

**Botulinum Toxin A in Tissue  
Expander Breast Reconstruction  
A Double-Blinded Randomized  
Controlled Trial**

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## Botulinum Toxin A in Tissue Expander Breast Reconstruction A Double-Blinded Randomized Controlled Trial

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**List of Abbreviations****LIST OF ABBREVIATIONS**

ADL	Activity of daily living
ADM	Acellular Dermal Matrix
AE	Adverse Event/Adverse Experience
BTX-A	Botulinum toxin A, BOTOX-A ®, Onabotulinum Toxin A
CFR	Code of Federal Regulations
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
DSMP	Data and Safety Monitoring Plan
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
HRQoL	Health-Related Quality of Life
IB	Investigator's Brochure
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
NPIS	Numeric Pain Intensity Scale
PHI	Protected Health Information
PI	Principal Investigator
PRCT	Prospective Randomized Controlled Trial
PRO	Patient-Reported Outcomes
QoL	Quality of Life
SAE	Serious Adverse Event/Serious Adverse Experience
SD	Standard Deviation
SOP	Standard Operating Procedure

**PRÉCIS (Study Summary)**

Title	<i>Botulinum Toxin A in Tissue Expander Breast Reconstruction – A Double-Blinded Randomized Controlled Trial</i>
Running Title	<i>Botulinum Toxin A in Tissue Expander Breast Reconstruction</i>
Protocol Number	<i>11-001687</i>
Phase	<i>Phase 3</i>
Methodology	<i>Placebo-controlled, double-blinded, prospective randomized trial</i>
Study Duration	<i>48 months</i>
Subject Participation Duration	<i>Estimated length of participation by each individual: 4-6 months Estimated study accrual time: 40 months</i>
Single or Multi-Site	<i>Single-site</i>
Objectives	<i>To investigate the effect of intra-muscular BTX-A injections on pain and physical well-being during the expansion period in women undergoing unilateral and bilateral mastectomies with immediate placement of subpectoral tissue expanders.</i>
Number of Subjects	<i>134</i>
Diagnosis and Main Inclusion Criteria	<i>Women at least 18 years of age, who will undergo immediate unilateral or bilateral tissue expander breast reconstruction.</i>
Study Product, Dose, Route, Regimen	<i>Botulinum Toxin A (BOTOX-A®, Allergan Inc.) Single dose of 100 units of BTX-A injected intra-operatively in the pectoralis major muscle. Maximum dose per patient is 100 units of BTX-A for unilateral tissue expander placement, and 200 units for bilateral tissue expander placement.</i>
Duration of Administration	<i>Single intra-operative dose</i>
Reference therapy	<i>Placebo control group (intra-operative saline injection)</i>
Statistical Methodology	<i>Outcome variables will be summarized overall and by treatment group with frequency counts and percentages or means, standard deviations, medians, and ranges, as appropriate. Variables will be compared by treatment group using two-sample t, Wilcoxon rank sum, Pearson's chi-square, or Fisher's exact tests.</i>

## Abstract

As part of an ongoing effort to improve quality of patient care in plastic surgery, we propose to investigate the effect of intra-muscular Botulinum toxin A (BTX-A) injections on pain and physical well-being in women undergoing mastectomies with immediate placement of tissue expanders. Implant-based breast reconstruction is frequently achieved in two-stages. The first stage consists of the placement of a tissue expander after mastectomy. This is followed by a period of tissue expansion lasting several months. In the second stage, the tissue expander is replaced with a permanent breast implant. Despite the well-recognized advantages of this successful technique, the subpectoral placement of a tissue expander is associated with significant pain and discomfort in the immediate post-operative period and during the phase of tissue expansion.<sup>1-11</sup> Although the potential for a therapeutic use of BTX-A as an analgesic agent is well established in various clinical problems<sup>12-23</sup>, there is a paucity of data assessing the effects of BTX-A in relieving pain associated with tissue expander breast reconstruction. We performed a systematic review of the literature, to identify existing data on the use of BTX-A with subpectoral tissue expanders.<sup>24</sup> The largest study of the effect of BTX-A on pain during tissue expansion was published by Layeeque et al.<sup>25</sup> In this retrospective study, the authors reported a significant reduction in pain postoperatively and during tissue expansion with the use of BTX-A. Since 2008, BTX-A has been routinely used at Mayo Clinic to alleviate pain associated with tissue expansion. A preliminary analysis of our pilot data confirms the findings previously reported by Layeeque et al. We propose to conduct a multi-center, double-blinded prospective randomized controlled trial (PRCT) of women undergoing unilateral and bilateral mastectomies with immediate placement of tissue expanders, to establish the efficacy of BTX-A on alleviating pain and on improving physical well-being during the expansion period.

## **1 Introduction**

This document is a protocol for a human research study. This study will be carried out in accordance with the applicable United States government regulations and Institutional research policies and procedures.

### **HUMAN SUBJECTS**

Institutional Review Board (IRB) approval will be obtained to conduct this study. Although no identifiable Protected Health Information (PHI) will be transmitted to the study's database, study staff will comply with HIPAA guidelines for the protection of PHI. All data gathered will be de-identified and kept in a secured location. Medical records will be assigned code numbers, so that names will not be connected to any information. The following safeguards will be used with respect to protection of human subjects:

**Recruitment and Informed Consent:** Eligible women will be invited to participate in the study by a member of the patient's surgeon/treatment team or the study's research coordinator at Mayo Clinic, at the time of their preoperative assessment by a plastic surgeon. Electronic medical records will be examined to confirm subjects' eligibility for study participation. Women who agree to participate will receive documents describing the purpose and details of the study, information about confidentiality and sharing of information within the research team, as well as an informed consent form. They will have a full opportunity to ask questions about the study and may decide at any point to decline participation in the study. Should patients have additional questions about the study, contact information for a project research coordinator will be provided in the informational documentation. If eligible women do not wish to participate in the study, no further effort will be made to contact them.

**Confidentiality Safeguards:** Participants will be notified that information collected during their participation in this study is considered confidential. All data gathered in the study will be de-identified and kept in a password-protected study-specific database at Mayo Clinic. Confidentiality of each participant's data will be protected with all data identified solely by a code number. Names will not be connected to any information. Only the authors of the study will have access to this information, and patient details will be filed under code number, not under patient name. A list matching subject names and code numbers will be maintained on a separate sheet of paper kept in locked storage; only the authors of the study will have access to this information. This information will be kept on a computer in a locked departmental office at all times. Information from any woman who is enrolled but later determined to be ineligible will be retained in a confidential electronic data base.

**Sources of Materials:** Participants' responses to the NPIS and the BREAST-Q© will be the main source of research material. Patient information will also be obtained from existing electronic medical records. These data will be collected for research purposes only and not used for any other purposes.

**Data Safety and Monitoring Plan (DSMP):** A Data Safety and Monitoring Plan (DSMP) will allow us to identify adverse events and accrual problems. A statistician will review the unblinded adverse event data after 1/2 of the target sample size has accumulated.

**Data Safety and Monitoring Board (DSMB):** An independent Data Safety and Monitoring Board will consist of Dr. Jamie Bakkum-Gamez, Dr. Judy Boughey, Dr. Martin Zielinski, Dr. Kellie Mathis, Debbie Dixon, Kent Bailey Ph.D. and a Mayo Clinic statistician who is not involved with this project. The Data Safety Monitoring Board will assess safety and efficacy at interim analysis (when 75 patients have been enrolled) and whenever necessary.

*Median Lethal Dose:*

The median lethal dose is estimated to be approximately 39 units of BTX-A per kilogram of body weight.<sup>26</sup>

*Safety, Risks and Adverse Effects:*

BTX-A has been used as a therapeutic agent since the late 1970s. Since then, it has been demonstrated to be a remarkably safe drug when used under medical supervision.<sup>27, 28</sup> The mechanism of action of BTX-A has been extensively studied and is known to be very specific. Thus, non-neuromuscular adverse effects and systemic side effects are very rare.<sup>29</sup> Local side effects of BTX-A injection include a flu-like syndrome that is generally short-lived, dysphagia, and xerostomia. Unintended weakness of the muscle injected or of nearby muscles could also be a negative effect. Other potential side effects include muscle soreness, rash, headaches, light-headedness, fever, chills, hypertension, diarrhea and abdominal pain.

