BACKGROUND The U.S. Food and Drug Administration has approved four distinct formulations of botulinum toxin (BoNT) serotypes A and B (BoNTA and BoNTB) for medical use. These four products are indicated for many medical applications, but the three BoNTA formulations are the most widely used worldwide and are the only products approved for aesthetic use. The latest approval of a BoNTA with no complexing proteins (incobotulinumtoxinA) necessitates a review and discussion of differences between available formulations and the effect that these differences may have on clinical practice.

OBJECTIVES To review the history, science, safety information, and current and emerging applications of BoNT in clinical and cosmetic practice and to compare commercially available BoNTA formulations.

METHODS AND MATERIALS Publications, clinical trials, and author experience were used as a basis for an up-to-date review of BoNT and its use in human medicine. The similarities and differences between formulations are presented, and diffusion, spread, equivalency ratios, stability, and storage are discussed.

RESULTS Each commercial formulation has unique characteristics that may influence its use in aesthetic medicine. Familiarity with the similarities and differences between products will aid physicians in making patient care decisions.

CONCLUSION New formulations, emerging uses, and continued research into the science and uses of BoNTA will lead to increasingly refined therapeutic approaches and applications. Continued education is important for physicians to optimize use of the agent according to the most current evidence and approaches.

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neuromuscular disorders, to name a few. In certain fields, BoNT has completely changed the standard of care, with perhaps no use garnering more attention than its applications in aesthetic medicine. From its first published mention as an aesthetic treatment for glabellar lines in 1992, the use of commercially available BoNT type A (BoNTA) has captivated healthcare professionals and lay people alike. Multispeciality statistics collected and reported annually by the American Society for Aesthetic Plastic Surgery demonstrate BoNTA’s exceptional popularity. Injection of BoNTA is the most prevalent aesthetic procedure in the United States; more than 2.6 million BoNTA procedures were reported in 2011 alone.

Recent expansion in the BoNT armamentarium provides an opportunity to explore the use of this powerful agent with a fresh perspective. Today’s aesthetic physician now has several BoNT formulations from which to choose, enhancing the possibilities for increasingly refined and personalized treatment approaches. Effective selection and use of each specific formulation requires an understanding of the basic science behind BoNT’s relevance to human medicine, the clinical similarities and differences between available formulations, and the unique qualities and practical characteristics inherent to each. In this section, we present essential background information about BoNT, review its efficacy and safety records, and discuss the data and experience behind available formulations to lay the foundation for understanding the agent’s optimal use in aesthetic medicine.

**Historical Perspectives: From Toxic to Therapeutic**

Dr. Justinus Kerner hypothesized the existence of BoNT in the early 1800s when he investigated a deadly outbreak of food poisoning from improperly prepared blood sausages. His extensive experiments improved the medical community’s understanding of the biologic basis for food poisoning, as well as the neurologic effects and potential therapeutic applications of the as-yet-unnamed agent that caused paralysis and death. In 1895, Belgian scientist Emile Pierre van Ermengem identified the causative bacterium for botulism, which he named *Bacterium botulinum*. Its name was subsequently changed to *Clostridium botulinum*. This discovery opened the doors to broader research into the bacterium, its toxin, and its effects on humans.

Dr. Herman Sommer first isolated BoNTA in purified form at the University of California at San Francisco. A pivotal point in BoNT research was the purification of BoNTA in crystalline form in 1946 at Fort Detrick. Dr. Edward Schantz commonly receives credit for this accomplishment, but Dr. Schantz credits Dr. Carl Lammana and colleagues with this milestone. Purification methods were improved over the next decade, and Dr. Schantz produced a batch of BoNTA at Fort Detrick that was selectively provided to government and educational researchers to contribute to the growing understanding of the agent’s characteristics and mechanism of action. During this same period, Dr. Vernon Brooks’ discovery that BoNTA blocks the release of acetylcholine from motor nerve endings when injected into a hyperactive muscle (thereby temporarily reducing the target muscle’s activity) was a critical breakthrough that led to increased focus on BoNT’s potential applications in medicine.

In the early 1970s, using BoNTA supplied by Dr. Schantz, Dr. Alan Scott, an eye surgeon at Smith-Kettlewell Eye Research Institute in San Francisco, California, studied the efficacy of chemical denervation of hyperactive muscles as a possible treatment for strabismus. His initial tests in monkeys were successful, and Dr. Scott and Dr. Schantz began to collaborate on the development of BoNT as a drug for medical purposes. Dr. Scott obtained Food and Drug Administration (FDA) approval for human testing in the late 1970s, and in 1979, Dr. Schantz (who had moved his research to the University of Wisconsin) successfully produced a 150-mg batch of
highly purified medical-grade toxin for use in humans—the famous batch 79–11.

