

IncobotulinumtoxinA: A Highly Purified and Precisely Manufactured Botulinum Neurotoxin Type A

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ABSTRACT

Aesthetic dermatologic applications of botulinum neurotoxin (BoNT), including treatment of glabellar lines, horizontal forehead lines, and crow's feet, were the most common non-surgical cosmetic procedures in the US in 2017, with high levels of subject satisfaction. Since the first BoNT type A (BoNT-A) formulation was approved in 1989, the number of formulations available on the world's commercial markets has increased and new approvals are expected. BoNT is produced by *Clostridium botulinum* in nature as part of a large protein complex. However, the unnecessary clostridial proteins, which dissociate from BoNT under physiological conditions with a half-life of <1 minute, have no role in clinical applications. Data demonstrate that BoNT administration can elicit an immunological response, leading to production of neutralizing antibodies that can be associated with reduced efficacy or treatment non-response. As repeat treatments are required to maintain efficacy, clinicians should be aware of the possibility of antibody development and choose a BoNT with the lowest risk of immunogenicity. IncobotulinumtoxinA is manufactured using advanced technology to precisely isolate the pure BoNT without unnecessary clostridial proteins, and with low immunogenicity and high specific activity. In incobotulinumtoxinA clinical studies, no previously BoNT-naïve subjects developed neutralizing antibodies, and there was no secondary non-response to incobotulinumtoxinA treatment. Here we review the role of unnecessary clostridial proteins in BoNT-A and discuss the unique incobotulinumtoxinA manufacturing and purification process with a focus on the implications for use in aesthetic medicine.

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INTRODUCTION

Since the first botulinum neurotoxin (BoNT) formulation was approved by the US Food and Drug Administration (FDA) in 1989,[1,2] the number of approved indications, and BoNT products commercially available worldwide, has increased. Aesthetic dermatologic applications of BoNT were the most common non-surgical cosmetic procedures in the US in 2017,[3,4] and have high levels of patient satisfaction.[5]

The three most widely used and commercially available BoNT type A (BoNT-A) formulations are: abobotulinumtoxinA (Dysport®/Azzalure®, Ipsen Biopharm),[6-8] incobotulinumtoxinA (Xeomin®/Bocouture®, Merz Pharmaceuticals GmbH),[9-11] and onabotulinumtoxinA (Botox®/Vistabel®, Allergan Inc.)[1,12,13] (approved indications differ by product and country, see individual product listings).[2] Several new BoNT-A formulations have recently been introduced in different countries,[14,15] however, incobotulinumtoxinA currently remains the only BoNT formulation approved in commercial markets worldwide that was intentionally designed to contain only the required therapeutic component, the pure BoNT, free from unnecessary clostridial proteins.[14] Here we discuss the role of these unnecessary proteins in BoNT-A, and the unique manufacturing and purification process for incobotulinumtoxinA, with a focus on the implications for use in aesthetic medicine.

Role of Unnecessary Clostridial Proteins

All BoNT-A products discussed here contain BoNT-A from a *C. botulinum* Hall strain,[2] which is produced in nature (its native form) as part of a larger multimeric complex with accessory proteins.[16,17] Three BoNT-A complexes are formed, comprised of BoNT-A and a non-toxic non-hemagglutinin (NTNHA) protein in the smaller 300 kDa M complex, with the addition of several hemagglutinin (HA) proteins in the larger 500 kDa L and 900 kDa LL complexes.[16,17] IncobotulinumtoxinA contains only the 150 kDa BoNT-A active therapeutic component purified from the therapeutically unnecessary clostridial proteins.[18] In contrast, abobotulinumtoxinA, onabotulinumtoxinA, and, the newer addition, prabotulinumtoxinA (Nabota®, Daewong Therapeutics, Korea/Evolus®, Evolus Inc., Europe, USA/Nuceiva®, Evolus Inc., Canada) all contain the HA and NTNHA proteins complexed with the 150 kDa BoNT.[2,14,19,20] Investigational drug candidate daxibotulinumtoxinA (RT002, Revance Therapeutics

Inc.) is composed of the 150 kDa BoNT-A with a peptide excipient, RTP-004, derived from the human immunodeficiency virus type 1 (HIV-1) transactivator of transcription (TAT) protein.[21]