## 1.1 Background

**Significance:** Each year, the number of breast cancer survivors who choose post-mastectomy breast reconstruction keeps rising. Among women who elect to pursue breast reconstruction, approximately 75% will choose prosthetic breast reconstruction.<sup>30</sup> By far, the most commonly employed surgical technique utilizes tissue expanders as a first stage. Tissue expansion is a well-established breast reconstruction technique characterized by high success rates and high patient satisfaction.<sup>9, 31-36</sup> However, women report significant amounts of pain and discomfort with this technique.<sup>1, 8, 11, 37</sup> Legeby et al. recently showed that women who underwent prosthetic breast reconstruction had higher pain scores and took more analgesics than those who did not choose post-mastectomy reconstruction.<sup>8</sup>

In the past 10 years, publications on the use of BTX-A for pain relief in a wide array of clinical conditions have increased tremendously. BTX-A is one of the neurotoxins produced by Clostridium botulinum bacteria. By reversibly inhibiting neurotransmitter release, BTX-A has both analgesic<sup>38</sup> and paralytic properties.<sup>28</sup> The presence of analgesic properties of BTX-A is increasingly supported by several clinical observations: pain relief with BTX-A injections has been reported for migraine headaches<sup>39</sup>, chronic pelvic pain<sup>15, 20, 40, 41</sup>, chronic tennis elbow<sup>21</sup>, and post-operative pain control for lower limb lengthening correction<sup>16, 42</sup>, among others.

Reduced pain and improved physical well-being are felt to contribute to an enhanced QoL for women who elect to pursue breast reconstruction. Moreover, clinically meaningful benefits of BTX-A on physical well-being and overall QoL of patients treated with this biologic agent have recently been highlighted in a systematic review.<sup>43</sup> Our contribution here is expected to provide high-level evidence of the efficacy of BTX-A on pain and physical well-being during the expansion phase of two-stage

breast reconstruction. In the current environment of rising healthcare costs and increased government scrutiny on marketing for off-label indications of BTX-A<sup>44</sup>, this study can provide exactly the type of data needed by the United States Food and Drug Administration (FDA) to expand approved uses of products and devices.

**Background:** Although there are presently nine FDA approved indications for the clinical use of BTX-A (1- Cervical dystonia, 2- Severe primary axillary hyperhidrosis, 3- Strabismus, 4- Blepharospasm, 5- Temporary improvement in the appearance of moderate to severe glabellar lines, 6- Chronic migraine, 7- Upper limb spasticity, 8- overactive bladder symptoms, 9- Crow's feet), off-label use of BTX-A has rapidly expanded beyond these approved clinical indications. One of the most rapidly expanding indications for BTX-A is in the treatment of various painful muscle spasms (e.g. paravertebral muscle spasm<sup>45</sup>, fibromyalgia-myofascial pain<sup>46</sup>, temporomandibular joint pain<sup>47</sup>, etc.). Pectoralis major muscle spasm is a frequently reported problem during tissue expansion. The neuromuscular and analgesic effects of BTX-A have been studied in a wide spectrum of spastic disorders, namely for the treatment of spasticity associated with disorders such as stroke<sup>19</sup>, multiple sclerosis, Parkinson's disease and cerebral palsy. Recently, emerging evidence has been published on the use of BTX-A for the treatment of migraine headaches<sup>14, 39</sup> and musculoskeletal disorders.<sup>48</sup> To the best of our knowledge, this aspect has never been studied in breast cancer survivors who elect to pursue breast reconstruction with tissue expanders. Furthermore, physical function outcomes are important to consider with BTX-A use because the link between temporary muscle paralysis and improvements in participation in daily activities is not a given.

Pain is the most commonly reported complication associated with the use of tissue expanders, and yet suboptimal pain control with the use of oral narcotics remains the current standard of care. Over the years, numerous methods to decrease pain associated with the process of tissue expansion have been tested and yielded unsatisfactory results.<sup>2-5, 10, 49</sup> For example, Sinow et al. experimented with intraluminal injections of lidocaine in tissue expanders, but were unable to demonstrate any reduction in pain when lidocaine was compared with placebo.<sup>2</sup> Moreover, in recent years, innovation in prosthetic breast reconstruction techniques has seen a rapid rise in popularity of acellular dermal matrix (ADM).<sup>50-53</sup> Many plastic surgeons have incorporated ADM in their surgical practice for one-stage and two-stage prosthetic breast reconstruction.<sup>54-56</sup> ADM is frequently used as a sling to cover the lower lateral pole of tissue expanders and implants in breast reconstruction.<sup>57</sup> Potential benefits may include: 1) improved control of the submuscular pocket and infra-mammary fold;<sup>57, 58</sup> 2) higher initial filling volumes of tissue expanders at the time of placement; 3) reduced overall number of expansions; and 4) decreased pain morbidity associated with the musculofascial dissection (rectus abdominus and serratus anterior). At the present time, possible benefits of ADM on postoperative pain alleviation have not been demonstrated in prospective clinical trials. The research proposed in this application is innovative, in our opinion, because it represents a new and substantive departure from the status quo in pain management following tissue expander breast reconstruction. An additional strength of this investigation is that it will include a newly developed patient-reported outcome (PRO) measure to study physical well-being. The BREAST-Q© was rigorously developed and validated with adherence to guidelines set by the Medical Outcomes Trust<sup>59</sup> and the FDA.<sup>60</sup>

## 1.2 Investigational Agent

Commercially available in the United States under the brand name BOTOX®, FDA approved BTX-A will be used as the investigational agent against a placebo. BTX-A will be obtained from the manufacturer, Allergan Inc. (Irvine, CA), and dispensed through the institutional research pharmacy.

BOTOX®, onabotulinumtoxin A or botulinum toxin type A, is one of the neurotoxins produced by the Gram positive, anaerobic *Clostridium botulinum* bacteria that causes botulism. It blocks neuromuscular transmission by binding to acceptor sites on motor or sympathetic nerve terminals, entering the nerve terminals, and inhibiting the release of acetylcholine. This inhibition occurs as the neurotoxin cleaves SNAP-25, a protein integral to the successful docking and release of acetylcholine from vesicles situated within nerve endings. When injected intramuscularly at therapeutic doses, BTX-A produces partial chemical denervation of the muscle resulting in a localized reduction in muscle activity. In addition, the muscle may atrophy, axonal sprouting may occur, and extrajunctional acetylcholine receptors may develop. There is evidence that reinnervation of the muscle may occur, thus slowly reversing muscle denervation produced by BTX-A. Using currently available analytical technology, it is not possible to detect BTX-A in the peripheral blood following intramuscular injection at the recommended doses.

BOTOX® for injection is a sterile, vacuum-dried purified botulinum toxin type A, produced from fermentation of a strain *Clostridium botulinum* type A, and intended for intramuscular and intradermal use. It is purified from the culture solution by dialysis and a series of acid precipitations to 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. One Unit of BTX-A corresponds to the calculated median intraperitoneal lethal dose (LD50) in mice. Each vial of BOTOX® contains either 100 Units of Clostridium botulinum type A neurotoxin complex, 0.5 mg of Albumin Human, and 0.9 mg of sodium chloride; or 200 Units of Clostridium botulinum type A neurotoxin complex, 1 mg of Albumin Human, and 1.8 mg of sodium chloride in a sterile, vacuum-dried form without a preservative.

## 1.3 Pre clinical data or other off label use

This is an FDA approved product in commercial distribution. Detailed information on specific warnings, precautions, contraindications and adverse reaction is available from the Package Insert. (See Attached)

In the late 1970s, the first therapeutic use of BTX-A was as a treatment for strabismus.<sup>61, 62</sup> By reversibly inhibiting neurotransmitter release, BTX-A has both analgesic and paralytic properties. When injected intramuscularly at therapeutic doses, BTX-A produces chemical denervation of the muscle, thus resulting in localized muscle weakness. This effect occurs within a few days to one week after injection, peaks within two weeks and lasts several weeks, before hitting a plateau and then gradually returning to baseline levels of muscle contraction.<sup>28</sup> The clinical effects last three to four months after each injection.<sup>28</sup> The analgesic action of BTX-A was initially thought to be related to its effects on muscular contraction. However, a recent in vitro study of embryonic rat dorsal neurons did

confirm that BTX-A inhibits release of substance P, a neurotransmitter associated with pain and inflammatory reactions.<sup>38</sup> Moreover, this analgesic property of BTX-A is increasingly supported by several clinical observations: pain relief with BTX-A injections has been reported for migraine headaches<sup>39</sup>, chronic pelvic pain<sup>15, 20, 40, 41</sup>, chronic tennis elbow<sup>21</sup>, and post-operative pain control for lower limb lengthening correction<sup>16, 42</sup>, among others. These observations suggest an anti-nociceptive action for BTX-A, which seems to be independent of its paralytic effect based on neuromuscular junction blockade. Only a few anecdotal reports exist that describe the positive effect of BTX-A on pain management in women with subpectoral tissue expanders and breast implants.<sup>63-67</sup>

The use of BTX-A in myocutaneous flaps has been studied in animals. A previous study indicated that BTX-A can prevent myocutaneous flap contraction, thus stabilizing pedicled muscle flaps.<sup>68</sup> Using an experimental animal model, Chenwang et al. studied the effect of BTX-A in tissue expansion. It was found that BTX-A could eliminate isometric muscle contractions and significantly increase the speed of expansion of myocutaneous flaps.<sup>69</sup>