In the early 1980s, Dr. Scott led the first large multicenter clinical trial of BoNT, involving more than 7,000 subjects, establishing its safety and efficacy as a treatment for strabismus. Research into the agent’s efficacy as a treatment for muscle-related eye disorders expanded, and in 1989, the FDA approved BoNT (BoNTA; trade name Oculinum) for the treatment of strabismus, blepharospasm, and hemifacial spasm in adults. In 1990, Allergan acquired Oculinum, marketing the product under a new trade name: Botox (onabotulinumtoxinA; BoNTA-ONA).

Dr. Scott’s work dramatically advanced our knowledge of BoNT and coincidentally planted the seed for its future cosmetic applications. Dr. Jean Carruthers, who worked with Scott on his trials of the ophthalmologic uses of BoNTA, noted an unexpected side effect in a patient with blepharospasm—diminished wrinkles in the glabellar region. She discussed her observations with her husband, Dr. Alastair Carruthers, a dermatologist treating this area with the earliest fillers, and their subsequent studies are credited as the first experiments with BoNTA for purely cosmetic purposes. Other researchers who had been conducting their own experiments for therapeutic purposes also noticed unusual but desirable side effects. The Carruthers’ publication of their findings seemed to trigger an explosion of interest in BoNTA’s cosmetic applications, prompting additional studies and considerable off-label use. So much interest was generated, that the original batch 79–11 finally ran out in 1997. A new manufacturing process producing consistent batches of BoNTA-ONA with reduced protein load has been in use since.

BoNTA received its first aesthetic approval in 2001, when health regulators in Canada
approved BoNTA-ONA’s use for treatment of glabellar lines. U.S. approval for glabellar lines followed in April 2002. BoNT’s dramatic progress in reaching this approval seems to have been only the beginning of the story. Today, barely 10 years later, BoNT is used globally for a broad range of therapeutic and aesthetic applications and is one of the most widely researched agents in the world (Figure 1).

Much remains to be discovered about BoNT. New agents with unique characteristics and proprietary manufacturing and purification processes provide valuable opportunities for increasingly refined approaches to patient care in therapeutic and aesthetic applications alike. We look forward to continuing to explore the unique benefits of BoNT.

**The Science of BoNT**

In preface to a discussion of commercial formulations, an understanding of BoNT’s basic science is useful for establishing a framework for evaluation of the similarities and differences between them. BoNT is a product of *Clostridium botulinum*, a species of anaerobic, rod-shaped, spore-forming bacteria. The various strains of *C. botulinum* produce at least seven distinct neurotoxins, denoted as types A through G. The human nervous system is susceptible to only five of the seven serotypes; type A appears to be the most naturally potent serotype to humans.

All BoNT serotypes demonstrate the same basic mechanism of action. Upon introduction into the human system, BoNT travels to the neuromuscular junction, where it binds to high-affinity presynaptic receptors, is internalized, and then cleaves a membrane protein responsible for acetylcholine exocytosis. This functionally blocks acetylcholine release, causing neuromuscular paralysis through chemical denervation. The intracellular targets vary among BoNT serotypes; BoNTA cleaves synaptosomal-associated protein 25, whereas BoNT type B (BoNT-B) cleaves a vesicle-associated membrane protein, or synaptobrevin. The effects of BoNT intoxication are not permanent in nature; similarly, the effects of BoNT injections in medicine are not permanent, with facial cosmetic treatment results typically lasting at least 3 months and in some cases 6 months or longer. During that time, normal muscle innervation and function are restored through axonal sprouting at a new neuromuscular junction. Evidence suggests that the original

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**Figure 2. BoNTA structure.**

neuromuscular junction is also restored in that time.\textsuperscript{7}

BoNT occurs naturally as a macromolecular protein complex (900 kDa) composed of the core neurotoxin (150 kDa) noncovalently bound to various hemagglutinins and a nontoxin nonhemagglutinin protein (Figure 2). This structure appears to have a protective function after ingestion, shielding the neurotoxin from acidic stomach conditions and thermal and pH stress.\textsuperscript{8–10} It has also been suggested that the natural complex acts as a shield for the antigenic epitopes on the 150-kD heavy chain\textsuperscript{11} and facilitates BoNTA transfer across the intestinal epithelium.\textsuperscript{12–15} In medicine, the effect and role of the complex’s size and composition are not clear and remain controversial. It is an interesting contemporary consideration, because each commercially available BoNTA formulation is unique. All commercial formulations contain the 150-kDa core neurotoxin, but the presence and amount of nontoxin proteins vary, yielding products with molecular weights ranging from 900 kDa for BoNTA-ONA to 150 kDa for incobotulinumtoxinA (BoNTA-INCO), which is composed only of the core neurotoxin protein. AbobotulinumtoxinA (BoNTA-ABO) falls somewhere in between; its estimated complex size is a variable 500 to 900 kDa, reflecting the presence of accessory proteins.\textsuperscript{16}