In naturally occurring BoNT, the NTNHA protein directly binds to BoNT, protecting it from low pH and proteolytic cleavage in the gastrointestinal tract,[22] while HA proteins help to transport the BoNT into the bloodstream through interactions with intestinal epithelial cells.[23-25] By protecting the BoNT from digestive destruction, the complexing proteins increase the toxicity of native BoNT compared with the purified BoNT when ingested via oral route.[26]

Contrary to their role as mediator for the oral toxicity of naturally occurring BoNT, complexing proteins have no role in clinical applications.[17,27] Stability of the commercial BoNT complex (300, 500, 900 kDa) is pH dependent and dissociates from the active 150 kDa BoNT with a half-life of <1 minute under physiological conditions.[17] Further, reconstitution of complex-containing products with saline (as recommended for all BoNT products) results in dissociation even under acidic conditions, such that ≥85% of onabotulinumtoxinA and all detectable BoNT in abobotulinumtoxinA is in the free, uncomplexed 150 kDa form prior to injection,[17] further suggesting unnecessary clostridial proteins have no role in stabilizing or otherwise contributing to clinical effect in BoNT in target tissues.

Furthermore, unnecessary clostridial proteins also do not appear to contribute to the stability of BoNT in the commercially manufactured vial. IncobotulinumtoxinA remained stable, with no effects on the content (neurotoxin or excipients) or the biological activity of the BoNT, when stored unreconstituted with refrigeration or at ambient temperatures for ≤48 months, and under elevated temperatures ≤60°C (140°F) for one month.[28] Efficacy was also retained when incobotulinumtoxinA was stored at 25°C (77°F) for one week post-reconstitution.[29]

IncobotulinumtoxinA is the only currently approved BoNT-A formulation that can be stored unreconstituted at ambient temperatures, which may be a benefit in everyday clinical practice. Because incobotulinumtoxinA does not require cold-chain storage, this can be advantageous during the summer months, in temperate countries, in practices with limited space, or in outpatient clinics where refrigeration may be problematic. Besides the practical and clinical implications, it should be taken into consideration that greenhouse gas emissions due to secondary shipping and handling of non-temperature-controlled versus temperature-controlled BoNT reduces our carbon footprint.[63]

Immunogenicity

Although unnecessary clostridial proteins play no role in clinical applications when injected,[17,27] or in stabilizing the molecule,[17,28] they may act as adjuvants, stimulating an immune response, thus potentially altering the response to BoNT therapy.[27] Two types of antibodies may be produced in response to injected BoNT; neutralizing antibodies against BoNT itself have been reported[30] and can lead to reduced efficacy or treatment non-response, even at the low doses indicated for aesthetic applications.[31,32] The non-neutralizing antibodies directed against the unnecessary clostridial proteins do not affect the biological activity of the neurotoxin, but the unecessary clostridial proteins may act as adjuvants.[30,33,34] Two pre-clinical studies suggest an adjuvant role for unnecessary proteins, increasing the antigenicity of injected BoNT.[33,35] Unnecessary clostridial proteins, but not pure BoNT-A, stimulated an immune response by modulating the inflammatory response.[33,36] However, results should be interpreted with caution as these studies included formaldehyde-fixed proteins, non-comparable dosing, and shorter injection intervals than those used in clinical practice.

Although the extent of non-response in aesthetics is unknown, reports of neutralizing antibody development are increasing.[32,37] Individuals are increasingly initiating aesthetic treatment earlier in life.[3] As BoNT treatment effects are temporary, and repeat injections are required to maintain efficacy,[32] clinicians should consider both the temporal extent of exposure[2] and BoNT protein load to reduce the risk of neutralizing antibody formation and treatment non-response.[32] Previous exposure for use in aesthetics may lead to non-response if BoNT-A were required for essential therapeutic treatment (eg, of post-stroke spasticity) later in life.

