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SPECIAL TOPIC

Neurotoxin Impurities: A Review of Threats to Efficacy

Je-Young Park, MD*
Owen Sunga, MD, MSc†
Rungsima Wanitphakdeedecha, MD, MA, MSc‡
Jürgen Frevert, PhD§

Summary: Recently launched esthetic botulinum toxin serotype A (BoNT/A) products include Nabota/Jeuveau, Meditoxin/Neuronox, and Botulax, which contain nontoxic accessory proteins and excipients. Clinical evidence supporting these formulations, including their purity and potential immunogenicity or their link to treatment failures, is limited. Any nonhuman protein, including nontoxin accessory proteins, can initiate immune reactions, especially if administered repeatedly, yet the issue of BoNT/A-induced immunogenicity is widely contested. However, there have been multiple reports of treatment failures and observations of BoNT/A-induced neutralizing antibodies. Compared with the purified formulation in Xeomin, these recently launched toxins contain higher total neurotoxin quantities, much of which is inactive and exposes patients to potentially immunogenic nontoxin proteins or inactive neurotoxins that increase their risk of developing treatment failure. Well-established products [especially abobotulinumtoxinA (Dysport), onabotulinumtoxinA (Botox) and Xeomin] are accompanied by comprehensive and long-ranging clinical evidence on safety and efficacy in esthetic facial indications, which still remains undisclosed for many of the recently introduced toxins. Clinicians need this information as patients will require repeated BoNT treatments and may be unnecessarily but cumulatively exposed to potential immunogens. To underscore the need for caution and further evidence, we review some of the issues surrounding BoNT/A-induced immunogenicity and antibody-induced treatment failures and argue that using highly purified toxins that do not negatively impact patient outcomes is a prudent clinical decision. *(Plast Reconstr Surg Glob Open 2020;8:e2627; doi: 10.1097/GOX.0000000000002627; Published online 24 January 2020.)*

INTRODUCTION

In 2018, an estimated 7 million botulinum toxin (BoNT) esthetic procedures were performed in the United States,[1] making this an extremely popular minimally invasive cosmetic procedure.[2,3] Established BoNT serotype A (BoNT/A) formulations approved for esthetic use include abobotulinumtoxinA[4] (Dysport and Azzalure; Ipsen Ltd, Slough, Berkshire, United Kingdom), onabotulinumtoxinA[5–9] (Botox, Vistabel, Vistabex; Allergan Inc, Irvine, Calif.), and incobotulinumtoxinA (Xeomin,[10–17] Bocouture; Merz Pharmaceuticals GmbH, Frankfurt am Main, Hessen, Germany). Recently launched toxins include Nabota (Daewoong Pharmaceutical, Seoul, Korea and approved in Korea; approved as Jeuveau in the United States and Nuceiva in Canada and the European Union); Relatox (Microgen, Moscow, Russia; approved in Russia); Regenox (approved in Korea; Hugel Pharma, Seoul, South Korea; approved as Botulax in Korea or Zentox in Thailand); Neuronox (approved in Korea and Russia; Medytox Inc., Ochang, South Korea; also approved as Meditoxin in multiple countries including

*From the *Apkoo-Jung Department, Oracle Dermatology Center, Seoul, Korea; †Merz Aesthetics Asia Pacific Pte Ltd, Singapore; ‡iSKY Innovative Skin & Laser Surgery Center, Bangkok, Thailand; and §Merz Aesthetics, Frankfurt, Germany.*

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List of products used in patient case report: Botox, Xeomin, and BoNT/A products from Korea (brand names unknown).

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Korea, Brazil, and Mexico; Botulift in Brazil, Cunox, or Siax); and CBTX-A (approved in China; Lanzhou Institute of Biological Products, China; also approved as Prosigne in Brazil and Lantox in Russia).[18,19] Although these products contain the same BoNT/A serotype, different manufacturing processes produce preparations with differing compositions, neurotoxin concentrations, toxin complex sizes, and immunogenic risks. Some commercial BoNT/A preparations also contain nontoxic accessory proteins, also known as "complexing proteins or neurotoxin-associated proteins (NAPs)," and excipients such as human serum albumin (HSA) (Table 1). The active neurotoxin dissociates completely from the complexing proteins on reconstitution. Therefore, complexing proteins do not influence the therapeutic effect of the core neurotoxin.[20,21]

SHOULD DOCTORS WORRY ABOUT IMMUNOGENICITY?