It has been proposed that complexing proteins enhance toxin activity, stabilize and protect the neurotoxin, and limit diffusion, but recent research has provided some insight into whether accessory proteins really aid in clinical effectiveness and safety. It has long been established that the neurotoxin must be freed from its complex before it can act; therefore, all BoNTA formulations must dissociate. Friday and colleagues evaluated the stability of three commercial toxin preparations (BoNTA: Botox, Dysport; BoNTB: Myobloc) under physiologic pH and temperature conditions.\textsuperscript{17} They found that, in all three formulations, the neurotoxin was released from its complex as soon as the preparations were exposed to physiologic conditions. Perhaps most interesting is that all formulations showed paralytic activity at zero incubation time. Eisele and colleagues found that BoNTA 900- and 500-kDa neurotoxin complexes dissociated in <1 minute after being exposed to a pH of 6.9 or greater.\textsuperscript{18} They also found that other environmental factors, such as dilution and changes in salt concentration, affect the dissociation process. Their experiments revealed that the dilution, drying, and reconstitution processes associated with the normal preparation of commercial toxins lead to complete dissociation of the 900-kDa complex. An alternative view questions the pH measurements in the Eisele experiments, suggesting that the pH was much higher than physiologic and therefore much more likely to induce decomplexing.

There appear to be differences between the toxins, suggesting that complexing proteins have an effect of some kind, but when reconstitution volume and dose are adjusted, it seems possible to produce similar effects with each of the commercially available BoNTAs. Further exploration of the influence of complexing proteins is necessary to establish their role clearly in clinical effect, but current knowledge supports the idea that the clinical effect of complexing proteins, if any, is likely to be short-lived.

Is there any reason to be cautious about the presence of complexing proteins? In general, the introduction of any foreign proteins can activate the human immune system and cause the formation of neutralizing antibodies. Neutralizing antibody formation has potentially significant implications for therapy and can diminish therapeutic response. Further study is warranted to assess whether and to what degree complexing proteins influence antibody development and how this correlates to clinical response.

**Overview of Commercially Available BoNT Formulations**

As of 2012, four distinct BoNT formulations are approved for human medical use in the United
States. BoNTA is available as onabotulinumtoxinA (BoNTA-ONA; Botox, Botox Cosmetic, Allergan, Inc., Irvine, CA), abobotulinumtoxinA (BoNTA-ABO; Dysport, Medicis Aesthetics Inc, Scottsdale, AZ), and incobotulinumtoxinA (BoNTA-INCO; Xeomin, Merz Aesthetics, Inc., San Mateo, CA). BoNTB is available as rimabotulinumtoxinB (BoNTB; Myobloc, Solstice Neurosciences LLC, South San Francisco, CA). At present, BoNTA formulations are the most widely used worldwide and are the only products with an approved indication for aesthetic use. Table 1 lists the BoNTA formulations currently approved worldwide and their trade names.

Additional botulinum products are poised for potential entry into the U.S. aesthetic market in the coming years. PurTox (BoNTA; Mentor Corporation, Santa Barbara, CA) has completed three phase III trials for treatment of glabellar lines. It may enter the U.S. market as early as the end of 2012 and, if approved, will be the second BoNTA formulation available with no complexing proteins. A novel topically applied BoNTA (RT001; Revance Therapeutics, Newark, CA) has completed phase II trials for use in lateral canthal lines; research has been promising and indicates that this method of delivering BoNTA may have clinical benefits. Although these future BoNTA products look interesting, our discussions in this monograph will focus on the formulations currently approved for aesthetic indications in the United States: onabotulinumtoxinA, abobotulinumtoxinA, and incobotulinumtoxinA.

**OnabotulinumtoxinA**

BoNTA-ONA has been used for therapeutic and aesthetic purposes for longer than 2 decades and is one of the most widely researched medicines in the world. It has been approved for more than 20 therapeutic indications in approximately 80 countries. In the United States, BoNTA-ONA is currently approved for the treatment of strabismus, blepharospasm, cervical dystonia, upper limb spasticity, chronic migraine, axillary hyperhidrosis, urinary incontinence, and glabellar lines. Although many aesthetic physicians are familiar with the clinical literature regarding this agent’s use in aesthetic medicine, a brief review of BoNTA-ONA’s aesthetic clinical program is helpful as preface to a discussion of more recently released agents.

