to Identify the Impact of OnabotulinumtoxinA (BOTOX®) on Patient Perceived Stress

DESIGN:
Prospective, single-center, randomized, proof-of-concept study

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Funded by: Center for Advanced Facial Plastic Surgery

Draft Number: 5.0
Version Date: 8 December 2024

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TABLE OF CONTENTS

STATEMENT OF COMPLIANCE

The trial shall be carried out in accordance with ICH-GCP and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812).

Investigators and clinical trial site staff who are responsible for the conduct, management, and/or oversight of this trial or any part there-of have completed Human Subjects Protection and ICH-GCP Training.

1 PROTOCOL SUMMARY

1.2 SCHEMA

1.3 SCHEDULE OF ACTIVITIES (SOA)

VISITS	Screening	Treatment	Follow-ups			End-of-Study
	--	1	2	3	4	5
	Day 0	Day 1 ± 3 Days	Week 2 ± 3 Days	Week 4 ± Days	Week 8 ± 5 Days	Week 12 ± 5 Days
Informed Consent *						
Inclusion Criteria *						
Exclusion Criteria *						
Demographics *						
Comprehensive Medical/Surgical History *						
Labs (CBC, CMP, urine hCG)						
Height/Weight/BMI						
Vital Signs						
Pertinent Physical Exam						
Randomization[2]						
BOTOX® administration/placebo (dosing)						
PSS-10 Questionnaire (patient reported outcome) *						
Concomitant Medication Review *						
Adverse Events						
Interval Medical/Surgical History *						

Notations:

1. PSS-10 **is to be conducted prior to dosing.**

2. Treatment allocation and r**andomization is to be conducted prior to study initiation.**

**Shall be conducted in written, visual, and/or verbal format based on participant preference(s) by qualified personnel*

2 INTRODUCTION

2.1 BACKGROUND

2.1.1 IMPACT OF MENTAL STRESS ON PHYSICAL HEALTH

Perceived stress is shaped by an individual's subjective interpretation of a stressor, meaning two people exposed to the same stressor may report different levels of stress. Therefore, psychological interpretation of a stressor is responsible for the significance of associated negative impact on health in a variety of domains. Increased psychological stress has been associated with effects on the immune system, including evidence of increased risk of developing a cold after rhinovirus exposure, faster progression to AIDS after HIV infection, and reactivation of herpes simplex virus infection.[1] Maternal stress during the prenatal period was found to be positively associated with infant illness.[2] These are all thought to be related to decreased cellular and humoral immune responses when the body experiences chronic stress. This is also one of the mechanisms by which stress seems to have an impact on malignancy.

By undermining our body's natural defense mechanisms, psychological stress can increase risk of cancer and affect the progression, relapse, and treatment of existing malignancies.[3] Stress has been associated with weakened cell cytotoxicity in combating human papillomavirus-related tumors such as cervical cancer, allowing reactivation and re-expression of Epstein-Barr virus (another oncogenic virus), and creating an environment conducive for tumor cell metastasis by compromising the blood brain barrier, promoting vascular endothelial growth factor expression, and compromising Natural Killer cell cytotoxicity.[4] Patients with post-traumatic stress disorder (PTSD) have been shown to have increased risk of developing conditions such as diabetes mellitus, metabolic syndrome, obesity, autoimmune disease, and cardiovascular disease.[5,6]

Stress' effects on the body are still under study and involve a complex interplay between the endocrine, nervous, and immune systems. Whether existing or anticipated, psychological stress have shown to elicit physiological responses that involve the neuroendocrine system. Activation of the hypothalamic-pituitary-adrenal (HPA) and sympathetic-adrenal-medullary (SAM) axes can indce rapid catecholamine and glucocorticoid (e.g. cortisol) release to provide an evolutionary advantage in the setting of perceived threats that affects the cardiovascular and respiratory systems and the immune system, respectively.[1,3,7] Furthermore, repeated or prolonged stressors and unresolved exposures to traumatic events can intensify individuals' perception of stress to disrupt these highly regulated neuroendocrine feedback mechanisms. Long-term persistence of elevated stress-hormones have been associated with systemic inflammation (i.e. PTSD patients), poor sleep, increased rates of cardiovascular deaths, and poorer overall health .[5,8-10] Stress-related sleep deprivation further impacts hormonal dysregulation and misestimation of energy stores, which have demonstrated blunting of diurnal (sleep-wake) cortisol levels.[12]

The effect of stress on metabolism and weight-gain is even more complex. The HPA axis is one mechanism through which stress seems to impact metabolism. Cortisol levels have been associated with increased body mass index (BMI), waist-to-hip ratio, fasting glucose, insulin, blood pressure, triglycerides, and cholesterol. Glucocorticoids also seem to have an impact on the body's appetite regulating hormones, including leptin, ghrelin, and neuropeptide-Y, although the exact relationship is unknown.[11] Stress also affects the reward and motivation neural circuitry. Specifically, stress activates

reward-seeking dopaminergic pathways and thus can lead to compulsive food seeking, even in those who are already satiated and overweight. High levels of stress can affect the prefrontal and limbic brain regions, which may explain why increased stress is also associated with increased risk for alcohol, smoking, and other drug abuse.[13]