The immunogenicity of BoNT/A and its complexing proteins are controversial. Although the relevance of immunogenicity in esthetics is debated, many reports suggest that it should be considered seriously.[22–33] Indeed, the immunogenic potential of BoNT/A products depends on multiple factors including their formulation, quantity of antigenic proteins (proteins that elicit immune responses and antibody production) and accessory proteins, and treatment-related factors such as total toxin dose, injection frequency, and previous exposure.[34] Immunogenicity describes a protein's ability to induce an immune response, and consequently, stimulate antibody formation.[35] The distinction should be made between primary nonresponse (no clinical response to initial and subsequent treatments) and secondary nonresponse or resistance (which develops only after initial successful clinical response to treatment). As with any nonhuman, foreign protein, commercial BoNT/A preparations can initiate immune reactions on injection, particularly when administered repeatedly.[36,37] Secondary treatment failure is caused by neutralizing antibodies (NABs) against the 150kD core neurotoxin (whether deactivated due to denaturation,[38] or nonactivated because of a failure to cleave the toxin). The presence of complexing proteins which, by their bacterial nature, increase the foreign protein load can therefore also increase the risk of inducing an immune response and producing NABs targeting the core neurotoxin.[39–41] This effectively blocks the toxin's pharmacologic action and renders it ineffective, with 13.9% of patients developing NABs in one study.[42] Different manufacturer's BoNT/A preparations also contain varying complexing protein quantities, which may increase the formulation's load of unnecessary bacterial proteins.[1,18,37,43]

Complexing proteins can thus potentially increase the immunogenic risk of NAB formation. Hemagglutinating (HA) and non-HA [nontoxin non-HA (NTNH)] proteins are NAPs found in toxin preparations.[18] NAP-associated BoNT/A elicits stronger immune responses than the 150 kDa core toxin alone.[39] For example, HA-33 is a highly immunoreactive NAP that activates dendritic cells to initiate immune responses,[44,45] and HA-33 removal can minimize immunogenicity. Antibody formation is a concern because repeated BoNT/A injections are required over the long term, which can lead to diminished efficacy over time or even treatment nonresponse.[46] For indications requiring significantly higher toxin doses, one study found NABs in over 15% of patients with cervical dystonia, other dystonias, and spasticity, all of whom had received Dysport and/or Botox.[42] Over a 10-year period, the NAB prevalence in these populations was estimated to be over 27%, 60%, and 47%, respectively. In 1997, Botox was reformulated with a higher specific potency and therefore reduced the amount of antigenicity, resulting in lower nonresponse rates.[47,48] However, even with this less-immunogenic formulation, antibody formation is still reported.[49,50] A direct comparison of immunogenicity between products has not been performed. However, the risk of developing an immune response may be affected by repeated exposure to foreign proteins, antigen quantity, cumulative dose, and the presence of impurities.[51–53]

Moreover, clinical responsiveness may occur in patients with NABs, whereas nonresponsiveness can develop in patients without detectable antibodies. It is unsurprising to find NABs in patients with good outcomes[54] as immune responses can mature over time, after boosters, because of genetic regulation, and even when treated with similar doses or protocols.[43,55] Unfortunately, such patients[56,57] may also have more progressive symptoms, require greater doses of BoNT and longer periods of treatment.[57] This highlights the fact that patient characteristics can influence the development of immunogenicity, especially

Table 1. Composition and Excipient Content of Botulinum Neurotoxin Type A Products

Product Name	Xeomin	Nabota/Jeuveau/Nuceiva	Meditoxin/ Neuronox	Botulax/ Regenox/Zentox	Relatox	CBTX-A/Prosigne/ Lantox
Manufacturer	Merz (Germany)	Daewoong Pharmaceuticals (South Korea)	Medytox Inc (South Korea)	Hugel Inc (South Korea)	Microgen (Russia)	Lanzhou Institute of Biological Products (China)
Composition	Purified toxin (150 kDa)	Complex (900 kDa)	Complex	Complex	Complex (900 kDa)	Complex (900 kDa)
Excipients	4.7 mg sucrose 1 mg HSA	0.5 mg HSA 0.9 mg NaCl	0.5 mg HSA 0.9 mg NaCl	0.5 mg HSA 0.9 mg NaCl	6 mg gelatin 12 mg maltose	Gelatin, dextran, sucrose
Clostridial protein per 100 U (pg)	416 pg	N/A	N/A	5,000 pg[8]	N/A	N/A

N/A, information not publicly available; NaCl, sodium chloride.
Modified from Frevert et al.[18]

those with existing antibodies from previous botulism or tetanus vaccinations.[37] Nonresponse in patients without NABs may be due to incorrect toxin placement, storage, dosing, handling, and even reconstitution.[33]