The U.S. clinical trial program for the use of BoNTA-ONA in glabellar lines was conducted over a 12-month period in two phases: a placebo-controlled phase (Period 1) followed by an open-label repeat-injection phase (Period 2). Period 1 consisted of two identical randomized, multicenter, double-blind, placebo-controlled studies, in which 405 subjects were randomly assigned to receive a single fixed-dose treatment of BoNTA-ONA. Responders were defined as subjects whose
glabellar line severity changed from moderate or severe at baseline to none or mild at follow-up. At day 30, approximately 80% of subjects had responded to treatment (physician’s assessment). Adverse events were similar to those of placebo, with the exception of blepharoptosis (3.2% in the active group vs 0% in the placebo group in Period 1). Patients enrolled in Period 2 were given the opportunity to receive two additional BoNTA-ONA treatments at 4-month intervals. In patients enrolled in both periods, the overall incidence of blepharoptosis decreased from 3.0% after the first treatment (Period 1) to 2.2% after the second (Period 2) and 0.8% after the third (Period 2). Dosing did not change between the treatments, and the lower incidence of blepharoptosis was possibly due to improvement in injector technique during the study period.

Beyond these initial aesthetic clinical trials, BoNTA-ONA has been the subject of numerous additional clinical studies and literature reports of uses spanning a broad array of therapeutic and aesthetic applications. It has a well-established presence in clinical practice, and more recent BoNT formulations are understandably subject to comparisons with this first-generation neurotoxin.

**AbobotulinumtoxinA**

BoNTA-ABO has been used worldwide for therapeutic purposes since 1991 and is approved for aesthetic use in more than 45 countries. It received FDA approvals for treatment of cervical dystonia and glabellar lines in 2009, becoming the second BoNT formulation approved for aesthetic use in the United States.

BoNTA-ABO’s U.S. aesthetic clinical trial program consisted of three randomized, multicenter, placebo-controlled, double-blind studies and two open-label, repeat-dose studies. Its efficacy was studied through three treatment protocols: a single fixed-dose treatment, repeat injections of a fixed dose, and a single treatment of a variable dose determined according to patient sex and muscle assessment. The day 30 response rate to a single fixed dose was approximately 90% (investigator’s assessment). Subjects were considered responders when their glabellar line severity grade changed from moderate or severe (at maximum frown) at baseline to none or mild at day 30. Day 30 response rate in the variable-dose protocol was approximately 85% (blinded evaluator), with a median duration of effect of 109 days. Study results indicated a median time to onset of 3 days. Adverse events across the clinical studies were similar to placebo, with the exception of blepharoptosis (2% in the active group vs <1% with placebo).

**IncobotulinumtoxinA**

BoNTA-INCO was FDA-approved for the treatment of cervical dystonia and blepharospasm in 2010, followed by approval in 2011 for the treatment of glabellar lines. It is a relative newcomer to the BoNT armamentarium, first registered in Germany in 2005. Despite its short history, BoNTA-INCO’s unique characteristics have made it the subject of extensive research and interest, and its market growth has been rapid, with more than 261,000 patients treated worldwide. BoNTA-INCO is approved in more than 20 countries for therapeutic indications (including cervical dystonia, blepharospasm, and upper limb spasticity) and in 15 countries for the treatment of glabellar lines. BoNTA-INCO is expected to become widely available in the United States in spring 2013.

BoNTA-INCO’s U.S. aesthetic clinical trial program consisted of two randomized, multicenter, placebo-controlled studies (GL-1 and GL-2) of identical design evaluating the safety and efficacy of a 20-U fixed-dose treatment in the glabella; 547 subjects with glabellar lines of at least moderate severity at maximum frown were randomized (2:1) to receive 20 U of BoNTA-INCO administered in five intramuscular injections of 4 U each or placebo. Patients were followed for 120 days and evaluated at days 7,
30, 60, 90, and 120. The primary efficacy endpoint was day 30 response rate. Efficacy was assessed according to a 4-point Facial Wrinkle Scale (FWS); responders were subjects who showed a minimum 2-point improvement from baseline on the FWS, as assessed by the investigator and the patient. Sixty percent of 184 subjects in GL-1 and 48% of 182 subjects in GL-2 met the responder criteria. Adverse events were similar to those with placebo. After the placebo-controlled period, 105 eligible subjects were enrolled in an open-label study evaluating the efficacy and safety of repeat treatments; 99% achieved at least a 1-point improvement on the FWS (investigator assessment) at 4 weeks after treatment (unpublished observations.) Adverse events were mild.