A final way in which stress can affect the human body is via action on telomeres. Telomeres are found on the ends of chromosomes and are designed to protect DNA during cellular replication. As cells divide, telomeres naturally shorten, but the enzyme telomerase can add repeat sequences to maintain telomere length. Studies of patients with mutations in telomere maintenance have demonstrated that shortened telomeres can lead to pathologies and premature aging processes such as loss of bone marrow progenitor cells, pulmonary fibrosis, liver cirrhosis, hair greying, changes in skin pigmentation, and overall increased human all-cause mortality.[14] Stress seems to play a role in causing decreased telomerase activity and shorter telomeres.[1,15] For example, one study found that increased maternal stress led to newborns with shorter telomeres.[16]

In summary, while not necessarily definitive, psychological stress has been associated with a variety of alterations in physical health, including impacts on immune, cardiovascular, and metabolic function. Stress thus predisposes people to multiple conditions including diabetes mellitus, obesity, autoimmune disease, and malignancy. As we continue to learn more about how stress can negatively impact the human body, it is critical to develop effective methods for managing and treating stress to mitigate these untoward effects. As we continue to learn more about how stress can negatively impact the human body, it is critical to develop effective methods for managing and treating stress to mitigate these untoward effects.

2.1.2 FACIAL FEEDBACK

The facial feedback hypothesis, which was described as far back as the 1870s by Charles Darwin, suggests that facial muscle expression can affect the psychological experience of said emotion. This effect has been shown in a variety of studies, including one by Kraft and Pressman, who had volunteers sorted into groups with either neutral or smiling expressions who were then subjected to stressful tasks including a cognitive task and a physical task. Volunteers who were in the smiling group demonstrated a lower heart rate during the post-task recovery period.[17] Another study by Richesin et al. found that when volunteers were asked to assume a “game face” expression for a stressing task, participants who had this expression were able to complete more of a cognitive puzzle task and had decreased skin conductance (a surrogate marker for sympathetic nervous system activation) compared to the control group.[18] Both studies suggest that specific facial expressions can impact the body’s physiologic stress response.

Recently, more studies have come to light regarding the use of botulinum toxin (BTX) to facial musculature for depression. Lewis and Bowler studied the injection of BTX to the forehead and glabella musculature and had patients fill out an Irritability-Depression-Anxiety Scale questionnaire. Patients in the treatment group scored lower in depression and anxiety but not irritability compared to patients seeking other cosmetic treatment.[19] Wollmer et al. performed a double-blinded randomized control trial comparing patients injected with BTX vs placebo to the face and found that in the treatment group, there was greater reduction in the Hamilton Depression Rating Scale that was statistically significant.[20]

Qian et al.'s systematic review and meta-analysis study of the use of BTX injections to the face for Major Depressive Disorder found that across 417 patients treated, there was overall a positive effect in the use of BTX reducing depression with no serious adverse events reported.[21] These findings suggest that BTX injections to facial musculature may have a role in treating depressive mood. Interestingly, in Lewis and Bowler's study, volunteers were also asked to rate their attractiveness before and after treatment, and there was no statistically significant difference between the groups that received BTX to the forehead and glabella versus those that received other therapies, suggesting that the improvement in depression and anxiety seen was not at all related to patient self-perception.[19]

It remains unclear what the mechanism is behind these findings. Hennenlotter et al. performed fMRI of volunteers during attempted imitation and observation of angry and sad expressions before and after BTX injections to the corrugator supercilii (a muscle in the glabella responsible for brow depression and medialization). They found that activation of the left amygdala was significantly reduced in the BTX-treated group compared to the control group during imitation of angry expressions. Additionally, they found reduced connectivity between the amygdala and dorsolateral pons (an area of the brain that participates in control of autonomic arousal) in the BTX subjects.[22] Li et al. suggests that the influence on mood by BTX may be related to levels of monoamines. They cite animal studies that showed that facial injections with BTX led to increased serotonin in the hippocampus, hypothalamus, and prefrontal cortex in chronically stressed mice, and that injections of BTX to whiskers resulted in increased norepinephrine in the striatum.[23] Both serotonin and norepinephrine are well-established neurotransmitters that are pharmacologically increased during treatment of depressive and anxiety disorders, and thus it is interesting to note that BTX injections resulted in increased levels in areas of the brain specifically associated with mood, emotion, and impulse regulation. Li et al. also note that it is possible that the mere prevention of facial expression associated with negative emotion via BTX injections may result in improved social connectivity, which in and of itself may improve mood.[23]

2.2 STUDY RATIONALE

Botulinum toxin (BTX) injections are one of the most popular and well-established ways to treat and/or prevent external signs of facial aging. As described above, there may be a role for facial BTX injections in management of depression. However, there have been no studies that specifically address whether BTX treatment can treat stress. Stress is increasingly associated with deleterious effects on the human body, as described above. The purpose of this study is to determine if facial BTX injections can also decrease the psychological experience of stress.

There is an established feedback loop between chronic stress and certain muscular activity of the face. Li et al. described that there is bidirectional signaling between facial musculature and emotional centers of the brain.[23] The most well-studied of these relationships has been elucidated between corrugator supercilii activity. This is a muscle located in the glabella that causes medialization and depression of the brow. Its activity has been associated with negative emotions including sadness, anger, fear, and distress.[19,24]

In Qian et al.'s systematic review and meta-analysis, of the five randomized control trials that were ultimately analyzed that demonstrated that BTX injections were effective at reducing depression, four of

these studies specifically involved injections to the glabellar musculature.[21] This demonstrates the potent role that decreasing activity of muscle movement in this area has on at least depression. Other muscles that may be related to the stress response include the frontalis, which can be seen with expressions of fear and sadness, the masseter, which is instrumental in jaw clenching, and the depressor anguli oris, which causes frowning.