MANY NEW TOXINS, LITTLE NEW EVIDENCE

Recently introduced BoNT/A formulations for esthetic indications all contain the BoNT protein as part of a unit with complexing proteins (NAPs). In contrast, Xeomin contains only the core neurotoxin protein without other nonfunctional components and is, therefore, distinct to other commercial preparations including Botox, Nabota/Jeuveau, Meditoxin/Neuronox, and Botulax. Prosigne/Lantox, an esthetic toxin from China, contains complexing proteins, although its exact composition is undisclosed. Medytox's third-generation toxin, Coretox, which contains only the core neurotoxin, also has the stabilizer polysorbate rather than HSA.[58–60] Coretox's exact composition is also undisclosed. Botulax, Nabota/Jeuveau, and Meditoxin/Neuronox may also include complexing proteins and the same excipients as Botox (0.5 mg HSA and 0.9 mg sodium chloride).[61,62] Botulax/Zentox preparations contain the 900 kDa BoNT/A protein with 0.5 mg of human albumin and 0.9 mg of sodium chloride.[18] Commercial documents[63] show that Nabota/Jeuveau contained much lower total protein content (0.75 ng/vial by Bradford assay or 4.6 ng/vial by ultraviolet absorbance) than other toxins from Asian companies, but these large differences were not explained. It should be stressed that these data are only calculations as a conclusive protein measurement of the neurotoxin or toxin complex in the final product by Enzyme-linked Immunosorbent Assay (ELISA) is not possible due to its excessive levels of HSA (0.5 mg/vial). Size exclusion chromatography of Nabota/Jeuveau on a G4000 column showed that it is "composed of 900 kDa (over 98%) and pure 300 kDa (impurities 0%)" [sic] proteins. Whether the 300 kDa component comprises NTNH proteins or other complexing proteins is undisclosed. Nabota/Jeuveau' actual antigenic protein load is unknown. However, considering that 150 kDa core protein comprises a sixth of the 900 kDa complex protein load, calculations by Daewoong state that each vial of Nabota/Jeuveau contains 0.12 ng/vial of the core 150 kDa toxin component and 0.75 ng/vial of antigenic protein. We previously showed that a neurotoxin preparations' level of antigenicity is equivalent to its clostridial protein content,[64] as NABs were generated following repeated Botox and Dysport injections, but not after repeated Xeomin injections. Therefore, unlike formulations devoid of nontoxin proteins, Nabota/Jeuveau may still pose some immunogenic risk.

These recently introduced toxins must also be stored refrigerated (2°C–8°C), have varying shelf lives [2 (Nabota/Jeuveau and Relatox) to 3 years (Meditoxin/Neuronox and Botulax)], and are "biosimilar" to Botox. Per 100 U vial, Botulax contains 844 ± 43 pg of toxin, whereas Nabota/Jeuveau contains 754 ± 11 pg of toxin, Meditoxin/Neuronox contains 575 ± 6 pg of toxin, and Relatox contains 578 ± 48 pg of toxin. Within each 100 U vial, the specific potency (toxin units per pg neurotoxin protein) of Botulax, Nabota/Jeuveau, Meditoxin/Neuronox, and Relatox is 0.118, 0.13, 0.174, and 0.173 U/pg, respectively.[18] These differences between the specific potency and total neurotoxin content indicate the presence of a high amount of inactive neurotoxin protein and, therefore, a low-specific potency. In contrast, the highly purified Xeomin formulation contains 416 ± 6 pg/100 U with the highest specific potency of 0.240 U/pg,[18,65–67] indicating that Xeomin contains no inactive neurotoxin. Xeomin can also be stored for 3 years at room temperature. Many studies have demonstrated equivalent efficacy and potency between Xeomin and Botox[42,68–73]; there is no rational need for these other products' higher neurotoxin quantities, which exposes patients to unnecessary and potentially immunogenic proteins. Ultimately, this increases their risk of antibody formation and future treatment failures.

Furthermore, the quantity of the core 150 kDa toxin found per 100 U of Xeomin, Botox, or Dysport, is now known to be 0.44, 0.73, and 0.65 ng, respectively.[74] Using a 1:1 dose ratio of Botox to Xeomin actually only delivers 0.44 ng/100 U of Xeomin compared with 0.73 ng/100 U of Botox, suggesting that in addition to the NAPs, Botox has inactive 150 kDa neurotoxin protein. A higher immunogenic risk would, therefore, be expected with Botox, without any accompanying increase in therapeutic advantage. Moreover, Prosigne/Lantox, which contains complexing proteins, was used to treat upper face wrinkles but has caused urticarial plaques.[75] This allergic reaction to Prosigne/Lantox was confirmed with subsequent intradermal testing and required corticosteroid and antihistamine treatments. Unlike other BoNT/A products that contain HSA, Prosigne contains bovine gelatin, which is potentially allergenic.[76]