Results from an international phase III active-comparator study demonstrated that 24 U of BoNTA-INCO was as effective as 24 U of BoNTA-ONA in the treatment of glabellar frown lines. Response rates and patient satisfaction scores were high in the BoNTA-ONA and BoNTA-INCO treatment groups; adverse events were similar in type and incidence in both groups. Comparative studies of the two formulations in the treatment of cervical dystonia and blepharospasm also demonstrated that BoNTA-INCO and BoNTA-ONA were equally effective.

### Similarities and Differences Between BoNTA Formulations

Understanding the similarities and differences between available BoNTA products enables more-educated decisions about their usefulness in practice. This section reviews current knowledge of the commonalities and unique qualities of commercially available BoNTA formulations. An overview of basic information about the three formulations can be found in Table 2.

#### Complex Structure

BoNTA-ONA, BoNTA-ABO, and BoNTA-INCO are similar in fundamental ways. They are all BoNTA

| TABLE 2. Commercially Available Botulinum Toxin Type A (BoNTA) Product Overview |
|---------------------------------|---------------------------------|---------------------------------|
| **OnabotulinumtoxinA**          | **AbobotulinumtoxinA**          | **IncobotulinumtoxinA**         |
| Brand Name                      | BOTOX, BOTOX Cosmetic, Vistabel, Vistabex | Dysport, Reloxin, Azzalure | Xeomin, Bocouture |
| Manufacturer                    | Allergan, Inc.                  | Ipsen                           | Merz Pharmaceuticals |
| Serotype & Strain               | Hall Strain                    | Ipsen Strain                    | Hall Strain         |
| Complex molecular weight, kD    | 900                            | -500–900                        | 150                 |
| Unit activity in relation to onabotulinumtoxinA | 1:1 | 1:2–1:4 | 1:1 |
| Stabilization                   | Vacuum-dried                   | Lyophilized                     | Lyophilized         |
| Storage before reconstitution   | Refrigerated (2–8°C)           | Refrigerated (2–8°C)            | Three storage options: |
|                                 |                                |                                | Room temperature    |
|                                 |                                |                                | (20–25°C)           |
|                                 |                                |                                | Refrigerated (2–8°C)| |
|                                 |                                |                                | Frozen (–20 to –10°C) |
| Shelf life before reconstitution| 36 months                      | Not specified                   | 36 months           |
| After reconstitution            | Store refrigerated for up to 24 hours | Store refrigerated for up to 4 hours | Store refrigerated for up to 24 hours |
| Packaging (U/vial)              | 100 or 50                      | 300                             | 100 or 50           |
and have the same basic mechanism of action. The proprietary manufacturing process for each product yields a unique formulation with intrinsic differences. Each formulation’s most basic difference is in complex size and structure. BoNTA-ONA and BoNTA-ABO are formulated as complexes, differing from one another in size and composition. BoNTA-INCO is unique in that it is the first BoNTA formulated with no complexing proteins.

**Diffusion and Spread**

Much of the interest in the differences in complexes between formulations is rooted in the premise that the complex affects toxin movement from the site of injection and thereby has a potential clinical effect. This movement has been referred to as diffusion or spread, and the availability of multiple BoNTA products has created much discussion regarding these terms and how different formulations compare.

To put it simply, all toxins diffuse upon injection, because diffusion is a natural process to attain equilibrium. Although a prevailing premise has been that smaller complexes allow greater diffusion, several studies have found no difference in diffusion rate between formulations. During diffusion, the toxin complex (if present) dissociates, and the neurotoxin proteins begin to bind.

Spread is a clinical consideration distinct from diffusion. Spread refers to the physical movement of the injected product due in part to controllable factors such as injection technique, choice of dilution volume, and needle length and gauge. Some research has found that there are potential differences in the spread of various formulations, regardless of controllable factors. A study of the three commercially available formulations determined that BoNTA-ONA and BoNTA-INCO have comparable spread (as determined by anhidrotic halos) and that BoNTA-ABO has a greater spread. Other studies that compared the spread of BoNTA-ABO and BoNTA-ONA have yielded varying results, with some finding comparable spread between the products and others finding that BoNTA-ABO has greater spread than BoNTA-ONA. Further research is necessary to clarify the differences between study results and the nuances of how formulations may differ in terms of spread.

**Unit Potency**

When it comes to comparing the different BoNTA formulations, perhaps the most common practical question has to do with units and dosages. Because BoNT use in medicine originated with BoNTA-ONA, the unit equivalencies of new formulations are established in the context of BoNTA-ONA potency, but simple conversions from one product to the next are not evident with every formulation. Because BoNTA formulations are unique products, the injector must be clear on the appropriate dose for the formulation being used.