Anecdotally, in my years of practice treating thousands of patients with BTX, including those with complex facial movement disorders related to facial paralysis, I have noticed a dramatic reduction of stress response in patients who received injections in one or more of the specific sites of treatment. I have also observed a direct relationship between number of sites injected and the extent of the effect on stress.

My hypothesis is that all these expression muscles are involved in the macro- and micro-expressions of grimace, which is a non-specific tensing of various facial muscles and is related to distress, or mirroring of the distress in those around us. In the same vein, isolated muscle treatments that chemically dampens the patient's ability to activate the muscles of grimace, may be related to stress reduction by short-circuit the neurological feedback loop stimulates the stress experience.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

Risks related to treatment: redness, pain, tenderness, firmness, swelling, lumps, bumps, bruising, itching, discoloration, allergic reaction, inflammation at the site of injection, numbness, infection at site of injection, skin rash, headache, vision abnormalities, flu-like symptoms, nausea, dizziness.

There is also currently a risk for COVID-19 infection due to the office visit for treatment.

Needle-related pain and/or anxiety may result in immediate vasovagal responses (including syncope, hypotension), which may require appropriate medical therapy. Localized pain, infection, inflammation, tenderness, swelling, erythema, bruising/bleeding may be associated with the injection and can occur immediately but may last several days or longer after injection. Local weakness of the injected muscle(s) represents the expected pharmacological action of botulinum toxin. However, weakness of nearby muscles may also occur due to spread of toxin. Specifically in this region of injection, there is risk for eyelid and/or brow ptosis. This effect may last several months or longer but is transient.

2.3.2 KNOWN POTENTIAL BENEFITS

- Improvement of facial wrinkles
- Migraine or headache relief
- Moderate platysma bands

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To determine if patients who are treated with facial BOTOX injections have improved stress levels.	The primary endpoint shall be a change in the patient-reported and validated PSS-10 questionnaire between screening to week 4, week 8, and week 12.	Other treatments that have reduced stress levels can lead to an enhancement of health related quality of life with improved vitality and greater social functioning. We expect to see similar results with OnabotulinumtoxinA.

4 STUDY DESIGN

4.1 OVERALL DESIGN

Structure: This shall be a prospective, single center, randomized, single-masked, proof-of-concept study.

Patient Population: Patients between the age of 18 to 65 who are toxin naïve within the past year who are seeking BOTOX® treatment for aesthetic purposes or are interested in the particular study and demonstrate increased stress (defined as PSS-10 scores above 13). Patients with a recent history of prior use of neuromodulators, fillers, alcohol, illicit drug abuse, and/or psychiatric diagnosis shall be excluded to avoid possible confounding factors. Other inclusion and exclusion criteria exist (see Section 5). Pretreatment visit will include history and physical examination with BMI calculations and routine lab tests (complete blood count, metabolic panel, pregnancy test) to rule out metabolic disorders such as hypothyroidism to avoid undiagnosed and untreated pre-existing conditions that can influence outcomes.

Study treatment groups/dose regimen: Patients shall be randomized to either placebo or BOTOX® arm. The injection sites are the glabella, lateral canthal region, and forehead. Participants will receive the following units in both the treatment or placebo arm: 20 units over five injection sites to the glabella, 24 units over six injection sites to the lateral canthal region (three on each side), and 20 units over five injection sites to the forehead.

Primary Endpoint: The primary endpoint shall be to assess the efficacy of BOTOX® by assessing the patient-reported, validated PSS-10 scores from the first screening encounter to week 12.

Anticipated number of subjects: 20 subjects – as this is a proof-of-concept study, a sample size determination pre-hoc analysis was not done.

Duration (12 weeks participation for each patient following enrollment): Patients will come in for a single treatment of BOTOX or placebo following enrollment. They will then have a follow-up visit at week 2, week 4, and week 12. BMI calculations shall be performed to ensure no major weight fluctuations occurred during treatment as this may introduce bias into the study. Participants shall be complete a PSS-10 questionnaire at each visit, and any adverse effects or medication changes will also be noted. In addition to these three visits, participants will also have a remote check-in at week 8 during which they will fill out a PSS-10 questionnaire and provide any updates on their medical health.
Anticipated Duration of Participant Enrollment: Patient enrollment will take approximately 6 -12 months.

Hypothesized Outcome: Participants in the BOTOX® arm would report a decrease in perceived stress relative to the initial screening visit.

4.2 JUSTIFICATION FOR DOSE**

The dosing proposed for this investigator-initiated trial (IIT) has been previously used for other pivotal Allergan studies using BOTOX®. These doses have been shown to be safe and efficacious in reducing the activity of the facial muscles with a predictable reduction in muscle strength and dynamic movement.

There is an established feedback loop between chronic stress and certain muscular activity of the face. The most well-studied of these relationships has been elucidated between corrugator supercilii activity – an area that connotes "concern" or "anxiety" – and stress. A decreased ability to move the corrugator muscles can lead to a reduction in stress and anxiety. In my experience, other head and neck muscles such as the frontalis, orbicularis oculi, masseter, depressor anguli oris, platysma, occipitalis, and trapezius may also be involved in this cascade. In this study, we wanted to focus on established regions of the face that have been previously studied for cosmetic reasons as a preliminary approach to determine what, if any, effect facial injections with BOTOX have on stress levels.