Clinical data on the safety and efficacy of Botulax, Nabota/Jeuveau, Meditoxin/Neuronox, and Coretox in medical esthetics are limited[77–80] (Table 2). To our knowledge, 2 esthetic trials with Botulax have been completed, the results of which have not yet been disclosed or published (ClinicalTrials.gov Identifier: NCT01791920[81] and NCT03641950[82]); 3 medical esthetic trials were completed with Meditoxin/Neuronox without publicly disclosed results or peer-reviewed publications (NCT01259557,[83] NCT03216473,[84] and NCT03216408[85]); and 5 medical esthetic trials were completed with Nabota/Jeuveau, only some of which were published or disclosed on ClinicalTrials.gov or CenterWatch[86] (NCT02568150,[87] NCT02947815,[88] NCT01629875,[89] NCT02334436,[90] and NCT02334423[91]). Although not all companies publish or disclose all of their sponsored studies, there are little data on these toxins' clinical efficacy and safety in esthetic indications. Patients will require repeat BoNT treatments and be cumulatively exposed to superfluous proteins. Company training literature cites phase III trials of Botulax against an undeclared US toxin for the treatment of nonesthetic indications (blepharospasm). This trial is not registered in conventional clinical study databases (ie, ClinicalTrials.gov)[92] but showed noninferiority for Botulax compared with the US toxin. However, platysmal injections of Botulax were associated with botulism-like, progressive

dysphagia, reinforcing the need for additional safety studies.[93] Since 2006, Meditoxin/Neuronox has been available in South Korea for blepharospasm treatment. To our knowledge, there are no data for Meditoxin/Neuronox in esthetic indications. Currently, 3 peer-reviewed publications for Nabota/Jeuveau are available.[80,84,95]

Concerns surrounding antibody-induced treatment failures and immunogenicity are legitimate, especially in patients seeking treatments to improve quality of life, mental health and body image issues and who may ultimately be exposed to higher toxin quantities,[96–98] or in those requiring long-term and repetitive BoNT/A use.[34] Using purified neurotoxins can reduce the risk of developing a secondary nonresponse.[99]

DIFFERENCES IN NONTOXIN CONSTITUENTS BETWEEN BONT/A PRODUCTS

Information is limited on the purity of the recently introduced Asian toxins, their immunogenicity and associated potential to cause treatment failure, but differences exist across all BoNT/A brands, including the established brands, in terms of the bacterial strain used and each company's proprietary purification methods. Although Botox is further ethanol and ammonium sulfate precipitated,[100] Dysport is purified through chromatography and dialysis.[101,102] Dysport's manufacturing process creates partially degraded complexing proteins and some contaminants, including flagellin and a *clp* protease involved in protein degradation.[66,103]

Flagellin initiates immune responses by interacting with the Toll-like receptor 5 (TLR5)[104] to trigger the proinflammatory nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway[105] and other innate and adaptive immune responses. It facilitates the development of adaptive immunity through dendritic cell maturation, cytokine expression, and co-stimulatory cytokine production.[106] Flagellin significantly increases the production of Immunoglobulin G1 (IgG1) and Immunoglobulin G2a (IgG2) antibody by T-cells. Because many other different immune cells (including monocytes, Langerhans

Table 2. Known Esthetic (Facial Indications) Trials for Commercially Available Esthetic Toxins