Although regulatory agencies emphasize that the units of different toxin preparations are not interchangeable because of proprietary manufacturing processes and median lethal dose assays, evidence from the literature clearly suggests that BoNTA-ONA and BoNTA-INCO have a clinical equivalency ratio of 1:1; that is, 1 U of BoNTA-INCO is equivalent to 1 U of BoNTA-ONA. This equivalence is convenient for injectors who are widely experienced and comfortable with the use of BoNTA-ONA in that it allows interchangeability of the two formulations with less concern about inadvertent overdosing.

Some research has called into question whether BoNTA-INCO’s potency is truly equivalent to that of BoNTA-ONA. A study by Moers-Carpi compared results obtained from 30 U of BoNTA-INCO with those from 20 U of BoNTA-ONA in the glabellar region and found comparable clinical results between the two treatments, but methodologic questions undermine the significance of this finding. In an earlier study, Carruthers and Carruthers tested BoNTA-ONA in the glabella and were unable to distinguish the results obtained from
administration of 20, 30, and 40 U. This suggests that the failure of the Moers-Carpi study to distinguish 20 U of BoNTA-ONA from 30 U of BoNTA-INCO may not be due to a true difference between the formulations’ potencies but rather to lack of sensitivity of the method. Substantial other research supports clinical equivalence at a 1:1 ratio between these two formulations. BoNTA-ABO’s unit potency is difficult to compare with those of other available formulations. The equivalency ratio of BoNTA-ONA to BoNTA-ABO is approximately 1:2 to 1:4, with expert consensus publications recommending that injectors unfamiliar with BoNTA-ABO apply a ratio of 1:2.5 to 1:3. The same ratio should be applied when attempting conversions between BoNTA-INCO and BoNTA-ABO. Regardless of formulation, optimal outcomes depend on the injector’s understanding of the product being used and the application of professional judgment to the development of a treatment plan that considers a range of product and patient factors.

Stability and Storage

A significant differentiating factor for BoNTA-INCO is its liberal storage requirement. Unopened vials may be stored for 36 months at room temperature, refrigerated, or frozen. Study of the formulation has demonstrated its stability for up to 4 years at room temperature and up to 6 months at 60°C. Unopened vials of BoNTA-ONA require refrigeration but may be stored for up to 36 months. Similarly, unopened vials of BoNTA-ABO require refrigeration; the manufacturer does not specify how long the vial may be kept before use.

All formulations require refrigeration after reconstitution. Manufacturer instructions state that BoNTA-ONA and BoNTA-INCO are to be used within 24 hours of reconstitution; BoNTA-ABO’s manufacturer specifies 4 hours. In practice, physician experts report that reconstituted BoNTA may be stored before use for longer periods with no evident reduction in potency or increase in adverse events. Studies confirm the clinical efficacy and safety of BoNTA when used weeks or months after reconstitution.

Safety of BoNT

BoNT has a remarkable efficacy and safety profile across many areas of medicine, but its popularity may cause some healthcare providers and consumers to forget that it is still a deadly neurotoxin—the most powerful yet discovered. A discussion of potential significant safety questions can help to reestablish an awareness of BoNTA as a potent therapeutic agent for use by educated physicians who understand the product and patient factors that may influence clinical outcomes.

Distant Spread

Distant spread is the unintended extension of BoNT effect into areas noncontiguous to the injection site. When considering issue, it is important to differentiate spread from distant spread. Although BoNTA injections can spread locally into areas adjacent to the injection site (due primarily to controllable factors, as discussed above), this rarely leads to significant clinical concerns. Distant spread is associated with higher-dose indications and may cause life-threatening illness with symptoms consistent with botulism. Hospitalizations and deaths attributed to botulism have been reported in ill and small-for-dates children with cerebral palsy treated with BoNT for muscle spasms and chronic spasticity, an indication that typically uses doses as high as 25 U/kg. Hospitalizations requiring ventilation have also been reported in adults treated with BoNT (BoNTA-ABO) for involuntary muscle movement and frequent neck spasms, another group that may require high doses. There have been no reports of distant spread after the aesthetic use of any of the approved BoNTA agents in normal healthy adults, but it is important to remember this possibility when treating patients with hyperhidrosis, for which the dose is significantly higher than that used cosmetically.
The potential safety outcomes of distant spread are serious—enough so that the FDA took steps to ensure that healthcare professionals and consumers were well-aware of the associated risks. In April 2009, the agency announced requirements for updated safety language in the prescribing instructions for all commercially available BoNT products, as well as the requirement that manufacturers of each formulation develop and implement Risk Evaluation and Mitigation Strategies (REMS).60 The updated prescribing instructions included the addition of a boxed warning about the risk of serious adverse events due to distant spread. The safety language and REMS are not indication dependent; they are required for all products and all indications. The FDA took additional steps to reduce the risk of dosing errors by issuing unique generic names for each commercially available product and emphasizing that BoNT products are not interchangeable.61