#### 1.4 Clinical Data to Date

Specific to the present application, our research team performed a systematic review of the literature, to identify existing data on the use of BTX-A with subpectoral tissue expanders and implants.<sup>24</sup> We identified a retrospective study published by Layeeque et al.<sup>25</sup> in *Annals of Surgery* in 2004, on the effect of BTX-A use on pain during tissue expansion. In this retrospective study, pain was scored using a visual analog scale ranging from 0 to 10, with higher scores indicating increased pain. Keeping in mind the biases inherent to retrospective studies, the authors did report a significant reduction in postoperative pain scores during the initial tissue expansion with the use of BTX-A (mean  $\pm$  standard deviation of  $1.95 \pm 1.88$  versus  $5.61 \pm 2.77$  in the BTX-A group compared with the non-BTX-A group, respectively). These data support our hypothesis that BTX-A has the potential to significantly improve pain morbidity associated with tissue expansion. Case reports and expert opinions comprised the remaining available data describing the positive effect of BTX-A on pain management in women with subpectoral tissue expanders and breast implants.<sup>63-67, 70</sup> However, it is difficult to draw any reliable conclusions from these reports as they all lack comparison with control groups. Thus, our systematic review has helped to identify a lack of prospective data on the efficacy of BTX-A in tissue expander breast reconstruction. Furthermore, no preliminary data was found on the impact of acellular dermal matrix (ADM) use on pain during tissue expansion. The preceding citations and critical analysis demonstrate the need for a prospective study on the efficacy of BTX-A use in reducing pain in women undergoing subpectoral tissue expander placement to guide an evidence-based practice, and this is precisely the focus of our specific aim. Since 2008, BTX-A has been routinely used at Mayo Clinic to alleviate pain and relieve muscle spasm associated with tissue expansion in women undergoing two-stage prosthetic breast reconstruction with and without the use of ADM. A preliminary analysis of our data supports the findings previously reported by Layeeque et al. Although this unpublished pilot data was not adequately powered and was lacking in the collection of possible confounding variables, the reduction in pain intensity scores was higher in women who underwent subpectoral tissue expander placement without the use of ADM. In this proposal, we will address these limitations in order to more rigorously evaluate pain and physical well-being outcomes in this larger, hypothesis-driven study.

### 1.5 Dose Rationale and Risk/Benefits

In our systematic review of the literature on the use of BTX-A with subpectoral breast implants<sup>24</sup>, the dose of BTX-A administered to each patient in various studies ranged from 75 units to 200 units per breast. BTX-A was injected into the pectoralis major muscle alone in four studies, while in the study published by Layeeque et al., the toxin was injected in the pectoralis major muscle, serratus anterior and rectus abdominis muscle insertion of each breast. Richards et al. injected BTX-A in the pectoralis major muscle and subcutaneous tissues of the breast.

Based on these data, we concluded that an injection of 100 units of BTX-A per breast is a prudent choice. This dosage is also the standard dose used for several clinical indications, such as strabismus. In one study, 500 units of BTX-A were injected in the pectoralis major muscle of patients suffering of spastic shoulder pain after a cerebrovascular accident, and according to the authors, no important adverse event occurred.<sup>18</sup>

We have opted for an intra-operative injection rather than a pre-operative injection prior to surgery to minimize a theoretical risk of tumor seeding from percutaneous needle injection. **The BTX-A injection will be performed intra-operatively under general anesthesia, after all irrigations of the surgical site are completed, BUT prior to the placement of the tissue expander under the pectoralis major muscle to avoid any inadvertent puncture of the prosthetic device. BTX-A will be given only once during surgery and will not be repeated postoperatively. The reason for giving the injection during the surgery is that it will allow the anti-nociceptive action of BTX-A to take effect immediately while the paralytic action will take about 7 to 10 days to take effect, thus coinciding with the start of tissue expansion.** Furthermore, since the pectoralis major muscle is large and easily accessible, intra-muscular injection will be ascertained during surgery as it will be performed under direct visualization of the muscle. The clinical effects of a single injection of BTX-A usually lasts 3-4 months.<sup>28</sup> On average, the duration of tissue expansion usually ranges between 1 and 4 months. Therefore, our study design is based on a single intraoperative injection of BTX-A, and no repeat injection will be performed on patients enrolled in this study.

#### *Safety, Risks and Adverse Effects:*

BTX-A has been used as a therapeutic agent since the late 1970s. Since then, it has been demonstrated to be a remarkably safe drug when used under medical supervision.<sup>27, 28</sup> The mechanism of action of BTX-A has been extensively studied and is known to be very specific. Thus, non-neuromuscular adverse effects and systemic side effects are very rare.<sup>29</sup>

Local side effects of BTX-A injection include a flu-like syndrome that is generally short-lived, dysphagia, and xerostomia. Unintended weakness of the muscle injected or of nearby muscles could also be a negative effect. Other potential side effects include muscle soreness, rash, headaches, light-headedness, fever, chills, hypertension, diarrhea and abdominal pain.

Potential risks with this therapy include a distant spread of BTX-A effect from the area of injection to produce symptoms consistent with systemic botulinum toxin effects. These symptoms have been reported hours to weeks after injection of BTX-A. Life threatening adverse events such as swallowing and breathing difficulties can occur, and there have been reports of death. The risk of these symptoms is probably greatest in children treated for spasticity, but the effects of distant toxin spread can also

occur in adults, particularly in those patients who have underlying conditions that would predispose them to these symptoms.

All serious adverse effects will be reported. The PI and the research team will continually review blinded data after each subject has study interaction, including a review of all adverse events, to determine seriousness and relation to study drug. Minor adverse events and unusual complications encountered during the period of tissue expansion will be carefully recorded. In the event of a serious adverse reaction to the treatment, the study participant will be treated accordingly depending on the reaction.

We consider that the risk level to subjects enrolled in this study is greater than minimal risk, yet not disproportionate in relation to the anticipated benefits and/or knowledge that might reasonably be expected from the results. Potential benefit for study participants is an improved pain management during the period of tissue expansion. BTX-A has been used as a therapeutic agent since the late 1970s. Since then, it has been demonstrated to be a remarkably safe drug when used under medical supervision.<sup>27, 28</sup> The median lethal dose is estimated to be approximately 39 units of BTX-A per kilogram of body weight.<sup>26</sup> The mechanism of action of BTX-A has been extensively studied and is known to be very specific. Thus, non-neuromuscular adverse effects and systemic side effects are very rare.<sup>29</sup>

By performing a study that accurately evaluates the effect of BTX-A on women's subjective pain and physical well-being assessments during the expansion period of implant-based breast reconstruction, information on a critical aspect of HRQoL will be obtained. If our expected outcomes are met, BTX-A could become part of the standard of care for women undergoing breast reconstruction with tissue expanders. Future studies in this continuum of research will include: 1) the determination of the minimal effective dose of BTX-A required for appropriate pain management during tissue expansion; 2) an economic evaluation of this therapy; and 3) a comprehensive evaluation of all aspects of patient satisfaction and HRQoL associated with the use of this therapy in tissue expansion, and the overall outcomes of breast reconstruction.

## **2 Study Objectives**

**Overall Aim:** The proposed study will be the first, multi-center, double-blinded PRCT evaluating the efficacy of BTX-A in alleviating pain and improving health-related quality of life (HRQoL) during the period of tissue expansion. Results from this study will provide preliminary information on the efficacy of BTX-A in this population. This base of knowledge will contribute to advances in quality of health care provision, will guide plastic surgeons in applying evidence-based practices, and support patients and surgeons in the process of shared decision-making.

### **Primary Objective**

**Specific Aim:** To determine the effect of BTX-A on pain and physical well-being during the expansion period in women undergoing subpectoral placement of tissue expanders for two-stage implant-based breast reconstruction.

**Hypothesis:** We hypothesize that intra-operative infiltration of BTX-A in the pectoralis major muscle reduces pain associated with tissue expansion, and improves women's physical well-being and HRQoL.

**Expected Outcomes:** Upon completion of the proposed study, it is our expectation that we may demonstrate an improvement in patient-reported outcomes with the use of BTX-A. We anticipate that the improvement gained with this therapy will outweigh the potential adverse events experienced. This would be in agreement with the documented findings in other areas.<sup>43</sup> More specifically, we believe that we may reveal the positive impact of BTX-A on patient-rated pain and a clinically meaningful benefit on physical well-being in this population, when compared with placebo (control group). Thus, important advances in the management of pain associated prosthetic breast reconstruction techniques could be expected, which could ultimately reduce the physical impact of implant-based reconstruction and improve QoL for breast cancer survivors.