ClinicalTrials.gov-listed Completed Phase III/IV Clinical Trials Using Commercial Botulinum Toxin A for Medical Esthetic Interventions with Disclosed Results, in Adult Patients (over 18 y)			
Toxin Name/Generic Name	**NCT Number**	**Title**	**Conditions**
Nabota/prabotulinumtoxinA Botulax/letibotulinum toxin A Neuronox Coretox	Results not posted on ClinicalTrials.gov		
Xeomin/incobotulinumtoxinA	NCT00770211	IncobotulinumtoxinA (Xeomin) Versus Placebo in the Treatment of Glabellar Frown Lines	Moderate to severe glabellar frown lines
	NCT00770029	IncobotulinumtoxinA (Xeomin) Versus Placebo in the Treatment of Glabellar Frown Lines No. 2	Moderate to severe glabellar frown lines
	NCT00406367	IncobotulinumtoxinA (Xeomin) Versus Placebo in the Treatment of Blepharospasm	Blepharospasm
	NCT00986570	Clinical Trial to Assess Efficacy, Safety and Tolerability of Botulinum Toxin A (Xeomin) in Treatment of Expression Wrinkles in the Upper Third of the Face	Skin aging
	NCT00777803	NT 201 (Xeomin /Bocouture) in Comparison With *Clostridium botulinum* Toxin Type A in the Treatment of Glabellar Frown Lines	Glabellar frown lines
	NCT01728337	Phase Iv Study On Muscle Activity Of Two Commercial Preparations Of Botulinum	Sun-induced wrinkles
	NCT01896895	Efficacy and Safety Study of Botulinum Toxin Type A Against Placebo to Treat Abnormal Contraction or Twitch of the Eyelid	BEB
	NCT01814774	A Retrospective Chart Review of BOTOX and Xeomin for the Treatment of Cervical Dystonia and Blepharospasm	Cervical dystonia/blepharospasm
	NCT02096081	The Treatment of Glabellar Frown Lines	Glabellar frown lines
	NCT00959907	Comparison of Two Commercial Preparations of Botulinum Toxin Type A	Wrinkles in frontal area
	NCT01271452	Safety and Efficacy of Two Types of Botulinum Toxin Type A For the Treatment of Glabellar Lines	Glabellar lines
	NCT01608659	An Observational Retrospective Study to Evaluate Treatment Patterns of Botulinum Toxin Type A	Facial rhytides
	NCT03048383	Comparison of Three Botulinum Neuromodulators for Management of Facial Synkinesis	Facial nerve injuries/facial paresis associated with facial nerve dysfunction/facial asymmetry/synkinesis
	NCT00761592	Comparison of Two Botulinum Type A Products in the Treatment of Blepharospasm	Blepharospasm
	NCT01014871	Comparison of Two Botulinum Toxins Type A on Forehead Wrinkles	Wrinkles

(*Continued*)

Table 2. (*Continued*)

ClinicalTrials.gov-listed Completed Phase III/IV Clinical Trials Using Commercial Botulinum Toxin A for Medical Esthetic Interventions with Disclosed Results, in Adult Patients (over 18 y)

Toxin Name/Generic Name	NCT Number	Title	Conditions
Botox/ OnabotulinumtoxinA	NCT02353871	Efficacy and Safety of *Clostridium botulinum* Toxin Type A to Improve Appearance of Moderate to Severe Glabellar Lines	Moderate to severe glabellar lines
	NCT01391312	Patient Satisfaction Study of BOTOX Cosmetic in the Treatment of Moderate to Severe Frown Lines	Glabellar frown lines
	NCT01269801	Study of BOTOX and JUVEDERM for Treatment of Moderate to Severe Facial Wrinkles and Folds	Wrinkles
	NCT02261467	A Safety and Efficacy Study of OnabotulinumtoxinA in Forehead and Glabellar Facial Rhytides	Forehead rhytides/glabellar rhytides
	NCT02261493	A Safety and Efficacy Study of OnabotulinumtoxinA in Upper Facial Rhytides	Facial rhytides/glabellar rhytides
	NCT02195687	BOTOX in the Treatment of Crow's Feet Lines in China	Lateral canthal lines/Crow's feet lines
	NCT02450526	Dysport in the Treatment of Glabellar Lines in Chinese Subjects	Glabellar lines
	NCT01777620	A Study of Subject Satisfaction With BOTOX Cosmetic Treatment in Facial Rhytides	Facial rhytides