**Immunoresistance**

The risk of true immunoresistance with aesthetic use of BoNTA is rare, but it behooves the injector to understand the evidence behind this to ensure safe long-term use of BoNTA products and to protect a patient’s “responder” status. BoNTA immunoresistance reports are largely based on the use of the original batch of BoNTA-ONA (in use through 1997). The current batch of BoNTA-ONA has a markedly lower protein load than the original and is associated with less immunogenicity and a lower rate of neutralizing antibody formation. Jankovic and associates compared the incidence of antibody formation between two groups of patients with cervical dystonia: 42 treated exclusively with the original batch of BoNTA-ONA and 119 treated exclusively with the current batch.62 Four (9.5%) patients treated with the original batch demonstrated blocking antibodies, versus none of the patients treated with the current batch \((p < .004)\).

Naumann and associates conducted a meta-analysis of antibody formation in five indications.63 The 16 clinical trials included in their analysis were conducted between 1999 and 2007 and thus exclusively used the current batch of BoNTA-ONA. Subjects included in the analysis were antibody negative at baseline. Across all indications, 11 of the 2,240 meta-analysis subjects (0.49%) converted from antibody negative to antibody positive (through mouse protection assay) at any follow-up visit, with four (0.2%) remaining antibody positive at the final posttreatment visit. Only three of the 11 antibody-positive patients were clinically unresponsive to BoNTA-ONA, highlighting the difficulty of correlating antibody status with clinical response.

A few recent case reports describe isolated instances of secondary nonresponse to BoNTA in the cosmetic setting. Borodic reported a case in which a patient did not respond to her fourteenth treatment with BoNT-ONA (current lot) after 13 successful treatments.64 Neutralizing antibodies were detected, and she subsequently responded to treatment with BoNTB. Similarly, Lee describes the case of a patient who did not respond to her fifth treatment with BoNTA-ONA (current lot) for masseteric hypertrophy; again, neutralizing antibodies were detected.65 Recently, Dressler reported four new cases of antibody formation in patients who had received BoNTA-ABO (2 patients), BoNTA-ONA (1 patient), or both (1 patient).66 Dressler noted that although three of the patients had risk factors for the development of secondary nonresponse (e.g., booster injections, increased immune system reactivity), one had no apparent risk factors.

Neutralizing antibodies to BoNTA can be difficult to detect using traditional assay methods because of varying levels of specificity and sensitivity,67 as results obtained during the evaluation of neutralizing antibody formation in subjects enrolled in the BoNTA-ABO U.S. clinical trials for glabellar use highlight. Five subjects (0.32% of study population) tested positive for neutralizing antibodies according to radioimmunoprecipitation assay; none tested positive according to the mouse protection assay.68
Antibody formation was assessed in all U.S. clinical studies of BoNTA-INCO for therapeutic indications. Twelve subjects (1.1%) who were antibody negative at baseline developed neutralizing antibodies during the course of their respective study. None was BoNT-naive upon study entry, complicating attempts to characterize the potential of antibody formation with this formulation. In addition, those who tested positive during the study had no detectable antibodies at the study’s conclusion.

**Contraindications**

Although BoNTA is safe, there are some contraindications to its use. All commercially available BoNTA products are contraindicated for use in patients with a known hypersensitivity to BoNTA or the components of the commercial formulation, and in patients with an active infection at the injection site. Beyond these absolute contraindications, BoNTA use is cautioned in patients with neuromuscular disorders (including diseases of the neuromuscular junction) and in those who are receiving concomitant treatment with agents that interfere with neuromuscular transmission. Manufacturers also caution against the possibility of drug interactions with anticholinergic drugs. All FDA-approved BoNTA products are classified pregnancy category C, and their use should be avoided in patients who are known to be pregnant or breastfeeding.

**A Final Note on Safety**

Serious safety problems associated with the aesthetic use of BoNTA are rare. Complications and adverse events after procedures are typically mild, transient, and often preventable. Effective patient assessment and pretreatment evaluation coupled with a precise injection strategy helps ensure safe and effective outcomes.

**Select Current and Emerging Uses of BoNT**

BoNT’s therapeutic applications have expanded rapidly since its first approval in 1989. In this section, we will briefly review some of its more common applications, as well as promising future uses.