In contrast to abobotulinumtoxinA and onabotulinumtoxinA, repeated incobotulinumtoxinA treatment in rabbits did not result in the formation of neutralizing antibodies, suggesting immunogenicity is lower with incobotulinumtoxinA.[38] Consistent with this, incobotulinumtoxinA is the only formulation with no subjects in clinical studies who have developed neutralizing antibodies and demonstrated a secondary lack of treatment response in the PI/SmPC product characteristics.[1,6,10] A recent analysis of the US FDA adverse event (AE) reporting system database found the incidence of AEs involving decreased therapeutic effect was 2.2% (15/689) for incobotulinumtoxinA; 9.2% (79/858) for abobotulinumtoxinA; and 11.6% (1247/10,733) for onabotulinumtoxinA. Reduced efficacy was more frequent among subjects on >1 year of treatment vs <1 year for both abobotulinumtoxinA (11.9% [36/302] vs 4.3% [11/257]) and onabotulinumtoxinA (19.6% [504/2577] vs 10.1% [539/5350]), but not incobotulinumtoxinA (0.0% [0/10] vs 4.5% [13/291] cases).[39]

IncobotulinumtoxinA: Advanced Manufacturing and Purification

IncobotulinumtoxinA is purified and precisely manufactured in a world-class German facility using advanced technology under Good Manufacturing Practice. The unnecessary clostridial proteins are removed in a refined process using step-wise chromatography to precisely isolate the therapeutic component

(Figure 1), followed by the addition of excipients (sucrose and human serum albumin) and lyophilization.[2] Stringent quality checks include visual inspection, vial weight checks, leak detection after 7-day hold, and ultraviolet light datametrics code application to confirm toxin identity.

The unique and precise purification process of incobotulinumtoxinA ensures that only the active 150 kDa neurotoxin, needed to achieve the clinical effect, is included.[18] IncobotulinumtoxinA contains no nucleic acid content, compared with onabotulinumtoxinA, which contains DNA fragments of the neurotoxin gene.[40] OnabotulinumtoxinA has a molecular weight of 900 kDa including unnecessary clostridial proteins and BoNT-A.[20] The exact molecular weight of the BoNT complex in abobotulinumtoxinA is unknown, but is accepted to be up to 900 kDa, with the 300 kDa protein complex as the most abundant.[2]

The specific activity of abobotulinumtoxinA, incobotulinumtoxinA, and onabotulinumtoxinA is reported as 154 U/ng, 227 U/ng, and 137 U/ng, respectively, based on the mean BoNT concentration in 100 U (0.65 ng, 0.44 ng, and 0.73 ng of the 150 kDa BoNT, respectively; Figure 2).[41] The high specific activity of incobotulinumtoxinA is consistent with no inactivation of the BoNT during purification.[41] In comparison, as onabotulinumtoxinA is reported to contain 0.73 ng of neurotoxin protein, the low specific activity of onabotulinumtoxinA suggests that a proportion of BoNT protein is inactive.[41]

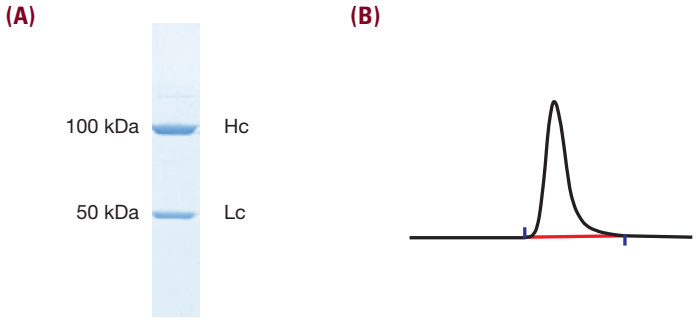
OnabotulinumtoxinA is manufactured by several precipitation and redissolution steps prior to the addition of excipients (sodium chloride [NaCl] and human serum albumin), and vacuum-dried,[2] resulting in a thin film of the product. An early study demonstrates that NaCl concentration may affect BoNT stability during freeze-drying, resulting in BoNT denaturation.[42] This may explain the lower specific activity of onabotulinumtoxinA compared with abobotulinumtoxinA and incobotulinumtoxinA.[41] Denatured BoNT may increase the risk of an immune response, subsequent development of neutralizing antibodies, and potential secondary non-response to further treatment.[2,43]