### 3 Study Design

#### 3.1 General Design

**EXPERIMENTAL DESIGN FOR SPECIFIC AIM#1:** To determine the effect of BTX-A on pain and physical well-being during the expansion period in women undergoing subpectoral placement of tissue expanders for two-stage implant-based breast reconstruction.

**Research Design:** The study design chosen to test our working hypothesis and answer our specific aim is a multi-center, double-blinded, placebo-controlled, prospective, randomized trial. Women at least 18 years of age, who will undergo immediate unilateral or bilateral tissue expander reconstruction, will be recruited to participate in the study.

Consecutively enrolled eligible women will be randomized into one of two different treatment groups, described in section 5.3. Expected duration of subject participation is 4 months.

#### Study Timeline and Feasibility:

PROPOSED PROJECT TIMETABLE				
AIMS/TASKS	YEAR 01	YEAR 02	YEAR 03	YEAR 04
Specific Aim #1:				→
• Enrollment			→	
• Follow-up			→	
Data Analysis			→	→

For this study, we plan to accrue up to 134 patients in order to obtain a sample size of 128 subjects (64 women per group). Based on annual operative volumes at Mayo Clinic Rochester, and considering that not all plastic surgeons performing tissue expander breast reconstruction will be contributing to

the study, we estimate that approximately 150 women will be eligible for recruitment over a 36-month period, ensuring the feasibility of adequate sample size recruitment (see table below).

**Annual numbers of breast reconstructions with tissue expanders at Mayo Clinic Rochester (2008-2010)**

<b>Year \ Procedure</b>	<b>Unilateral tissue expander breast reconstruction</b>	<b>Bilateral tissue expander breast reconstruction</b>	<b>TOTAL</b>
2008	98	123	221
2009	74	111	185
2010 (10 months)	70	107	177

We anticipate that these numbers will remain approximately constant. It is estimated that subjects will be recruited over a 40-month period, and each participant will be followed for 4 months with data collected longitudinally over this period. The last women enrolled in year 1 will be followed for 4 months into year 2. Data analysis will commence in year 2.

### 3.2 Primary Study Endpoints

The primary study endpoint is to determine the efficacy of a single intra-operative injection of BTX-A in the pectoralis major muscle on pain and physical well-being in women undergoing tissue expansions following mastectomy and immediate tissue expander breast reconstruction.

The outcome measures selected to test our working hypothesis and answer our specific aim #1 are a numeric pain intensity scale (NPIS)<sup>71</sup>, and the Physical Well-Being scale of the BREAST-Q®: Reconstruction Module. The NPIS is a reliable and valid tool that is widely used as a measure of pain intensity (See Appendix for complete scale). To assess physical well-being, we selected the 16-item Physical Well-Being scale from the BREAST-Q®: Reconstruction Module. The BREAST-Q® is a newly-developed and validated PRO measure designed to measure patients' perception of outcomes following breast surgery.<sup>72</sup> The Reconstruction Module consists of scales in six domains related to satisfaction and QoL (See Appendix for complete scale). The Physical Well-Being scale has items that relate to issues surrounding chest and upper body symptoms, and how these impact on physical function and participation in activities before and after breast reconstruction. Study participants will answer how often they have experienced each symptom during the previous two-week period, using a 5-point Likert scale (1 – none of the time, 5 – very often). Answers from these questions will be combined to provide a total physical well-being score for each patient at each follow-up visit. Average scores for both study groups will be compared. These two measures will be administered at the pre-operative visit, the first postoperative visit (1-2 weeks) and subsequent Plastic Surgery appointments/expansion visits until the end of study is reached. After much consideration, we have made the decision not to ask enrolled subjects to record the amount of oral analgesic medication taken

daily during the course of the study, as this method is often inaccurate due to missing data entries, and would therefore yield unreliable results. Furthermore, the randomization process in this study should ensure a random distribution of patients with different oral analgesics intakes. It was also felt that not recording the amount of oral analgesic medication would reduce the burden of study participants.

### **3.3 Secondary Study Endpoints**

Secondary study endpoints will include:

- 1) The initial percent volume expansion (defined as initial intraoperative fill volume divided by manufacturer recommended total tissue expander volume);
- 2) The rate of tissue expansion between the BTX-A group and the placebo group, with or without the use of ADM;
- 3) Rate of reconstructive failure (tissue expander removal for any reasons, such as infection or uncontrolled pain).

### **3.4 Primary Safety Endpoints**

The primary safety endpoints of this study is the incidence of side effects attributable to the injection of BTX-A at doses of 100 units and 200 units, in women undergoing immediate post-mastectomy breast reconstruction with tissue expander(s).

Patients will be advised to inform the Principal Investigator and the study coordinator immediately if they develop any unusual symptoms (including difficulty with swallowing, speaking, or breathing), or if any existing symptom worsens. Patients will also be counseled that if loss of strength, muscle weakness, blurred vision, or drooping eyelids occur, they should avoid driving a car or engaging in other potentially hazardous activities.

At each follow-up visit, the study coordinator will question study subjects about occurrence of adverse events. All serious adverse experiences will be collected and reported. Local side effects of BTX-A injection include a flu-like syndrome that is generally short-lived, dysphagia, and xerostomia. Unintended weakness of the muscle injected or of nearby muscles could also be a negative effect. Other rare potential side effects include muscle soreness, rash, headaches, light-headedness, fever, chills, hypertension, diarrhea, abdominal pain, swallowing and breathing difficulties. Furthermore, the status of wound healing and tissue integrity will be performed at the first postoperative visit for all patients (i.e., at one to two weeks following surgery).

## **4 Subject Selection Enrollment and Withdrawal**

### **4.1 Inclusion Criteria**

Eligible women must be able to give appropriate consent or have an appropriate representative available to do so, for participation in the study:

1. Women at least 18 years of age, who will undergo immediate unilateral or bilateral tissue expander breast reconstruction following therapeutic skin-sparing or nipple-sparing mastectomy
2. Women at least 18 years of age, who will undergo immediate bilateral tissue expander breast reconstruction following risk-reduction (prophylactic) skin-sparing or nipple-sparing mastectomy.

#### **4.2 Exclusion Criteria**

1. Subjects who are unable to read or speak English
2. Breast reconstruction using the latissimus dorsi flap combined with a tissue expander
3. Documented diagnosis of chronic pain, upper limb spasticity, cervical dystonia, axillary hyperhidrosis, strabismus or blepharospasm
4. Hypersensitivity to any botulinum toxin preparation or to any of the components in the formulation;
5. Infection at the proposed site of injection
6. Pre-existing neuromuscular disorders (including diagnosed myasthenia gravis, Eaton-Lambert syndrome, or amyotrophic lateral sclerosis)
7. Aminoglycosides intake at the time of surgery (these antibiotics can potentiate the effect of BTX-A)
8. Women who are pregnant or breast-feeding
9. Presence of implants from previous breast surgery (could result in higher probability of intraoperative placement of direct implant or subcutaneous placement of tissue expanders)
10. Reported use of Botox within 4 months prior to planned surgical date (could impact the occurrence of adverse events)

#### **4.3 Subject Recruitment, Enrollment and Screening**

Potential research subjects will be identified by a member of the patient's treatment team, the principal investigator, or research team at Mayo Clinic. If the investigator is a member of the treatment team, s/he will screen their patient's medical records for suitable research study participants and discuss the study and their potential for enrolling in the research study.

During the initial conversation between the investigator/research staff and the patient, the patient may be asked to provide certain health information that is necessary to the recruitment and enrollment process. The investigator/research staff may also review portions of their medical records at Mayo Clinic in order to further assess eligibility. They will use the information provided by the patient and/or medical record to confirm that the patient is eligible, and to contact the patient regarding study enrollment. If the patient turns out to be ineligible for the research study, the research staff will destroy all information collected on the patient during the initial conversation and medical records review, except for any information that must be maintained for screening log purposes. In most cases, the initial contact with the prospective subject will be conducted either by the treatment team,

investigator or the research staff working in consultation with the treatment team. The recruitment process outlined presents no more than minimal risk to the privacy of the patients who are screened and minimal PHI will be maintained as part of a screening log.

Eligible women will be invited to participate in the study by a member of the patient's surgeon/treatment team or the study's research coordinator at Mayo Clinic, at the time of their preoperative assessment by a plastic surgeon. Electronic medical records will be examined to confirm subjects' eligibility for study participation. During this visit with the plastic surgeon, women who agree to participate will receive information about the study, confidentiality and sharing of information within the research team, as well as an informed consent form. They will have a full opportunity to ask questions about the study and may decide at any point to decline participation in the study. Should patients have additional questions about the study, contact information for a project research coordinator will be provided in the informational documentation. If eligible women do not wish to participate in the study, no further effort will be made to contact them. Prior to enrollment, study participants will undergo a urine pregnancy test to confirm their eligibility.