A larger future study may involve treatment to other areas including the masseter, depressor anguli oris, platysma, occipitalis, trapezius, and other related muscles.

** *Since equivalent dose must be administered to all participants to reduce variability, we were able to demonstrate consistency by using specifically calculated dosages. Due to anatomical differences between genders, this study only includes female participants.*

4.3 END OF STUDY DEFINITION

A completed participant is one who has completed all phases of the study including the follow-up visits. The end of the study is defined as the last participant's last visit.

5.1 INCLUSION CRITERIA

1. Subjects capable of giving informed consent in the English language
2. Females 18-65 years of age
3. PSS-10 score ≥ 14 at screening. (For details, see Appendix 1)
4. Participants who have been toxin-naive for ≥1 year
5. Female subjects willing to minimize the risk of inducing pregnancy for the duration of clinical study
6. Subjects in good physical and mental health and not on any prescription psychiatric medications
7. Subjects willing to not undergo any other aesthetic or skin treatments for the duration of the study

5.2 EXCLUSION CRITERIA

TO BE ELIGIBLE, PATIENTS MUST NOT MEET ANY OF THE FOLLOWING CRITERIA:

1. Participants with history of facial (including periorbital) surgery within the last 12 months
2. Use of neuromodulators in the past ≤ 12 months

3. History or known alcohol and/or illicit drug abuse
4. Participants with psychiatric diagnosis
5. Body Mass Index (BMI) at Screening ≥ 30 kg/m2.
6. Participants with metabolic disorders (e.g., hypothyroidism and hyperparathyroidism)
7. Undiagnosed, unstable, or preexisting conditions that in the opinion of the investigators, would interfere with the course and conduct of the study. These include but are not limited to inflammatory disorders, diseases that affect muscles and/or nerves such as myasthenia gravis and Lambert-Eaton syndrome, high blood pressure, heart disease, and/or stroke that could result in a life-threatening response when treated with BOTOX or placebo.
8. Participant has any laboratory abnormality (at Screening) that, in the opinion of the investigators, is clinically significant, has not resolved at baseline and could jeopardize or would compromise the participant's ability to participate in this study.
9. Prior use of isotretinoin
10. Active skin disease or infection at or near injection sites
11. Active use of tobacco or nicotine products in any form via any route (e.g. inhalation, ingestion, absorption)
12. Chronic marijuana or tetrahydrocannabinol exposure
13. If the investigators feels that the subject is not a good candidate for the study
14. Females of childbearing potential not using a reliable means of contraception (see Appendix 2 for definition and acceptable methods of contraception).
15. Female subjects must not be breastfeeding
16. Any known previous hypersensitivity reactions to BOTOX
17. Current or recent (within therapeutic window) use of neuromuscular medications that may pose additional risk with treatment with BOTOX, such as but not limited to aminoglycosides and anticholinergic drugs

5.3 SCREEN FAILURES

A screening score of 13 or less on the PSS-10 shall disqualify participants from enrolling in the study, and shall be interpreted as screen failures.

5.4 STRATEGIES FOR RECRUITMENT AND RETENTION

Participants shall primarily be recruited using Web-based advertisements on social media platforms (e.g. Instagram, Facebook, LinkedIn) and/or websites. Only IRB approved advertisements shall be disseminated for participant recruitment purposes.

Recruits meeting study criteria will self-identify remotely and/or in-person, on enrollment, before signatures are rendered.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 DOSING AND ADMINISTRATION

Participants shall be randomized 3:1 to BOTOX® or placebo. Participants will receive a single treatment of BOTOX® or placebo as intramuscular (IM) injections into three facial muscles/regions. The recommended treatment dose is 64 Units administered IM using a sterile 30-gauge, 0.5-inch needle as follows: 20 units in the Glabella (includes the Procerus and Corrugator Supercilli muscles), 24 units in Orbicularis Oculi muscles (12 units to each side), and 20 units in Frontalis muscle.

6.1.2 PLACEBO

The placebo administered shall be sterile, non-preserved 0.9% Sodium Chloride injection, which is the same diluent used to prepare the treatment (see section 6.2.4 for details). A volume equivalent amount of saline shall be injected to the same areas in the placebo group.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

While the PI is ultimately responsible, study treatment accountability, reconciliation, and record maintenance (i.e. receipt, reconciliation and final disposition records) can be designated to the Pharmacist or other staff.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

OnabotulinumtoxinA (BOTOX®) is Botulinum Toxin Type A Purified Neurotoxin Complex manufactured by Allergan Pharmaceuticals Ireland, a subsidiary of Allergan, Inc., and is a sterile, vacuum-dried, purified botulinum toxin type A, produced from fermentation of Hall strain *Clostridium botulinum* type A grown in a medium containing casein hydrolysate, glucose, and yeast extract. It is purified from the culture solution by dialysis, with a series of acid precipitations into a complex consisting of the neurotoxin, and several accessory proteins. The complex is dissolved in sterile sodium chloride solution containing Albumin (Human) and is sterile filtered (0.2 microns) prior to filling and vacuum-drying.