**Ophthalmology**

BoNT has been used routinely in ophthalmology for 2 decades, mainly for the treatment of certain forms of strabismus and benign essential blepharospasm. Although surgery remains a cornerstone of strabismus therapy, evidence suggests that BoNT and surgery are comparable in their efficacy and safety.

BoNT is a first-line therapy for blepharospasm associated with facial dystonia. A 2005 literature review of studies designed to evaluate the efficacy and safety of BoNTA for blepharospasm found that this therapy benefited approximately 90% of patients to whom it was administered. A 2008 review found BoNT to be the treatment of choice for blepharospasm and hemifacial spasm.

**Dystonia**

BoNT is considered one of the mainstays of treatment for most focal dystonias. One of the earliest and most common uses of BoNT in clinical practice was to treat the symptoms of cervical dystonia. In 1990, Jankovic and Schwartz found that 71% of 205 patients treated with injections of BoNTA-ONA for cervical dystonia noted substantial improvement in neck movement. Of the 89 patients who reported pain, 76% reported that the pain was almost completely relieved. Most patients improved within 1 week of the injection. BoNTA is the first-choice treatment for primary cranial or cervical dystonia and for other dystonias such as writer’s cramp.

**Aesthetic**

Perhaps no use of BoNT has garnered more attention than its use in aesthetic medicine. What started as a simple treatment for glabellar lines has grown into a flexible tool capable of producing dramatic change in almost every area of the face. BoNTA is also extremely effective in combination with other aesthetic modalities, such as dermal...
fillers, laser, skin resurfacing, and surgery. Specific aesthetic uses of BoNTA will be discussed in Part II of this monograph.

**Hyperhidrosis**

Primary focal hyperhidrosis, particularly for the axillae, palms, feet, and face, is one of the most common conditions treated with BoNT. BoNTA has been shown to safely and effectively reduce focal sweating without major side effects and to markedly improve quality of life. BoNTA-ONA is approved for treatment of hyperhidrosis in the United States.

**Headache/Migraine**

BoNT has been extensively studied as a possible therapy for headache and migraine. Efficacy has not been conclusively demonstrated with BoNT as a treatment for tension-type headaches, but research into migraine has yielded promising results. In the first of two large-scale, phase III double-blind, placebo-controlled clinical trials (Phase III REsearch Evaluating Migraine Prophylaxis Therapy (PRE-EMPT)-1), Aurora and colleagues demonstrated the efficacy of BoNTA-ONA injections as a prophylactic treatment in patients with chronic migraines. Patients were randomized to receive BoNTA-ONA (155–195 U) or placebo injections into the head and neck muscles. Results showed no significant difference for headache episodes, but there were statistically significant reductions in multiple headache symptoms (headache day frequency, severity of headache, and duration of headache) and improvements in quality of life. A second clinical trial (PREEMPT-2) of identical design also reported improvements in headache day frequency and other outcomes. BoNTA-ONA is approved in the United States for prophylactic treatment of headaches in adults with chronic migraines.

**Urology**

One of the most promising new indications for BoNT is for the treatment of overactive bladder, which affects approximately 16% of men and 17% of women in the United States. Studies suggest that BoNT injections significantly improve symptoms of idiopathic overactive bladder by weakening the activity of the neurogenic detrusor muscle or by reducing afferent stimulation signals. A multicenter, randomized, placebo-controlled trial with 275 subjects demonstrated that BoNTA-ONA significantly reduced urinary incontinence in patients with neurogenic detrusor overactivity due to spinal cord injury or multiple sclerosis. BoNTA-ONA was recently approved by the FDA for treatment of urinary incontinence in this population.

BoNT injections are also considered a potentially effective therapy for benign prostatic hyperplasia (BPH). In a 2008 literature review, Boy and colleagues concluded that BoNTA injections into the prostate improve peak flow rate, postvoid residual volume, and prostate volume in a majority of patients. Improvement was seen in patients with varying degrees of BPH, and results lasted up to 12 months. No systemic side effects were reported, nor did BoNTA affect sexual function. Several large placebo-controlled trials of BoNTA in BPH are under way.

**Conclusion**

Despite BoNT’s history and extensive use in medicine for decades, it is our belief that we have only scratched the surface of its potential. New formulations, emerging uses, and continued research into the science and uses of BoNTA will usher in increasingly refined therapeutic approaches, as well as exciting new applications that are perhaps currently just a fantasy. Continued education is important for physicians to optimize use of the agent according to the most current evidence and approaches. Part II of this piece, authored by our colleagues Jean Carruthers, Nathalie Fournier, Martina Kerscher, Javier Ruiz-Avila, and Ada Trindade de Almeida, provides valuable insight into contemporary approaches to aesthetic medicine with BoNT.
References


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