#### **4.4 Early Withdrawal of Subjects**

##### **4.4.1 When and How to Withdraw Subjects**

Study participants may be withdrawn from the study prior to the subject completing all of the study related procedures, for the following reasons:

- Ineligibility unknown at the time of enrollment (e.g., inability to proceed with tissue expander reconstruction at the time of mastectomy)
- Nonadherence of enrolled subjects to protocol requirements
- Occurrence of competing events (e.g., patient's best interests)

##### **4.4.2 Data Collection and Follow-up for Withdrawn Subjects**

Data collected on study subjects up to the time of withdrawal will remain in the study database and will not be removed, in order for the study to be scientifically valid. If a subject withdraws from the study, removal of already collected data would undermine the scientific, and therefore the ethical, integrity of the research. Such removal of data could also put enrolled subjects, future subjects, and eventual users of marketed products at an unreasonable risk.

Reasonable attempts to follow-up with study participants will be made by the research team. The Principal Investigator will ask each enrolled woman who is withdrawing whether she wishes to provide continued follow-up and further data collection subsequent to her withdrawal from the interventional portion of the study. Under this circumstance, the discussion with the subject would distinguish between study-related interventions and continued follow-up of associated clinical outcome information, such as successful completion of breast reconstruction, obtained through non-invasive chart review, and address the maintenance of privacy and confidentiality of the subject's information.

If a subject withdraws from the interventional portion of a study and does not consent to continued follow-up of associated clinical outcome information, the principal investigator will not access for purposes related to the study the subject's medical record or other confidential records requiring the subject's consent. However, an investigator will review study data related to the subject collected prior to the subject's withdrawal from the study, and will consult public records, such as those establishing survival status.

## 5 Study Drug

### 5.1 Description

BOTOX® (onabotulinumtoxinA) for injection is a sterile, vacuum-dried purified botulinum toxin type A, produced from fermentation of Hall strain *Clostridium botulinum* type A, and intended for intramuscular and intradermal use. It is purified from the culture solution by dialysis and a series of acid precipitations to 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.

One Unit of BOTOX® corresponds to the calculated median intraperitoneal lethal dose (LD50) in mice. The method utilized for performing the assay is specific to Allergan's product, BOTOX®. Due to specific details of this assay such as the vehicle, dilution scheme, and laboratory protocols for the various mouse LD50 assays, Units of biological activity of BOTOX® cannot be compared to nor converted into Units of any other botulinum toxin or any toxin assessed with any other specific assay method. Therefore, differences in species sensitivities to different botulinum neurotoxin serotypes preclude extrapolation of animal-dose activity relationships to human dose estimates. The specific activity of BOTOX® is approximately 20 Units/nanogram of neurotoxin protein complex.

Each vial of BOTOX® (Botulinum Toxin Type A) contains 100 units (U) of *Clostridium botulinum* toxin type A, 0.5 mg albumin (human), and 0.9 mg sodium chloride in a sterile, vacuum-dried form without a preservative. For the purpose of this study, one and two vial(s) of 100 units will be used for the unilateral and bilateral breast reconstructions, respectively. Prior to injection, the research pharmacy service will reconstitute each vacuum-dried vial of BTX-A with non-preserved 0.9% Sodium Chloride Injection USP for a final BTX-A concentration of 2 units/0.1 mL. Thus, **the total volume of solution (BTX-A or placebo) injected in each pectoralis major muscle will be 5 mL (100 units) of BTX-A or 5 mL of 0.9% Sodium Chloride).**

### 5.2 Treatment Regimen

A single intramuscular injection of 100 units of BTX-A will be done intra-operatively in the pectoralis major muscle. This injection will not be repeated during the course of the study. Thus, women undergoing unilateral tissue expander placement will receive a dose of 100 units of BTX-A, whereas women undergoing bilateral tissue expander placement will receive a dose of 200 units of BTX-A for the purpose of this study.

### 5.3 Method for Assigning Subjects to Treatment Groups

**Methods:** Consecutive women who elect to pursue breast reconstruction with tissue expanders will be approached to participate in this study. All consenting subjects will be randomized to receive either a single dose of 100 units of BTX-A per breast, or a placebo. Study participants will not be blinded regarding the use of ADM at the time of surgery.

**Treatment group assignment:**

STUDY ARMS	ASSIGNED TREATMENT
1	BTX-A +/- ADM
2	Placebo +/- ADM

**Randomization and Masking:** Treatment blinding and randomization will be carried out in the hospital's Research Pharmacy Service using a program to generate random numbers. The injected solutions of BTX-A and placebo (0.9% Sodium Chloride Injection USP) will be prepared by the pharmacy service to ensure masking. At the time of injection in the pectoralis major muscle, neither the treating surgeon nor the study participants will be aware of group allocation with regards to the use of BTX-A or placebo. All research team members involved in the assessments and analysis of the data will be blinded. Thus, only the pharmacy personnel preparing the syringes will be aware of group assignment (Group A or B).

### 5.4 Preparation and Administration of Study Drug

BTX-A is supplied as a freeze-dried crystalline complex, in single-use vials of 100 Units. The preparation also contains 0.5 mg of human albumin and 0.9 mg of sodium chloride. For the purpose of this study, vials of 100 units will be used for the unilateral and bilateral breast reconstructions, respectively. Prior to injection, the Research Pharmacy at Mayo Clinic will reconstitute each vacuum-dried vial of BTX-A with non-preserved 0.9% Sodium Chloride Injection USP for a final BTX-A concentration of 2 units/0.1 mL. Thus, the total volume of solution (BTX-A or placebo) injected in each pectoralis major muscle will be 5 mL (or 100 units of BTX-A). The study drug and placebo will be sent to the operating room in single-use vials and administered by the plastic surgeon.

Prior to injection, each vacuum-dried vial of BOTOX® will be reconstituted with sterile, non-preserved 0.9% Sodium Chloride Injection USP. The proper amount (5 mL) of diluent will be drawn in an appropriate size syringe, and the diluent will then be slowly injected into the vial. The vial will be discarded if a vacuum does not pull the diluent into the vial. The BOTOX® will then be mixed gently with the saline by rotating the vial. The date and time of reconstitution will be recorded on the space on the label. BOTOX® will be administered within 24 hours after reconstitution. During this time period, reconstituted BOTOX® will be stored in a refrigerator (2° to 8°C).

**Injection protocol:**

The surgical team will receive one single-use vial (for unilateral cases) or two single-use vials (for bilateral cases) containing the study drug from the research pharmacy. The contents of each vial will be transferred into one or two appropriate size sterile syringes using an 18-gauge needle. A 22-gauge spinal needle will be used to perform the injections.

**The injection of the study drug will occur once the creation of the subpectoral pocket is completed, and AFTER ALL IRRIGATIONS OF THE SURGICAL FIELD to avoid aspiration of the study drug with the suction.** The plastic surgeon will inject 1 mL of solution (BTX-A or placebo) at five equidistant points in the inferior third of the pectoralis major muscle.

### **5.5 Subject Compliance Monitoring**

To assure adherence to the one-time intraoperative study drug intervention, the study coordinator will review documentation of intraoperative medications. Monitoring adherence of timely questionnaire completion at each tissue expansion visit will also occur. For patients who will undergo tissue expansions at Mayo Clinic, the study coordinator will ensure that the questionnaires have been fully completed by the study participants before they leave the premises.

In some instances, study participants will not return to Mayo Clinic for each tissue expansion visit. The study coordinator will prepare a packet for these women that will include 7 questionnaires and 7 prepaid return envelopes by post. The study participants are to fill out and return one copy of the questionnaire at each tissue expansion visit performed at an outside institution. To maximize the response rate, we will use the methodology recommended by Dillman<sup>73</sup>. If the questionnaire is not returned within one week after the planned tissue expansion visit, the study coordinator will contact the patient, and complete the questionnaire by phone. A telephone script will be used for this purpose. If the patient cannot be reached by phone, a reminder will be sent along with one additional copy of the questionnaire.

### **5.6 Prior and Concomitant Therapy**

As part of the standard of care at Mayo Clinic, patients are offered optional regional anesthesia in the form of a thoracic paravertebral block (PVB) on the morning of surgery, in addition to the routine procedures. The PVB technique<sup>74, 75</sup> used includes multilevel injections of 1% to 0.5% ropivacaine with 1:400,000 epinephrine into the ipsilateral paravertebral space at six levels from T1 through T6. This technique can improve postoperative pain control for 4 to 8 hours after performance of the PVB. The data on PVB will be collected as it can act as a potential confounding variable.

Depending on individual surgeon practice and preference, some patients may receive other forms of short-term intraoperative local analgesia such as Exparel, an injectable suspension of bupivacaine liposome which can provide pain relief for up to 72 hours. Data on Exparel will also be collected as it can act as a potential confounding variable.