BOTOX® is supplied in a single-use vial in the following sizes:
50 Units NDC 0023-3920-50
100 Units NDC 0023-1145-01
200 Units NDC 0023-3921-02

Vials of BOTOX® have a holographic film on the vial label that contains the name "Allergan" within horizontal lines of rainbow color. To see the hologram, rotate the vial back and forth between your fingers under a desk lamp or fluorescent light source. (Note: the holographic film on the label is absent in the date/lot area.) If you do not see the lines of rainbow color or the name "Allergan", do not use the product, and contact Allergan for additional information at 1-800-890-4345 from 7:00 AM to 3:00 PM Pacific Time.

6.2.3 PRODUCT STORAGE AND STABILITY

All study treatments shall be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labelled storage conditions, with access limited to the investigators and authorized site staff.

Unopened vials of BOTOX® should be stored in a refrigerator (2° to 8°C) for up to 36 months for the 50 Units and 100 Units vials or up to 24 months for the 200 Units vial. Do not use after the expiration date on the vial. Administer BOTOX® within 24 hours of reconstitution; during this period reconstituted BOTOX® should be stored in a refrigerator (2° to 8°C). Reconstituted BOTOX® should be clear, colorless, and free of particulate matter.

All vials, including expired vials or equipment used with the drug should be disposed of carefully, as is done with all medical waste.

6.2.4 PREPARATION

Only authorized site staff delegated by the PI may prepare, handle, supply or administer study treatment. BOTOX® is supplied in single-use 50 Units, 100 Units, and 200 Units vials. Prior to injection, reconstitute each vacuum-dried vial of BOTOX® with sterile, non-preserved 0.9% Sodium Chloride Injection USP. Draw up the proper amount of diluent in the appropriate size syringe (Dilution Table), and slowly inject the diluent into the vial. Discard the vial if a vacuum does not pull the diluent into the vial. Gently mix BOTOX® with the saline by rotating the vial. Record the date and time of reconstitution on the space on the label.

BOTOX® should be administered within 24 hours after reconstitution, during which the reconstituted BOTOX® should be stored in a refrigerator (2° to 8°C).

Diluent* Added to 50 Unit Vial	**Resulting Dose Units per 0.1 mL**	**Diluent* Added to 100 Unit Vial**	**Resulting Dose Units per 0.1 mL**	**Diluent* Added to 200 Unit Vial**	**Resulting Dose Units per 0.1 mL Vial**
1 mL	5 Units	1 mL	10 Units	1 mL	20 Units
1 mL	2.5 Units	2 mL	5 Units	2 mL	10 Units

4 mL	1.25 Units	4 mL	2.5 Units	4 mL	5 Units
8 mL	1.25 Units	8 mL	2.5 Units	10 mL	2 Units

*Preservative-free 0.9% Sodium Chloride Injection, USP Only

Note: These dilutions are calculated for an injection volume of 0.1 mL. A decrease or increase in the BOTOX® dose is also possible by administering a smaller or larger injection volume - from 0.05 mL (50% decrease in dose) to 0.15 mL (50% increase in dose).

An injection of BOTOX® is prepared by drawing into an appropriately sized sterile syringe an amount of the properly reconstituted toxin slightly greater than the intended dose. Air bubbles in the syringe barrel are expelled and the syringe is attached to an appropriate injection needle. Patency of the needle should be confirmed. A new, sterile needle and syringe should be used to aspirate BOTOX® from the vial on each occasion.

Reconstituted BOTOX® should be clear, colorless, and free of particulate matter. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration and whenever the solution and the container permit.

At the study testing center, the dilution of 2.5 mL of diluent into 100 units of BOTOX® shall be used. This is equivalent to 4 units of BOTOX in every 0.1 mL. Participants who are randomized into the treatment arm will receive 1.6 mL volume for a total of 64 units. Participants who are randomized into the placebo arm shall receive an equivalent volume (1.6 mL) of preservative-free 0.9% sodium chloride. The placebos shall be prepared, stored, and handled in a similar fashion as the treatment arm.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Participants shall be randomized 3:1 of BOTOX® or placebo (if there are a total of 20 participants, 15 shall receive treatment with BOTOX® and 5 to receive placebo).

Participants will be randomized using the Sealed Envelope platform by block randomization due to the small sample size. Likewise, treatment allocation shall be concealed and the materials shall be prepared (with exception of reconstitution) prior to study initiation by the co-investigator or other delegated staff member.

All injections (treatment and placebo) shall be performed only by the PI to minimize potential risks of bias. Statistical analysis shall be performed by the co-investigator or delegated staff member.

6.4 CONCOMITANT THERAPY

Any concomitant medications or supplements shall be documented in the study records and shall include the dosages, dates, number of times administered/consumed, as well as their indications.

7 STUDY INTERVENTION, DISCONTINUATION, AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Subjects may withdraw from the study at any time at their discretion. If the subject withdraws consent for future, the sponsor may use any data collected before the withdraw of consent. If subjects withdraw from the study after 4 weeks, we will utilize the most recent primary and secondary endpoint measures.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

If a participant is prematurely discontinued from investigational product(s), the PI shall make every effort to perform an Early Termination Visit.

7.3 LOST TO FOLLOW-UP

In cases where the participant does not return for the rescheduled visit or cannot be reached to reschedule the missed visit, the site should make every effort to regain contact with the participant (3 documented telephone calls and if necessary, a certified letter to the participant's last known mailing address) so that they can appropriately be withdrawn from the study with a primary reason of "Lost to Follow-up".