Implications for Use in Aesthetic Medicine

The clinical and real-world efficacy of incobotulinumtoxinA is established in >200 peer-reviewed publications.[44,45] IncobotulinumtoxinA is proven to effectively reduce upper facial lines for up to 4 months post-treatment,[46-49] with a high level of subject satisfaction[47,48] and no treatment-related serious AEs in a Phase III trial leading to upper facial lines approval in Europe.[47] The safety profile of incobotulinumtoxinA was further confirmed in a pooled analysis of 13 prospective multicenter studies in aesthetic indications of crow's feet, glabellar lines, and upper facial lines. Overall, the frequency of treatment-related AEs was low and analysis of repeat-dose studies suggested the incidence of AEs may decrease with repeated treatments over time.[50]

In incobotulinumtoxinA clinical studies, no previously BoNT-naive subject developed neutralizing antibodies[51-55] or demonstrated secondary lack of treatment response,[50,52] even with doses up to 800 U in the treatment of upper-limb spasticity,[52] consistent with the low immunogenicity of incobotulinumtoxinA. In the published literature, all subjects who developed neutralizing antibodies and secondary non-response after incobotulinumtoxinA treatment had received treatment with another BoNT formulation.[51,56] IncobotulinumtoxinA may be the best choice for patients seeking long-term treatment with BoNT due to the lower risk of neutralizing antibodies, such as treatment-naive patients. Moreover, in some cases, the low immunogenicity of incobotulinumtoxinA may offer a renewed therapeutic effect in subjects with antibody-induced BoNT-A treatment non-response.[57,58]

FIGURE 1. Protein content of incobotulinumtoxinA. **(A)** Representative SDS-polyacrylamide gel electrophoresis of the active pharmaceutical ingredient of incobotulinumtoxinA showing the heavy chain (Hc, ≈100kDa) and the light chain chain (Lc, ≈ 50kDa). In the native form of the neurotoxin Hc and Lc are linked by a disulfide bond, which is cleaved in this analysis. **(B)** Representative size exclusion chromatography of the active pharmaceutical ingredient of incobotulinumtoxinA. The neurotoxin is eluted in a volume corresponding to a molecular weight of ≈ 150kDa.