	NCT02493946	Efficacy and Safety of Botulinum Toxin Type A Haemagglutinin Complex Next Generation (BTX-A-HAC NG) in Glabellar Lines	Glabellar lines
	NCT01586819	Lateral Canthal Rhytides With Medium Depth Chemical Peel With or Without Pretreatment With Botulinum Toxin A	Wrinkles
	NCT01189747	Safety and Efficacy Study of Botulinum Toxin Type A for the Treatment of Crow's Feet Lines	Lateral canthus rhytides/ Crow's feet lines
	NCT01797094	BOTOX in the Treatment of Upper Facial Lines in Japan	Upper facial rhytides/Crow's feet lines/glabellar lines/ frown lines
	NCT01814670	Treatment With Botulinum Toxin Type A (BOTOX) in Chinese Patients With Moderate to Severe Frown Lines	Glabellar rhytides
	NCT00959907	Comparison of Two Commercial Preparations of Botulinum Toxin Type A	Wrinkles in frontal area
	NCT01189760	Safety and Efficacy Study of Botulinum Toxin Type A for the Treatment of Crow's Feet Lines and Frown Lines	Facial rhytides/Crow's feet lines/glabellar lines
	NCT01224015	Safety and Efficacy Study of Botulinum Toxin Type A for the Treatment of Crow's Feet Lines and Frown Lines	Facial rhytides/Crow's feet lines/glabellar lines
	NCT01271452	Safety and Efficacy of Two Types of Botulinum Toxin Type A For the Treatment of Glabellar Lines	Glabellar lines
	NCT00989768	Field of Effects of Two Commercial Preparations of Botulinum Toxin Type A	Wrinkles in frontal area
	NCT01797081	BOTOX in the Treatment of Crow's Feet Lines in Japan	Lateral canthus rhytides/ Crow's feet lines
	NCT00777803	NT 201 (Xeomin /Bocouture) in Comparison With *Clostridium botulinum* Toxin Type A in the Treatment of Glabellar Frown Lines	Glabellar frown lines
	NCT01728337	Phase Iv Study On Muscle Activity Of Two Commercial Preparations Of Botulinum	Sun-induced wrinkles
	NCT02176356	Patient Satisfaction Study of Combined Facial Treatment With BOTOX Cosmetic, JUVÉDERM and LATISSE (HARMONY Study)	Facial rhytides/Crow's feet lines/glabellar lines/ nasolabial fold
	NCT00856414	Patient Satisfaction With Treatment of BOTOX Cosmetic for the Temporary Correction of Moderate to Severe Glabellar Lines	Skin aging
	NCT00986570	Clinical Trial to Assess Efficacy, Safety and Tolerability of Botulinum Toxin A (Xeomin) in Treatment of Expression Wrinkles in the Upper Third of the Face	Skin aging
	NCT01529203	Subjects' Satisfaction on Pan Facial Aesthetic Enhancement After Treatment With Azzalure and the Restylane Range	Aging
	NCT02718118	Comparison of Dysport Reconstitution at 1.5 mL and 2.5 mL for the Treatment of Moderate to Severe Glabellar Lines	Glabellar lines/wrinkles
	NCT02096081	The Treatment of Glabellar Frown Lines	Glabellar frown lines
	NCT00761592	Comparison of Two Botulinum Type A Products in the Treatment of Blepharospasm	Blepharospasm
	NCT01896895	Efficacy and Safety Study of Botulinum Toxin Type A Against Placebo to Treat Abnormal Contraction or Twitch of the Eyelid	BEB
	NCT00770211	IncobotulinumtoxinA (Xeomin) Versus Placebo in the Treatment of Glabellar Frown Lines	Moderate to severe glabellar frown lines
	NCT00770029	IncobotulinumtoxinA (Xeomin) Versus Placebo in the Treatment of Glabellar Frown Lines No. 2	Moderate to severe glabellar frown lines
	NCT00406367	IncobotulinumtoxinA (Xeomin) Versus Placebo in the Treatment of Blepharospasm	Blepharospasm
	NCT02334436	A Phase III Study to Demonstrate the Safety and Efficacy of DWP-450 to Treat Glabellar Lines - EV-002	Glabellar Frown lines
	NCT02334423	A Phase III Study to Demonstrate the Safety and Efficacy of DWP-450 to Treat Glabellar Lines - EV001	Glabellar frown lines