8 STUDY AVTIVITIES, ASSESSMENTS, AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

The primary endpoint of this proof-of-concept study is changes in stress level measured by the PSS-10, which was determined to be more reliable than the originally developed PSS-14 (see Appendix 1 for further details). The maximum score on the PSS-10 is 40, and the normative study population used to test the validity of this scale, as described in Appendix 1- citation 2, showed a mean PSS-10 score of 13.02. Thus, participants with screening scores greater than 13 suggest increased stress and will be included in the study. The questionnaire shall be administered at four time points during the study (see Section 1.3 for details). Decreased perceived stress would be evident with decreased PSS scores.

8.2 SAFETY AND OTHER ASSESSMENTS

Safety will be evaluated by assessment of clinical laboratory tests, physical examinations, vital signs measurements, at various time points during the study, and by the documentation of AEs.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether considered intervention-related or not (21 CFR 312.32 (a)).

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigators or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- Mild – Events require minimal or no treatment and do not interfere with the participant's daily activities.

- Moderate – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating.

  Of note, the term "severe" does not necessarily equate to "serious".

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) shall have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product shall always be suspect.

- Related – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.
- Not Related – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

8.3.3.3 EXPECTEDNESS

The PI and/or qualified Sub-/Co-Investigators shall be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review during QA.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study shall be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

Delegated study staff will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigators will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.3.5 ADVERSE EVENT REPORTING

Adverse events following use of BOTOX® should be reported to the Pharmacovigilance Department, Allergan Inc. (1-800-433-8871). Adverse events may also be reported to the U.S. Department of Health and Human Services (DHHS) Adverse Event Reporting System. Report forms and reporting requirement information can be obtained from Adverse Event Reporting System (AERS) through a toll-free number 1-800-822-7967.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

The study clinician will immediately report to the sponsor any serious adverse event, whether or not considered study intervention related, including those listed in the protocol or investigator brochure and shall include an assessment of whether there is a reasonable possibility that the study intervention caused the event. Study endpoints that are serious adverse events (e.g., all-cause mortality) shall be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis). In that case, the investigators shall immediately report the event to the sponsor.

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigators deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by the Data Coordinating Center (DCC)/study sponsor and should be provided as soon as possible.

The study sponsor will be responsible for notifying the FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsors initial receipt of the information. In addition, the sponsor shall notify FDA and all participating investigators in an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting.

8.3.7 REPORTING EVENTS TO PARTICIPANTS

If any mild or moderate adverse events occur, future participants in the study will be notified on their screening visit during the informed consent process of the risk for these adverse events. If any severe adverse events occur and are deemed to be related to the study intervention, all enrolled participants, regardless of whether they have completed the study, will be notified via phone of said event. If participants are unable to be reached, a HIPAA-compliant certified letter may be sent to the last known

mailing address. Attempts will be made by the PI to ensure the safety of patients including referrals for additional labs, specialist visits, and/or hospitalization.

8.3. 8 REPORTING OF PREGNANCY

Administration of BOTOX® is not recommended during pregnancy. There are no adequate and well-controlled studies of BOTOX® in pregnant women. When pregnant mice and rats were injected intramuscularly during the period of organogenesis, the developmental NOEL (No Observed Effect Level) of BOTOX® was 4 Units/kg. Higher doses (8 Units/kg or 16 Units/kg) were associated with reductions in fetal body weights and/or delayed ossification.

In a range finding study in rabbits, daily injection of 0.125 Units/kg/day (days 6 to 18 of gestation) and 2 Units/kg (days 6 and 13 of gestation) produced severe maternal toxicity, abortions and/or fetal malformations. Higher doses resulted in death of the dams. The rabbit appears to be a very sensitive species to BOTOX®.

If the patient becomes pregnant after the administration of this drug, the patient should be apprised of the potential risks, including abortion or fetal malformations that have been observed in rabbits.

8.4 UNANTICIPATED PROBLEMS (UP)

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers UP those that involve risks to participants or others that include – in general – any incident, experience, or outcome that meet all the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research);
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4.2 UNANTICIPATED PROBLEM REPORTING

The investigators will report unanticipated problems (UP) to the reviewing IRB and to the Data Coordinating Center (DCC)/lead PI. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;

- An explanation of the basis for determining that the event, incident, experience, or outcome represents a UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.
- UPs that are serious adverse events (SAEs) will be reported to the IRB and to the DCC/study sponsor within 15 days of the investigators becoming aware of the event.
- Any other UP will be reported to the IRB and to the DCC/study sponsor within one month of the investigators becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the OHRP within one month of the IRB's receipt of the report of the problem from the investigators.

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

If any unanticipated problems arise, the PI will provide written notification to all participants of the study. This includes any identification of serious adverse events. If any changes in the protocol design are required due to these unanticipated problems, this will be detailed to any active participants undergoing treatment.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

Primary Efficacy Endpoint(s):

The primary endpoint will be a change in the PSS questionnaire (see Appendix 1) from screening (prior to treatment) to 4, week 8 and week 12 to assess the duration of stress relief, if any. Statistical analysis will be performed to observe for scoring trends with clinical and/or statistical differences between the treatment groups. We hypothesize that (1) the treatment group PSS scores will decrease, along with stress levels, when compared to the initial screening/pre-treatment questionnaire, and that (2) the treatment group will have lower PSS scores/aggregate scores compared to the placebo group.