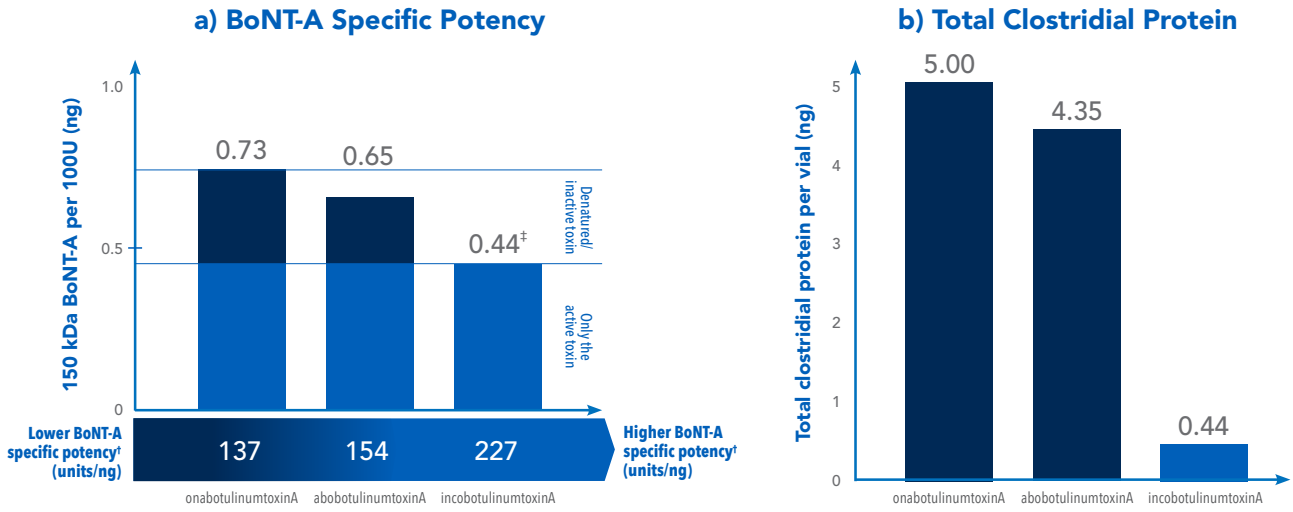


New Entrants to the BoNT Commercial Market

The number of BoNT-A products available on the commercial markets worldwide continues to increase and two new entrants currently under review are planning to file FDA Biologics License Applications. PrabotulinumtoxinA is a similar version of onabotulinumtoxinA in terms of pharmacological development and manufacturing compared with currently approved formulations.[19] The specific potency of prabotulinumtoxinA was recorded as 133 U/ng compared with 240 U/ng for incobotulinumtoxinA, with a high percentage of inactive neurotoxin,[14] consistent with inactivation of the BoNT due to the presence of NaCl during drying,[42] and the manufacturing process of onabotulinumtoxinA discussed above.

DaxibotulinumtoxinA is a new BoNT-A formulation currently in clinical development for aesthetic (glabellar lines) and therapeutic (cervical dystonia and plantar fasciitis) indica-

FIGURE 2. Specific potency and clostridial protein content of BoNT formulations.



†Unique manufacturing technology of incobotulinumtoxinA isolates the therapeutic component and has allowed for the highest specific BoNT-A potency: 100 U / 0.44 ng = 227 U/ng.
‡Lower neurotoxin protein in incobotulinumtoxinA than previously reported may be due to higher sensitivity in the ELISA assay used for analysis.
Abbreviations: BoNT-A, botulinum neurotoxin type A; ELISA, enzyme-linked immunosorbent assay; ng, nanograms; pg, picograms; U, units

tions.[59,60] DaxibotulinumtoxinA contains the purified 150 kDa BoNT-A without unnecessary clostridial proteins or human albumin in a lyophilized powder, and is stable at room temperature,[60] like incobotulinumtoxinA.[28] DaxibotulinumtoxinA includes an excipient peptide, RTP-004, consisting of the protein transduction domain sequence from the HIV-1 TAT protein at each end, separated by a peptide consisting of 35 positively charged lysine residues. The lysine residues form a core that is claimed to noncovalently bind the BoNT.[21] The protein was previously believed to be useful as a carrier for a transdermal BoNT formulation (RT001)[21] and is now postulated to play a role in increased duration of efficacy compared with onabotulinumtoxinA.[59] The greater efficacy and duration of response with daxibotulinumtoxinA 40 U vs onabotulinumtoxinA 20 U (median, 23.6 vs 18.8 weeks) is likely due to a doubling of the dose administered rather than the excipient peptide, as no consistent significant difference in efficacy or duration of response was noted between daxibotulinumtoxinA 20 U vs onabotulinumtoxinA 20 U,[59] and a similar duration of response was recently reported for onabotulinumtoxinA 40 U (median, 24.0 weeks).[61] A strong dose-response has also been observed in a recently conducted randomized, double-blind study with incobotulinumtoxinA 20–100 U, with duration of treatment effect up to 9 months in some subjects and no unexpected safety findings.[62] The long-term consequences of the addition of polylysine structures to BoNT are not known.

CONCLUSIONS

BoNT-A treatment has been available for several decades and is among the most common non-surgical cosmetic procedures worldwide, with good efficacy and safety profiles, and high levels of patient satisfaction. Because patients are seeking aesthetic treatments at increasingly younger ages, both neurotoxin protein load and protein load over time can increase the risk of diminished efficacy or treatment non-response due to neutralizing antibodies over a young individual's lifetime. This may also impact essential treatment for therapeutic indications later in life. Clinicians should therefore consider the least immunogenic BoNT-A formulation to meet individual treatment requirements, thus minimizing potential for lack of efficacy due to neutralizing antibodies for future treatment options. IncobotulinumtoxinA currently remains the only BoNT formulation approved in commercial markets worldwide that was intentionally designed to contain only the required therapeutic BoNT component. The unique and precise purification of incobotulinumtoxinA represents innovative advances in BoNT manufacturing. The data reviewed here suggest incobotulinumtoxinA offers an advantage over other BoNT-A formulations, due to its lower potential to provoke an immune response when used clinically.