(*Continued*)

Table 2. (*Continued*)

ClinicalTrials.gov-listed Completed Phase III/IV Clinical Trials Using Commercial Botulinum Toxin A for Medical Esthetic Interventions with Disclosed Results, in Adult Patients (over 18 y)			
Toxin Name/Generic Name	**NCT Number**	**Title**	**Conditions**
Dysport/ AbobotulinumtoxinA	NCT01529203	Subjects' Satisfaction on Pan Facial Aesthetic Enhancement After Treatment With Azzalure and the Restylane Range	Aging
	NCT01896895	Efficacy and Safety Study of Botulinum Toxin Type A Against Placebo to Treat Abnormal Contraction or Twitch of the Eyelid	BEB
	NCT00761592	Comparison of Two Botulinum Type A Products in the Treatment of Blepharospasm	Blepharospasm
	NCT00406367	IncobotulinumtoxinA (Xeomin) Versus Placebo in the Treatment of Blepharospasm	Blepharospasm
	NCT01777620	A Study of Subject Satisfaction With BOTOX Cosmetic Treatment in Facial Rhytides	Facial rhytides
	NCT01189760	Safety and Efficacy Study of Botulinum Toxin Type A for the Treatment of Crow's Feet Lines and Frown Lines	Facial rhytides/Crow's feet lines/glabellar lines
	NCT01224015	Safety and Efficacy Study of Botulinum Toxin Type A for the Treatment of Crow's Feet Lines and Frown Lines	Facial rhytides/Crow's feet lines/glabellar lines
	NCT02176356	Patient Satisfaction Study of Combined Facial Treatment With BOTOX Cosmetic, JUVEDERM and LATISSE (HARMONY Study)	Facial rhytides/Crow's feet lines/glabellar lines/ nasolabial fold
	NCT02261493	A Safety and Efficacy Study of OnabotulinumtoxinA in Upper Facial Rhytides	Facial rhytides/glabellar rhytides
	NCT02261467	A Safety and Efficacy Study of OnabotulinumtoxinA in Forehead and Glabellar Facial Rhytides	Forehead rhytides/glabellar rhytides
	NCT01391312	Patient Satisfaction Study of BOTOX Cosmetic in the Treatment of Moderate to Severe Frown Lines	Glabellar frown lines
	NCT00777803	NT 201 (Xeomin /Bocouture) in Comparison With *Clostridium botulinum* Toxin Type A in the Treatment of Glabellar Frown Lines	Glabellar frown lines
	NCT02096081	The Treatment of Glabellar Frown Lines	Glabellar frown lines
	NCT02334436	A Phase III Study to Demonstrate the Safety and Efficacy of DWP-450 to Treat Glabellar Lines - EV-002	Glabellar frown lines
	NCT02334423	A Phase III Study to Demonstrate the Safety and Efficacy of DWP-450 to Treat Glabellar Lines - EV001	Glabellar frown lines
	NCT02450526	Dysport in the Treatment of Glabellar Lines in Chinese Subjects	Glabellar lines
	NCT02493946	Efficacy and Safety of Botulinum Toxin Type A Haemagglutinin Complex Next Generation (BTX-A-HAC NG) in Glabellar Lines	Glabellar lines
	NCT01271452	Safety and Efficacy of Two Types of Botulinum Toxin Type A For the Treatment of Glabellar Lines	Glabellar lines
	NCT02718118	Comparison of Dysport Reconstitution at 1.5 mL and 2.5 mL for the Treatment of Moderate to Severe Glabellar Lines	Glabellar lines/wrinkles
	NCT01814670	Treatment With Botulinum Toxin Type A (BOTOX) in Chinese Patients With Moderate to Severe Frown Lines	Glabellar rhytides
	NCT02195687	BOTOX in the Treatment of Crow's Feet Lines in China	Lateral canthal lines/Crow's feet lines
	NCT01189747	Safety and Efficacy Study of Botulinum Toxin Type A for the Treatment of Crow's Feet Lines	Lateral canthus rhytides/ Crow's feet lines
	NCT01797081	BOTOX in the Treatment of Crow's Feet Lines in Japan	Lateral canthus rhytides/ Crow's feet lines
	NCT00770211	IncobotulinumtoxinA (Xeomin) Versus Placebo in the Treatment of Glabellar Frown Lines	Moderate to severe glabellar frown lines
	NCT00770029	IncobotulinumtoxinA (Xeomin) Versus Placebo in the Treatment of Glabellar Frown Lines No. 2	Moderate to severe glabellar frown lines
	NCT02353871	Efficacy and Safety of *Clostridium botulinum* Toxin Type A to Improve Appearance of Moderate to Severe Glabellar Lines	Moderate to severe glabellar lines
	NCT00986570	Clinical Trial to Assess Efficacy, Safety and Tolerability of Botulinum Toxin A (Xeomin) in Treatment of Expression Wrinkles in the Upper Third of the Face	Skin aging
	NCT00856414	Patient Satisfaction With Treatment of BOTOX Cosmetic for the Temporary Correction of Moderate to Severe Glabellar Lines	Skin aging
	NCT01728337	Phase Iv Study On Muscle Activity Of Two Commercial Preparations Of Botulinum	Sun-induced wrinkles
	NCT01797094	BOTOX in the Treatment of Upper Facial Lines in Japan	Upper facial rhytides/Crow's feet lines/glabellar lines/ frown lines
	NCT01269801	Study of BOTOX and JUVEDERM for Treatment of Moderate to Severe Facial Wrinkles and Folds	Wrinkles
	NCT01586819	Lateral Canthal Rhytides With Medium Depth Chemical Peel With or Without Pretreatment With Botulinum Toxin A	Wrinkles
	NCT00959907	Comparison of Two Commercial Preparations of Botulinum Toxin Type A	Wrinkles in frontal area
	NCT00989768	Field of Effects of Two Commercial Preparations of Botulinum Toxin Type A	Wrinkles in frontal area

Trials on nonesthetic indications on nonfacial areas, such as upper limb spasticity, are not relevant to our discussion and are excluded.
BEB, bilateral blepharospasm; NCT, national clinical trial.

cells, and natural killer cells) express TLR5 on their surfaces,[107–113] flagellin may regulate the immune system. Flagellin has been shown to enhance the regulatory activity of regulatory T-cells, block T-cell receptor-mediated activation of regulatory T-cells,[114] activate human memory CD4+ and CD8+ T-cell proliferation and cytokine production,[109] and stimulate CD4+ T-cell proliferation.[115] Taken together, flagellin thus has a proven capacity for immunostimulation,[116,117] but whether it interacts with TLR5 to induce immune reactions when used as an esthetic toxin is a topic for further study because this may contribute to treatment failures observed with Dysport. In contrast, all clostridial proteins are removed through a stepwise chromatographic purification during Xeomin production.[10,118]