9.2 SAMPLE SIZE DETERMINATION

This proof-of-concept study did not necessitate sample size determination.

9.3 STATISTICAL ANALYSES

9.3.1 GENERAL APPROACH

The material in this section is the basis for statistical analysis for this study. This plan may be revised during the study to adapt to unexpected issues that may impact planned analyses.

All statistical tests will be two-sided. All summary tables of quantitative parameters will include mean, standard deviation, range, and notation of missing data (if relevant).

9.3.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

The primary endpoint is determined by deceased stress levels defined by scores on the PSS-10 questionnaire (see Appendix 1). The maximum and minimum scores on this are 40 and 0, respectively. Normative data from original publications on this scale demonstrated an average score of 0-13. Thus, scores ≥14 shall be used as an indication of increased perceived stress.

PSS-10 questionnaires shall be collected at each participant visit. Total scores and subscale scores (Perceived Helplessness and Perceived Self-Efficacy) shall be calculated after appropriate reverse-coding of positive items. Changes from baseline shall be evaluated at each time point. A paired t-test shall be used to compare aggregate PSS-10 scores between time points, with a two-tailed significance level of 0.05. Higher scores indicate greater perceived stress, with scores ≥10 representing moderate to high stress levels.

The analysis shall account for:

- Total PSS-10 score (range 0-40)
- Perceived Helplessness subscale (items 1, 2, 3, 6, 9, 10)
- Perceived Self-Efficacy subscale (items 4, 5, 7, 8, reverse-coded)

9.3.3 SAFETY ANALYSES

Any adverse or unexpected events (see section 8.3) shall be collected and/or reassessed at each time-point in the study (see section 1.3).

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

Consent forms describing in detail the study intervention, study procedures, and risks shall be provided to the participant. Written documentation of informed consent is required prior to starting intervention/administering study intervention.

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks shall be provided to the participant. Written documentation of receiving and understanding the California Experimental Subject's Bill of Rights and the informed consent is required prior to starting intervention/administering study intervention.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms shall be IRB-approved, and the participant shall be asked to read and review the document.

The investigators shall explain the research study to the participant and answer any questions that arise. A verbal explanation shall be provided in terms suited to the participant's comprehension, including the purposes, procedures, potential risks, and their rights. Participants shall have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates and consider participation before agreeing to participate. The participant will sign the informed consent document prior to the initiation of any study-related procedures.

Participants shall be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. The participant shall receive a copy of the signed informed consent document. The informed consent process shall be conducted and documented in the source documents with the consent form signed and dated before the participant undergoes any study-specific procedures. The rights and welfare of participants shall be protected by emphasizing that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification documenting the reason for study suspension or termination shall be provided by the suspending or terminating party to the PI. If the study is prematurely terminated or suspended, the PI shall promptly inform study participants, the IRB, and sponsor, providing the reason(s) for the termination or suspension. Study participants shall be contacted and be informed of changes to their study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance with protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

The study may resume once concerns about safety, protocol compliance, and data quality are addressed to the satisfaction of the sponsor, IRB and/or FDA.

10.1.3 CONFIDENTIALITY AND PRIVACY

Participants' confidentiality and privacy shall be strictly maintained by all investigators, research personnel, and the sponsor. This confidentiality extends to all study-related information, including medical records, biological samples, and research data. The study protocol, documentation, data, and all generated information shall be held in strict confidence. Access to study information shall be limited to authorized personnel directly involved in the study unless authorized by the sponsor in writing.

All in-person research activities shall be conducted in the most private setting possible. Remote sessions may be offered for research activities that can be adequately and invariably conducted in written, visual, and/or verbal format based on the investigators' professional judgement.

The study monitor, an authorized representatives of the sponsor, IRB, and state and/or federal regulatory agencies holding appropriate jurisdiction may inspect required documents and records maintained by the investigators, including but not limited to, medical (administrative, clinic, or hospital) and pharmaceutical records associated with the study participants. The clinical study site will grant access to such records as necessary.

Study participants' contact information shall be securely stored at each clinical site for internal use during the study period. All records shall be maintained in a secure location after study completion for the duration required by the reviewing IRB, institutional policies, or sponsor requirements, whichever period is longest.

Research data collected for statistical analysis and scientific reporting shall be stored at the Center for Advanced Facial Plastic Surgery. Each participant shall be assigned a unique identification number, excluding contact or identifying information. All study data entry and management systems shall utilize secure, password-protected platforms. Upon study completion, all health record databases shall be de-identified using validated software or by authorized personnel and archived at the Center for Advanced Facial Plastic Surgery.

10.1.4 QUALITY ASSURANCE AND QUALITY CONTROL

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation, and completion. An individualized quality management plan shall be developed to describe a site's quality management.

Quality control (QC) procedures shall be implemented beginning with the data entry system and data QC checks that shall be run on the database shall be generated. Any missing data or data anomalies shall be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted – with data generated and biological specimens collected – documented (recorded), and reported in compliance with this protocol, ICH, and GCP, and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.1.5 DATA HANDLING AND RECORD KEEPING

10.1.5.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the co-investigator. The investigators are responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data recorded and/or reported. All study data shall be recorded as a single point data capture electronically via the sites Clinical Trial Management System (CTMS), Clinical Research io (CRIO) a 21 CFR Part 11 compliant system. Any paper source documents i.e., informed consent forms, patient questionnaires etc., that require the study participant to complete and/or sign shall be scanned into the system and filed in the forms section within the study visit it pertains to clinical data e.g., concomitant medications, adverse events, serious adverse events, lab results etc., will also be recorded withing CRIO.