DISCLOSURES

M. Kerscher has conducted clinical trials for Galderma/Q-Med, Ipsen, and Merz Pharmaceuticals GmbH (as Head of the Division of Cosmetic Sciences, University of Hamburg, Germany), and has acted as a speaker for Galderma/Q-Med and Merz Pharmaceuticals.

R. Wanitphakdeedecha has no conflicts of interest to disclose.

A. Trindade de Almeida is an advisor for Allergan, Galderma/Q-Med, Lupin, Mantecorp, Merz Pharmaceuticals, and Sinclair, has participated in clinical trials for Allergan, has received re-

search support from Merz Pharmaceuticals, and is a speaker for Allergan, Merz Pharmaceuticals, and Theraskin.

C. Maas is an investigator for Merz Pharmaceuticals GmbH and Allergan, and has acted an advisor to Merz.

J. Frevert is a former employee of, and current consultant for, Merz Pharmaceuticals GmbH.

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REFERENCES

1. Allergan Inc. Highlights of prescribing information - Botox®. 2017. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/103000s5302lbl.pdf. Accessed 2 October 2018.
2. Frevert J. Pharmaceutical, biological, and clinical properties of botulinum neurotoxin type A products. *Drugs R D.* 2015;15(1):1-9.
3. American Society for Dermatologic Surgery. Survey on dermatologic procedures. 2017. Available from: https://www.asds.net/portals/0/PDF/procedure-survey-results-presentation-2017.pdf. Accessed 20 September 2018.
4. American Society of Plastic Surgeons. Plastic Surgery Statistics Report. 2017. Available from: https://www.plasticsurgery.org/documents/News/Statistics/2017/plastic-surgery-statistics-full-report-2017.pdf. Accessed 2 October 2018.
5. Fagien S, Carruthers JD. A comprehensive review of patient-reported satisfaction with botulinum toxin type a for aesthetic procedures. *Plast Reconstr Surg.* 2008;122(6):1915-1925.
6. Ipsen Biopharm Ltd. Highlights of prescribing information - Dysport®. 2017. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/125274s109lbl.pdf. Accessed 2 October 2018.
7. Galderma (U.K.) Ltd. Azzalure® Summary of Product Characteristics. 2018. Available from: https://www.medicines.org.uk/emc/product/6584/smpc. Accessed 2 October 2018.
8. Ipsen Biopharm Ltd. Dysport® 500 U Summary of Product Characteristics. 2017. Available from: https://www.medicines.org.uk/emc/medicine/32114. Accessed 2 October 2018.
9. Merz Pharma UK Ltd. XEOMIN® (50/100/200) Summary of Product Characteristics. 2017. Available from: http://www.medicines.org.uk/emc/medicine/24582. Accessed 2 October 2018.
10. Merz Pharmaceuticals LLC. Highlights of prescribing information – Xeomin®. 2018. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/125360s073lbl.pdf. Accessed 2 October 2018.
11. Merz Pharma UK Ltd. Bocouture® Summary of Product Characteristics. 2018. Available from: https://www.medicines.org.uk/EMC/medicine/23251/SPC/Bocouture/. Accessed 2 October 2018.
12. Allergan Ltd. Botox® 100 U Summary of Product Characteristics. 2017. Available from: http://www.medicines.org.uk/EMC/medicine/112/SPC/. Accessed 2 October 2018.
13. Allergan Ltd. Vistabel® Summary of Product Characteristics. 2018. Available from: https://www.medicines.org.uk/emc/product/187/smpc. Accessed 2 October 2018.
14. Frevert J, Ahn KY, Park MY, Sunga O. Comparison of botulinum neurotoxin type A formulations in Asia. *Clin Cosmet Investig Dermatol.* 2018;11:327-331.
15. Evolus Inc. Nuceiva product monograph. 2018. Available from: https://pdf.hres.ca/dpd_pm/00046932.PDF. Accessed 11 October 2018.
16. Inoue K, Fujinaga Y, Watanabe T, et al. Molecular composition of Clostridium botulinum type A progenitor toxins. *Infect Immun.* 1996;64(5):1589-1594.
17. Eisele KH, Fink K, Vey M, Taylor HV. Studies on the dissociation of botulinum neurotoxin type A complexes. *Toxicon.* 2011;57(4):555-565.
18. Frevert J. Xeomin: an innovative new botulinum toxin type A. *Eur J Neurol.* 2009;16 (Suppl. 2):11-13.
19. Wanitphakdeedecha R, Ungaksornpairote C, Kaewkes A, Sathaworawong A, Vanadurongwan B, Lektrakul N. A pilot study comparing the efficacy of two formulations of botulinum toxin type A for muscular calves contouring. *J Cosmet Dermatol.* 2018:[Epub ahead of print].
20. Zhang L, Lin WJ, Li S, Aoki KR. Complete DNA sequences of the botulinum neurotoxin complex of Clostridium botulinum type A-Hall (Allergan) strain. *Gene.* 2003;315:21-32.