Additional oral analgesic intake by study participants will not be monitored as this data collection is often incomplete and of poor quality, making it difficult or impossible to draw any reliable conclusions. Thus, oral analgesic intake will not be restricted during the course of this study.

## **5.7 Packaging**

Allergan, Inc. will provide 180 vials of BOTOX® (100 unit vials) for the purpose of this study. As part of this agreement, Allergan, Inc. will ship 50% of the study drug upon:

1. Full execution of the Letter of Agreement by authorized representatives of the parties;
2. Confirmation that the study has been registered on ClinicalTrials.gov;
3. Approval of the study protocol by the appropriate IRB (if required);
4. The FDA has not placed the IND on clinical hold (if applicable);
5. At least 30 days have elapsed since FDA's official receipt date of the filed IND (if applicable)

The remaining 50% of the study drug will be shipped by Allergan upon enrolling 50% of the target enrollment. If for storage and shelf-life reasons less materials should be sent than indicated above, shipments can be divided up to the maximum-contributed materials amount.

BOTOX® will be supplied in a single-use vial in the following size:

- 100 Units NDC 0023-1145-01

Vials of BOTOX® have a holographic film on the vial label that contains the name "Allergan" within horizontal lines of rainbow color. In order to see the hologram, the vial should be rotated back and forth between the fingers under a desk lamp or fluorescent light source. (Note: the holographic film on the label is absent in the date/lot area.) If the lines of rainbow color or the name "Allergan" cannot be seen, the product will not be used and Allergan will be contacted immediately for additional information at 1-800-890-4345 from 7:00 AM to 3:00 PM Pacific Time.

## **5.8 Blinding of Study Drug**

Treatment blinding and randomization will be done by each site specific Mayo Clinic Research Pharmacy. The research pharmacy will prepare BOTOX® or placebo (0.9% Sodium Chloride Injection USP) according to the randomization. The BOTOX® and placebo will look identical in the syringe after preparation. The labels used for both products are also identical. The final prepared product that will be provided to the surgeon will be labeled "For Investigational Use Only".

## **5.9 Receiving, Storage, Dispensing and Return**

### **5.9.1 Receipt of Drug Supplies**

Study treatment supplies will be sent from Allergan, Inc. to each site specific Mayo Clinic Research Pharmacy.

Upon receipt of the study treatment supplies, an inventory will be performed and a drug receipt log filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipping invoice. Any discrepancies, damaged or unusable study drug in a given shipment (active drug or comparator) will be documented in the study files. The Principal Investigator will notify the study sponsor immediately of any discrepancies, damaged or unusable products.

### **5.9.2 Storage**

Unopened vials of BOTOX® will be stored in a refrigerator (2° to 8°C) for up to 36 months for the 100 Units vial, in each site specific Mayo Clinic Research Pharmacy. The vials will not be used after the expiration date that appears on the vial. BOTOX® will be administered within 24 hours of reconstitution; during this period, reconstituted BOTOX® will 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 will be disposed of carefully, as is done with all medical waste.

### **5.9.3 Dispensing of Study Drug**

Regular study drug reconciliation will be performed to document drug assigned, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, and signed and dated by the study team.

### **5.9.4 Return or Destruction of Study Drug**

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be documented and investigated, prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

## 6 Study Procedures

### TABLE OF VISITS:

	Research activity	Subject activity
<b>Preoperative evaluation/Surgical listing appointment</b>	Study Coordinator (SC) reviews the study with eligible patients and obtains informed consent	Complete the BREAST-Q and Numeric Pain Intensity Scale
		•
<b>Surgery</b>	Randomization by site specific research pharmacy and intra-operative injection of study drug by plastic surgeon	
<b>First post-operative visit:</b> <ul style="list-style-type: none"> <li>Week 1- 2</li> </ul>	SC facilitates completion of questionnaire and assesses for adverse events	Complete the BREAST-Q and Numeric Pain Intensity Scale
<b>Subsequent Plastic Surgery Appointments/Expansion Visits</b> <ul style="list-style-type: none"> <li>Beginning at week 2-3 and intervals thereafter as determined by the plastic surgeon</li> </ul>	SC facilitates completion of questionnaire and assesses for adverse events	Complete the BREAST-Q and Numeric Pain Intensity Scale
<ul style="list-style-type: none"> <li><b>End of Study</b></li> <li>The last plastic surgery appointment prior to removal of the TE, or the date of TE removal (whichever occurs last within the 4 month postoperative period)</li> </ul>		

**Tissue expander placement:** The surgical procedure for submuscular placement of the tissue expander will be performed in the usual manner by each participating plastic surgeon.

**BTX-A Injection Protocol:** see Section 5.4

**Outcome Assessment:** The outcome measures selected to test our working hypothesis and answer our specific aim #1 are a numeric pain intensity scale (NPIS)<sup>71</sup>, and the Physical Well-Being scale of the BREAST-Q®: Reconstruction Module. These two measures will be administered to study participants at the pre-operative visit, the first postoperative visit (1-2 weeks), and subsequent Plastic Surgery appointments/expansion visits until the end of the study is reached.

## 7 Statistical Plan

### 7.1 Sample Size Determination

The *primary study endpoint* is to determine the efficacy of a single intra-operative injection of BTX-A in the pectoralis major muscle on pain and physical well-being in women undergoing tissue expansions following mastectomy and immediate tissue expander breast reconstruction.

*Secondary study endpoints* include:

- 1) The initial percent volume expansion (defined as initial intraoperative fill volume divided by manufacturer recommended total tissue expander volume);
- 2) The rate of tissue expansion between the BTX-A groups and the placebo and control groups, with or without the use of ADM;
- 3) Rate of reconstructive failure (tissue expander removal for reasons such as infection or uncontrolled pain).

The *primary safety endpoints* of this study is the incidence of side effects attributable to the injection of BTX-A at doses of 100 units and 200 units, in women undergoing immediate post-mastectomy breast reconstruction with tissue expander(s).

**Power and Sample Size:** The only available preliminary data for the effect of BTX-A use on pain during initial tissue expansion were obtained from Layeeque et al, *Annals of Surgery* 2004. In this retrospective study, pain was scored using a visual analog scale ranging from 0 to 10, with higher scores indicating increased pain. Layeeque et al reported a mean  $\pm$  standard deviation (SD) pain score during initial tissue expansion of  $1.95 \pm 1.88$  for the BTX-A group compared with  $5.61 \pm 2.77$  for the non- BTX-A group.

Sample size estimates were obtained assuming 80% power and a significance level of 0.05 for a two-sample t-test to test the effects of BTX-A use on pain during initial tissue expansion. The table below (entitled Sample Size Estimates) summarizes the sample size needed in each group to achieve approximately 80% power. We calculated the sample sizes needed to detect a 50% decrease in the mean pain scores (assuming mean pain scores for the BTX-A and non-BTX-A groups of 2.0 and 4.0) and a 25% decrease in mean pain scores (assuming mean pain scores for the BTX-A and non-BTX-A groups of 3.0 and 4.0). Sample sizes were calculated assuming low, moderate, and high SDs of 2.0, 2.5, and 3.0, respectively. For example, if mean pain scores for the BTX-A and non-BTX-A groups

were 3.0 and 4.0, respectively, with a moderate SD of 2.5, 100 patients in each group, for a total of 200, would be required to detect this difference as statistically significant.

In order to minimize cost while still maintaining scientific integrity, our goal is to accrue up to 134 patients in order to obtain 128 complete data sets (64 patients per study arm). If the accrual rate was slower than anticipated, we would extend the enrollment period and continue to accrue patients until sample size requirements were met. If the required number of enrolled subjects was not met for any reason, we would end the study and analyze the data using the same techniques. An appropriate cautionary statement would be added to the discussion section of any publication.

**SAMPLE SIZE ESTIMATES**

<b>BTX-A</b>	<b>Mean Pain</b>	<b>SD</b>	<b>N per Group</b>
No	4.0	2.0	17
Yes	2.0		
No	4.0		64
Yes	3.0		
No	4.0	2.5	26
Yes	2.0		
No	4.0		100
Yes	3.0		
No	4.0	3.0	37
Yes	2.0		
No	4.0		143
Yes	3.0		

**7.2 Statistical Methods****Descriptive Statistics:**

Demographic and medical characteristics will be analyzed for all enrolled subjects. Descriptive statistics will be calculated for all baseline variables. Univariate descriptive statistics and frequency distributions will be calculated, as appropriate for all variables. Baseline values for demographic, clinical, and outcome variables (primary and secondary) will be tabulated separately for the treatment groups. These analyses will help identify whether the randomization was successful in equalizing distributions of these prognostic variables across treatment groups or if confounding variables need to be included as covariates in sensitivity analyses. Putative prognostic variables that will be investigated through these descriptive analyses include variables such as age, body mass index, smoking status, diabetes mellitus, coronary artery disease, hypertension, clinical breast cancer stage, mastectomy weight, initial percent volume expansion (defined as initial intraoperative fill volume divided by manufacturer recommended total tissue expander volume), sentinel lymph node biopsy, axillary dissection, adjuvant therapy factors (e.g., previous and postoperative chemotherapy and radiation), operative time, ADM use.