To see if clostridial DNA was present among either the pure 150 kD, core neurotoxin, or to complexing proteins, Botox and Xeomin were analyzed by polymerase chain reaction (PCR) [see figure, Supplemental Digital Content 1, which displays Samples from reconstituted vials of Botox and Xeomin were analyzed by PCR on a Roche LightCycler 480 thermocycler to generate amplification curves (top). Sigmoidal curves (pink, blue and green) show the presence of clostridial DNA in Botox samples. Xeomin samples (red, yellow, and purple) did not produce amplification curves, indicating the absence of clostridial DNA. (Bottom) Electropherogram of NTNH (left) and HA34 (right) after PCR of reconstituted Botox and Xeomin samples provides visual evidence of these clostridial DNA contaminants in Botox. L indicates 100 bp ladder; 1 and 7—positive control (genomic DNA of *Clostridium botulinum* type A); 2 and 8—Botox batch C2525C3; 3 and 9—Botox batch C0919C2; 4 and 10—Xeomin batch 21140; 5 and 11—Xeomin batch 20317; 6 and 12—negative control (water). The black arrow denotes primer dimers, a by-product of the PCR that indicates background or "noise" and does not negatively affect protein identification here, http://links.lww.com/PRSGO/B306]. Botox preparations were found to contain 5.8 -12.6 pg (per vial) of clostridial DNA, nontoxic nonhemagglutinin (NTNH) and hemagglutinin HA34 DNAs, whereas Xeomin preparations had none. As bacterial DNA contains sequences that allow binding to TLR9 on DCs and immune activation, products containing bacterial DNA may be immunogenic and promote antibody production against the 150 kDa complex.[119]

BONT/A SECONDARY TREATMENT FAILURES

As the number of BoNTs entering the East Asian market increases, physicians are correspondingly observing a worrying increase in cases of toxin nonresponsiveness.[23,25] We have observed cases of partial secondary treatment failures and estimate an incidence of approximately 10% of our patients to be affected. Anecdotally, we also note an increasing incidence of this in the last few years, and now see more patients wishing to resolve previous treatment failures.

The use of high single doses, short inter-injection intervals and booster injections, aging of patients' immune systems, and toxin immunogenicity are all risk factors for toxin nonresponsiveness. East Asian treatment strategies have evolved from using low toxin doses (≈50 U/session) for conventional facial muscle relaxation and dynamic line corrections, to using relatively high doses (≈100–400 U/session) to reduce muscle volume for facial or body shape contouring.[120,121] In larger body areas such as large calves, contouring treatments may be required twice yearly for 3 years, with cumulative toxin doses of 2,400 U (400 U/session). Thus, physicians should expect to diagnose partial or complete secondary treatment failure.

Of 27 patients suffering various dystonic syndromes and diagnosed with complete treatment failure due to NABs, 81% had previous partial responses.[122] Physicians must consider the possibility of immunogenicity if low clinical responses are observed, especially after repetitive treatments. Once antibodies have formed, increasing injection doses may be ineffective and may increase antibody titers. Because the neurotoxin in the different formulations is very similar, switching between brands does not produce a positive outcome, although some reports have demonstrated positive responses following Xeomin treatment of secondary nonresponders.[123–125] Using other BoNT serotypes (eg, type B) fails to sustain responses and can induce serotype-B immunogenicity.[43] The most prudent approach is to prevent NAB formation from the start. To lower the risks for nonresponsiveness, we recommend formulations with the lowest protein load, no adjuvant proteins, and only the active neurotoxins without inactive components.

CONCLUSIONS

A lack of clinical data prevents a direct cause-and-effect link being drawn between the presence of clostridial protein contaminants in commercial BoNT/A preparations and negative treatment outcomes. However, physicians must exercise caution when injecting formulations with potentially immunogenic foreign proteins. Nonneurotoxin components can act as adjuvants that promote antibody formation and cause immune reactions that lead to treatment nonresponse and compromise outcomes. Robust and long-term clinical data are still needed on the newer toxins emerging from Asia, which may be inexpensive[126–128] and lead to unnecessarily frequent injections. Using highly purified BoNT/A preparations containing only the highly purified, 150 kDa core neurotoxin protein, without any known contaminants or impurities, will ensure effective, durable, and well-tolerated treatment outcomes.

Owen Sunga, MD
Merz Asia Pacific Pte. Ltd
21 Biopolis Rd, #06-03/04 North Tower
Nucleos, Singapore 138670
E-mail: owen.sunga@merz.sg

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