21. Glogau RG, Waugh JM. Preclinical transcutaneous flux experiments using a macromolecule transport system (MTS) peptide for delivery of botulinum toxin type A. Poster presented at the 66th Annual Meeting of the American Academy of Dermatology; February 1–5, 2008; San Antonio, TX. Revance Therapeutics, Inc. 2008. Available from: http://www.revance.com/pdfs/Preclinical-transcutaneous-flux-experiment-type-a.pdf. Accessed 12 September 2018.
22. Gu S, Rumpel S, Zhou J, et al. Botulinum neurotoxin is shielded by NTNHA in an interlocked complex. *Science.* 2012;335(6071):977-981.
23. Fujinaga Y, Matsumura T, Jin Y, Takegahara Y, Sugawara Y. A novel function of botulinum toxin-associated proteins: HA proteins disrupt intestinal epithelial barrier to increase toxin absorption. *Toxicon.* 2009;54(5):583-586.
24. Matsumura T, Sugawara Y, Yutani M, et al. Botulinum toxin A complex exploits intestinal M cells to enter the host and exert neurotoxicity. *Nat Commun.* 2015;6:6255.
25. Ghosal KJ, Patel K, Singh BR, Hale ML. Role of critical elements in botulinum neurotoxin complex in toxin routing across intestinal and bronchial barriers. *PLoS One.* 2018;13(7):e0199524.
26. Cheng LW, Onisko B, Johnson EA, et al. Effects of purification on the bioavailability of botulinum neurotoxin type A. *Toxicology.* 2008;249(2-3):123-129.
27. Frevert J, Dressler D. Complexing proteins in botulinum toxin type A drugs: a help or a hindrance? *Biologics.* 2010;4:325-332.
28. Grein S, Mander GJ, Fink K. Stability of botulinum neurotoxin type A, devoid of complexing proteins. *Botulinum J.* 2011;2(1):49-57.
29. Soares DJ, Dejoseph LM, Zuliani GF, Liebertz DJ, Patel VS. Impact of postreconstitution room temperature storage on the efficacy of incobotulinumtoxinA treatment of dynamic lateral canthus lines. *Dermatol Surg.* 2015;41(6):712-717.
30. Joshi SG, Elias M, Singh A, et al. Modulation of botulinum toxin-induced changes in neuromuscular function with antibodies directed against recombinant polypeptides or fragments. *Neuroscience.* 2011;179:208-222.
31. Dressler D, Wohlfahrt K, Meyer-Rogge E, Wiest L, Bigalke H. Antibody-induced failure of botulinum toxin a therapy in cosmetic indications. *Dermatol Surg.* 2010;36 (Suppl. 4):2182-2187.
32. Torres S, Hamilton M, Sanches E, Starovatova P, Gubanova E, Reshetnikova T. Neutralizing antibodies to botulinum neurotoxin type A in aesthetic medicine: five case reports. *Clin Cosmet Investig Dermatol.* 2014;7:11-17.
33. Lee J-C, Yokota K, Arimitsu H, et al. Production of anti-neurotoxin antibody is enhanced by two subcomponents, HA1 and HA3b, of *Clostridium botulinum* type B 16S toxin-haemagglutinin. *Microbiology.* 2005;151(Pt 11):3739-3747.
34. Göschel H, Wohlfarth K, Frevert J, Dengler R, Bigalke H. Botulinum A toxin therapy: neutralizing and nonneutralizing antibodies – therapeutic consequences. *Exp Neurol.* 1997;147(1):96-102.
35. Kukreja R, Chang T-W, Cai S, et al. Immunological characterization of the subunits of type A botulinum neurotoxin and different components of its associated proteins. *Toxicon.* 2009;53(6):616-624.
36. Wang L, Sun Y, Yang W, Lindo P, Singh BR. Type A botulinum neurotoxin complex proteins differentially modulate host response of neuronal cells. *Toxicon.* 2014;82:52-60.
37. Stengel G, Bee EK. Antibody-induced secondary treatment failure in a patient treated with botulinum toxin type A for glabellar frown lines. *Clin Interv Aging.* 2011;6:281-284.
38. Blümel J, Frevert J, Schwaier A. Comparative antigenicity of three preparations of botulinum neurotoxin type A in the rabbit. *Neurotox Res.* 2006;9:238.
39. Kazerooni R. Decrease in therapeutic effect among botulinum neurotoxin type A agents: analysis of the FDA adverse event reporting system database. Paper presented at: TOXINS 2019. Basic science and clinical aspects of botulinum and other neurotoxins; 16-19 January 2019.
40. Frevert J, Groenewald C. Presence of clostridial DNA in botulinum toxin products. *Toxicon.* 2015;93 (Suppl.):S28.
41. Frevert J. Content of botulinum neurotoxin in Botox®/Vistabel®, Dysport®/Azzalure®, and Xeomin®/Bocouture®. *Drugs R D.* 2010;10(2):67-73.
42. Goodnough MC, Johnson EA. Stabilization of botulinum toxin type A during lyophilization. *Appl Environ Microbiol.* 1992;58(10):3426-3428.
43. Jankovic J, Schwartz K. Response and immunoresistance to botulinum toxin injections. *Neurology.* 1995;45(9):1743-1746.
44. Jost WH, Benecke R, Hauschke D, et al. Clinical and pharmacological properties of incobotulinumtoxinA and its use in neurological disorders. *Drug Des*