All recorded data in CRIO shall be subject to independent data validation checks for consistency and completeness. All study-related activities and documents shall be subject to validation to determine whether these activities were conducted, and data were collected, recorded, analyzed, and accurately reported according to the protocol, GCP guidelines of the ICH, and any applicable regulatory requirements. The PI should contact the Sponsor immediately if contacted by a regulatory agency about an inspection.

The CTMS includes password protection and an audit trail of any data changes made.
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10.1.5.2 STUDY RECORDSS RETENTION

All source data, clinical records, and laboratory data relating to the study shall be retained for a minimum of 2 years after the last approval of a marketing application or, until there are no pending or contemplated marketing applications, or for at least 2 years after the formal discontinuation of clinical development of

the investigation product. These documents should be retained for a longer period, however, if required by local regulations.

10.1. 6 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, ICH-GCP, or MOP requirements. Noncompliance may be either on the part of the participant, the investigators, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH-GCP:
4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
5.1 Quality Assurance and Quality Control, section 5.1.1
5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigators to use continuous vigilance to identify and report deviations within 3 working days of identification of the protocol deviation, or within [specify number] working days of the scheduled protocol-required activity. All deviations shall be addressed in study source documents and be sent to the reviewing IRB per their policies. The site investigators are responsible for understanding and adhering to the reviewing IRB requirements.

10.2 ABBREVIATIONS

AE	Adverse Event
ANCOVA	Analysis of Covariance
BMI	Body mass index
BOTOX®	OnabotulinumtoxinA
BTX	Botulinum toxin (any subtypes, various chemical structures)
CBC	Complete blood count
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Complete Metabolic Panel
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services

DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
EC	Ethics Committee
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Financial Report
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
hCG	Human Chorionic Gonadotropin
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IIT	Investigator Initiated Trial
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
LSMEANS	Least-squares Means
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
OHRP	Office for Human Research Protections
PI	Principal Investigator
PSS	Perceived Stress Scale (see appendix 1)

PTSD	Post-traumatic stress disorder
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
UP	Unanticipated Problem
US	United States

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APPENDIX 1: PERCEIVED STRESS SCALE (PSS)

Primary Reference (for PSS-14): Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A global measure of perceived stress. *Journal of Health and Social Behavior*, 24, 385-396.

Additional Reference (for PSS-10): Cohen, S., & Williamson, G. (1988). Perceived stress in a probability sample of the United States. In S. Spacapam & S. Oskamp (Eds.), *The social psychology of health: Claremont Symposium on applied social psychology*. Newbury Park, CA: Sage.
(Key points: While originally a PSS-14 scale was developed, the PSS-10 had better internal reliability with equivalent correlations to outcomes as the original PSS-14, and thus the PSS-10 is recommended for use in research. The mean PSS-10 for the studied population was 13.02.)

Purpose: To assess the degree to which people perceive their lives as stressful. High levels of stress are associated with poor self-reported health, elevated blood pressure, depression, and susceptibility to infection.

Description: Subjects indicate how often they have found their lives unpredictable, uncontrollable, and overloaded in the last month.

Psychometrics:

Reliability: alpha = .78
Validity: Correlates in a predicted way with other measure of stress
(Job Responsibilities Scale, life events scales).

Questionnaire:

0 = Never 1 = Almost Never 2 = Sometimes 3 = Fairly Often 4 = Very Often

1.	In the last month, how often have you been upset because of something that happened unexpectedly?	0	1	2	3	4
2.	In the last month, how often have you felt that you were unable to control the important things in your life?	0	1	2	3	4
3.	In the last month, how often have you felt nervous and "stressed"?	0	1	2	3	4
4.	In the last month, how often have you felt confident about your ability to handle your personal problems?	0	1	2	3	4
5.	In the last month, how often have you felt that things were going your way?	0	1	2	3	4
6.	In the last month, how often have you found that you could not cope with all the things that you had to do?	0	1	2	3	4
7.	In the last month, how often have you been able to control irritations in your life?	0	1	2	3	4
8.	In the last month, how often have you felt that you were on top of things?	0	1	2	3	4
9.	In the last month, how often have you been angered because of things that were outside of your control?	0	1	2	3	4
10.	In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?	0	1	2	3	4

Scoring:

Reversed Items: 4, 5, 7, 8

Total Perceived Stress: Sum Items: 1, 2, 3, 4R, 5R, 6, 7R, 8R, 9, 10

APPENDIX 2: CONTRACEPTION GUIDANCE

Female subjects of childbearing potential are eligible to participate in the study if they agree to use an acceptable method of contraception consistently and correctly, as listed below:

Failure Rate	Method of Contraception

Below 1% per year	• Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal) • Progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, intrauterine) • Bilateral tubal occlusion • Vasectomized partner • Sexual abstinence
More than 1% per year	• Progestogen-only oral hormonal contraception, where inhibition of ovulation is not the primary mode of action • Male or female condom, with or without spermicide • Cap, diaphragm, or sponge with spermicide • A combination of male condom with either cap, diaphragm, or sponge with spermicide (double barrier methods)