**Handling of Missing Data**

Handling of missing data will be done in a number of ways including complete-case analysis and multiple imputations via nearest neighbor, mean value, last value, and zero value carried forward

approaches. Multiple approaches are used so that the sensitivity of results to alteration in multiple imputational assumptions may be assessed.

### **Primary Hypotheses:**

- Pain scores will be at least 25% less, in those who received BTX-A, during the immediate post-operative period compared to those who received saline injections
- Pain scores will be at least 25% less, in those who received BTX-A, during the period of tissue expansion, compared to those who received saline injections
- Physical Well-Being scores during tissue expansion will be higher in those who received BTX-A compared to those who received saline injections

There are two primary outcomes: pain intensity measured by the NPIS, and physical well-being using the BREAST-Q®. The primary analysis will be performed during the post-operative period and during the expansion phase. Outcome variables will be summarized with frequency counts and percentages or means, standard deviations, medians, and ranges, as appropriate. The mean, median, standard deviation, and range of each variable and its change from baseline, measured on a continuous scale, will be presented by treatment and visit. Frequency tables will be provided for categorical variables by treatment and visit. Any differences among the study groups will be evaluated using two-sample t, Wilcoxon rank sum, Pearson's chi-square, or Fisher's exact tests.

### **Secondary Hypotheses:**

- 1) There will be no difference in the initial percent volume expansion between groups (defined as initial intraoperative fill volume divided by manufacturer recommended total tissue expander volume);
- 2) The rate of tissue expansion will be higher in the BTX-A groups compared to the placebo group, with or without the use of ADM;
- 3) There will be no difference in the rate of reconstructive failure (tissue expander removal for reasons such as infection or uncontrolled pain).

The volume of tissue expansion anticipated and the rate of tissue expansion between the BTX-A groups and the placebo and control groups will be calculated both as an absolute value as well as a percentage. It is hypothesized that the use of BTX-A may increase the rate of tissue expansion, which may lead to fewer postoperative tissue expansion visits. This will allow measurement of effect size between the intervention and the placebo groups. Two-sample t and Wilcoxon rank sum tests will be used for the secondary analyses.

## **7.3 Subject Population(s) for Analysis**

The subject populations whose data will be subjected to the study primary and secondary analyses will include all randomized populations.

## 8 Safety and Adverse Events

### 8.1 Definitions

**Adverse Event:** An untoward or undesirable experience associated with the use of a medical product (i.e. drug, device, biologic) in a patient or research subject.

Note: An adverse event (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An adverse event can arise from any use of the drug (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

**Suspected Adverse Reaction:** Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

**Reasonable Possibility:** There is evidence to suggest a causal relationship between the drug and the adverse event.

**Serious:** An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator 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 patient or subject 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.

### Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)

Any unanticipated problem or adverse event that meets the following three criteria:

- **Serious:** Serious problems or events that results in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others

(including individuals who are not research subjects). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, **AND**

- **Unanticipated:** (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, **AND**
- **Related:** A problem or event is "related" if it is possibly related to the research procedures.

### **Adverse Event Reporting Period**

The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is considered the four month postoperative period following study drug administration.

### **Preexisting Condition**

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

### **General Physical Examination Findings**

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

### **Post-study Adverse Event**

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the local investigator should instruct each subject to report, to the local investigator, any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The local investigator should notify the study regulatory sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the local investigator should become aware of the development of problems, cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

### **Hospitalization, Prolonged Hospitalization or Surgery**

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and evaluated for relationship to the study drug/placebo. If there is a reasonable possibility that it is related to the study drug/placebo then will be reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should **not** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

The expected adverse events from surgery include post-operative pain, nausea, vomiting, general anesthesia risks, hemorrhage, surgical site infection, mastectomy skin necrosis, seroma, surgical site paresthesia.

The table below will be used for collecting and grading serious adverse events associated with BTX-A injection.

Adverse Event	Grade				
	1	2	3	4	5
Cardiac dysrhythmia	Asymptomatic; intervention not indicated	Symptomatic; medical intervention indicated	Symptomatic and incompletely controlled medically, or controlled with device (e.g., pacemaker)	Life-threatening consequences; urgent intervention indicated	Death
Myocardial infection		Asymptomatic and cardiac enzymes minimally abnormal and no evidence of ischemic ECG changes	Severe symptoms; cardiac enzymes abnormal; hemodynamically stable; ECG changes consistent with infarction	Life-threatening consequences; hemodynamically unstable	Death
Dysphagia	Symptomatic, able to eat regular diet	Symptomatic and altered eating/swallowing	Severely altered eating/swallowing; tube feeding or TPN or hospitalization indicated	Life-threatening consequences; urgent intervention indicated	Death
Anaphylaxis			Symptomatic bronchospasm, with or without urticaria; parenteral intervention indicated; allergy-related edema/angioedema; hypotension	Life threatening consequences; urgent intervention indicated	Death
Seizure	Brief partial seizure; no loss of consequences	Brief generalized seizure	Multiple seizures despite medical intervention	Life-threatening; prolonged repetitive seizures	Death
Respiratory depression/		Decreased oxygen saturation with	Decreased oxygen saturation at rest	Life-threatening airway compromise;	Death

hypoxia		exercise; intermittent supplemental oxygen		urgent intervention indicated	
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## 8.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event section of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic, laboratory or procedure results should be recorded in the source document.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. Serious adverse events that are still ongoing at the end of the study period must be followed up, to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be at least possibly related to the study treatment or study participation should be recorded and reported immediately.

Adverse events will be monitored and recorded during the four month postoperative time period.

## 8.3 Reporting of Serious Adverse Events and Unanticipated Problems

When an adverse event has been identified, the study team will take appropriate action necessary to protect the study participant and then complete the Study Adverse Event form and log. The sponsor-investigator will evaluate the event and determine the necessary follow-up and reporting required.

### 8.3.1 Sponsor-Investigator reporting, notifying the Mayo IRB

When an adverse event has been identified, the study team will take appropriate action necessary to protect the study participant and then complete the Study Adverse Event form and log. The sponsor-investigator will evaluate the event and determine the necessary follow-up and reporting required.

The sponsor-investigator will report to the Mayo IRB any UPIRTSOs and Non-UPIRTSOs according to the Mayo IRB Policy and Procedures.

Any serious adverse event (SAE) must be reported to the Mayo IRB as soon as possible but no later than 5 calendar days. The adverse event report will contain the following information:

Fields populated from the clinical research database:

- Subject's name
- Medical record number

- Disease/histology (if applicable)
- Protocol number

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the adverse event was expected
- The severity of the adverse event
- The intervention

The PI's signature and the date it was signed are required on the completed report. For this protocol, only directly related SAE's will be reported to the IRB.

### **8.3.2 Sponsor-Investigator reporting: Notifying the FDA**

The sponsor-investigator will report to the FDA all unexpected, serious suspected adverse reactions according to the required IND Safety Reporting timelines, formats and requirements.

Unexpected fatal or life threatening suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 7 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Other unexpected serious suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Any clinically important increase in the rate of serious suspected adverse reactions over those listed in the protocol or product insert will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Findings from other studies in human or animals that suggest a significant risk in humans exposed to the drug will be reported. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigators initial receipt of the information about the event.

## **8.4 Unblinding Procedures**

In case of an adverse event, un-blinding of the study participant will be carried out immediately. The event will be analyzed in the same manner as any severe adverse drug reaction. This will be reported to the appropriate agencies with the analysis, conclusions and recommendations. The study will then

be re-instituted if given approval to continue. This study subject will be categorized as a treatment failure and imputed the most negative score on all outcomes.

All serious adverse events (SAE) will be reported, and un-blinding will be part of managing an SAE. The PI and the research team will continually review blinded data after each subject has study interaction, including a review of all adverse events, to determine seriousness and relation to study drug. Minor adverse events and unusual complications encountered during the period of tissue expansion will be carefully recorded. In the event of a serious adverse reaction to the treatment, the study participant will be treated accordingly depending on the reaction.

Any AE that occurs during the course of the study will be disclosed to the Mayo Clinic IRB. All SAE will be reported to the FDA as well as to Allergan within 15 calendar days after its first knowledge. The Principal Investigator will provide written notice to Allergan within 15 calendar days of any occurrence(s) of either (i) pregnancy or (ii) lactation during the time period of effect of BOTOX®, which can last three to four months.

In cases where un-blinding was not associated with an SAE, such actions will be reported in a timely manner. Notification of sponsor will be performed within 24 hours by phone or fax, followed by a written narrative of the event within 48 hours. The statistician conducting the interim analysis will contact Research Pharmacy personnel to proceed with un-blinding not associated with an SAE.

## 8.5 Stopping Rules

The study will be stopped immediately if any patient suffers *at least one* of the following events during the course of the study, unless it can be shown that this event was unrelated to participation in the clinical trial:

- 1) Death
- 2) Upper limb paralysis or weakness
- 3) Bulbar weakness
  - a. Blurred vision
  - b. Facial paralysis
  - c. Dysarthria
- 4) Anaphylactic reaction
- 5) Any severe adverse drug reaction

The study will be stopped if the absolute difference in the surgical site infection (SSI) rate is 15% higher in one treatment group as compared to the other treatment group. The study statistician will compare the SSI rates in a blinded fashion on a periodic basis and notify the Principle Investigator of this information. The study team will review the data and notify the DSMB accordingly.

## 8.6 Medical Monitoring

It is the responsibility of the Sponsor-Investigator to oversee the safety of the study. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted

above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10 "Study Monitoring, Auditing, and Inspecting"). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

### **8.6.1 Internal Data and Safety Monitoring Plan**

A Trial Steering Committee will include the Principle Investigator and site specific study representatives. The statistician, other study personnel and collaborators will be welcome to contribute and will be included as needed. The committee will meet every other month during data collection and as needed until the trial is completed. These meetings will be used to discuss data management and analysis, evaluate progress of the trial, review study recruitment and to troubleshoot any issues and/or administrative problems as they arise.

An internal Data Safety and Monitoring Plan (DSMP) will allow identification of adverse events and accrual problems. A Mayo Clinic statistician to be named who is not involved with this project will review the unblinded adverse event data to assess safety, integrity of the data collected and efficacy after 1/2 of the target sample size has accumulated. At the interim analysis, the pooled PRO data will be reviewed in order to assess sample size and determine if sample size adjustment is necessary. At this time, any safety concerns or grounds for early stopping also will be reviewed. In addition, patient safety will be protected by open communication and reporting of patient safety concerns by any personnel involved in the study to the principal investigator or Trial Steering Committee members during the course of patient participation in the study. If significant issues arise they will be reported to the institutional IRB for further consideration.

### **8.6.2 Independent Data and Safety Monitoring Board**

Data Safety and Monitoring Board (DSMB): An independent Data Safety and Monitoring Board will consist of Dr. Jamie Bakkum-Gamez, Dr. Judy Boughey, Dr. Martin Zielinski, Dr. Kellie Mathis, Debbie Dixon, Kent Bailey Ph.D. and a Mayo Clinic statistician who is not involved with this project. The Data Safety Monitoring Board will assess safety and efficacy at interim analysis (when 75 patients have been enrolled). The Board will be empowered to recommend continuation, modification, or early closure of the trial. Recommendations of the Data Safety Monitoring Board will be reviewed by the Steering Committee who will make the final decision to terminate accrual early. In addition, patient safety will be protected by open communication and reporting of patient safety concerns by any personnel involved in the study to the principal investigator or Trial Steering Committee members during the course of patient participation in the study. If significant issues arise they will be reported to both institutional IRBs for further consideration.

## **9 Data Handling and Record Keeping**

### **9.1 Confidentiality**

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period.

**Confidentiality Safeguards:** Participants will be notified that information collected during their participation in this study is considered confidential. All data gathered in the study will be de-identified and kept in a password-protected study-specific database at Mayo Clinic. Confidentiality of each participant's data will be protected with all data identified solely by a code number. Names will not be connected to any information. Only the authors of the study will have access to this information, and patient details will be filed under code number, not under patient name. A list matching subject names and code numbers will be maintained on a separate sheet of paper kept in locked storage; only the authors of the study will have access to this information. This information will be kept on a computer in a locked departmental office at all times. Information from any woman who is enrolled but later determined to be ineligible will be destroyed.

### **9.2 Source Documents**

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

### **9.3 Case Report Forms**

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO

NOT ERASE OR USE WHITE OUT FOR ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

### **Data Management**

A study coordinator will be assigned to the study. The responsibilities of the study coordinator will include project compliance, data collection, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team. Quality of data will be the responsibility of the study coordinator. Questionnaires filled out by study participants will be reviewed by the study coordinator to ensure completeness. Using a telephone script, the study coordinator will contact enrolled noncompliant subjects to remind them to complete the questionnaires when tissue expansions are performed at an outside institution. Upon survey receipt by mail, the study coordinator will ensure survey completeness and contact patients by telephone immediately when there is missing data. The missing elements will be completed by telephone.

### **Data Processing**

Clinical data will be collected and stored in paper and electronic format in locked/secure storage, with access limited to the Principle Investigator, the study statistician(s), and the study coordinator(s).

### **Data Security and Confidentiality**

Patient demographic data, treatment variables and questionnaire responses will be recorded in a secure REDcap database. The REDCap is a secure, web-based application for building and managing online databases. This institutional database will be used to obtain this dataset. All data collected will remain at Mayo Clinic. Source documentation will be available to support the computerized patient record.

### **Data Quality Assurance**

Monthly registration reports for enrolled subjects will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Quality of questionnaire completion will be performed as soon as they are completed or turned in to identify missing information, unanswered questions. The study coordinator will contact the subject as soon as possible to try to resolve these discrepancies. Accrual rates and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action.

### **Data Clarification Process**

Electronic data transmission will allow critical safety data and data queries at the time of interim analysis or at any other point in time during the course of the study.

## **9.4 Records Retention**

It is the investigator's responsibility to retain the required records and reports for 2 years after a marketing application is approved for the drug; or, if an application is not approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and FDA has

been so notified. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

## **10 Study Monitoring, Auditing, and Inspecting**

### **10.1 Study Monitoring Plan**

This study will be monitored on a routine basis during the conduct of the trial. The Mayo Clinic Office of Research Regulatory Support will provide clinical monitoring support for the trial as a service for the Sponsor-Investigator. Clinical trial monitoring requires review of the study data generated throughout the duration of the study to ensure the validity and integrity of the data along with the protection of human research subjects. This will assist sponsor-investigators in complying with Food and Drug Administration (FDA) regulations.

The ORRS support may include the following:

- On site or remote monitoring visits according to established processes.
- Tools and checklists for the sponsor-investigator to perform self-monitoring activities.
- Guidance based on study team monitoring needs or requests.
- Regulatory submission support.

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

### **10.2 Auditing and Inspecting**

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, and government regulatory agencies, of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance offices.

## **11 Ethical Considerations**

This study is to be conducted according to United States government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted local Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this

study. The investigator should provide a list of IRB members and their affiliate information to the sponsor.

All potential participants will be informed as to their rights as volunteers in a research study. The right to refuse or withdraw at any point during the study, without compromising medical and other care will be explained. The purpose of the study and potential risks and benefits associated with it will be stated. An informed consent will be obtained from all study participants. Consent will be obtained prior to any research related activities.

## **12 Study Finances**

### **12.1 Funding Source**

As of April 9, 2011, Allergan Sales, LLC, has agreed to provide support to Mayo Clinic for this study by means of an unrestricted research grant, with a contribution of 180 vials of BOTOX® (100U vials). The remaining of the study will be funded internally at Mayo Clinic.

**Financial costs:** There will not be any additional cost to study participants. Because ADM is an accepted treatment modality used in implant-based breast reconstruction, costs associated with its use will be charged to study participants' health insurers as in usual clinical care.

### **12.2 Conflict of Interest**

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study.

### **12.3 Subject Stipends or Payments**

No payment or subject stipends will be given to study participants.

## **13 Publication Plan**

The Principal Investigator will hold the primary responsibility for publication of the results of this study, and will have sole and complete rights (e.g., publication, presentation, distribution, etc.) to all data resulting from this study. The results of this study will be submitted for presentation at the annual meeting of the American Society of Plastic Surgeons. A manuscript will be submitted to the Plastic and Reconstructive Surgery Journal. Allergan will be given the opportunity to review and provide input regarding consolidated data as contained in a manuscript, abstract, presentation, etc. The Principal Investigator will wait thirty calendar days prior to any submission and/or presentation of any publication, including manuscripts, to allow Allergan the right to (i) address any factual inaccuracies with the publisher with regards to the materials, or (ii) request the redaction of Allergan's confidential information. If the results of this study are published, whether the publication is funded by Allergan or

independently, the Principal Investigator will acknowledge that Allergan has provided support for this study. This study will be registered on ClinicalTrials.gov prior to subject recruitment and enrollment.

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