Devel Ther. 2015;9:1913-1926.
45. Kerscher M, Yutskovskaya Y, Flynn TC. IncobotulinumtoxinA in esthetics. *J Drugs Dermatol.* 2013;12(6):e111-120.
46. Kerscher M, Rzany B, Prager W, Turnbull C, Trevidic P, Inglefield C. Efficacy and Safety of IncobotulinumtoxinA in the Treatment of Upper Facial Lines: Results From a Randomized, Double-Blind, Placebo-Controlled, Phase III Study. *Dermatol Surg.* 2015;41(10):1149-1157.
47. Streker M, Luebberding S, Krueger N, Harrington L, Kerscher M. Patient-reported outcomes after incobotulinumtoxinA treatment for upper facial wrinkles. *Dermatol Surg.* 2015;41 (Suppl. 1):S29-38.
48. Kane MA, Gold MH, Coleman WP, 3rd, et al. A randomized, double-blind trial to investigate the equivalence of incobotulinumtoxina and onabotulinumtoxinA for Glabellar Frown Lines. *Dermatol Surg.* 2015;41(11):1310-1319.
49. Prager W, Wissmuller E, Kollhorst B, Williams S, Zschocke I. Comparison of two botulinum toxin type A preparations for treating crow's feet: a split-face, double-blind, proof-of-concept study. *Dermatol Surg.* 2010;36 (Suppl. 4):2155-2160.
50. Coleman WP, III, Sattler G, Weissenberger P, Hast MA, Hanschmann A. Safety of incobotulinumtoxinA in the treatment of facial lines: Results from a pooled analysis of randomized, prospective, controlled clinical studies. *Dermatol Surg.* 2017;43:S293-S303.
51. Evidente VGH, Fernandez HH, LeDoux MS, et al. A randomized, double-blind study of repeated incobotulinumtoxinA (Xeomin®) in cervical dystonia. *J Neural Transm.* 2013;120(12):1699-1707.
52. Wissel J, Bensmail D, Ferreira JJ, et al. Safety and efficacy of incobotulinumtoxinA doses up to 800 U in limb spasticity: The TOWER study. *Neurology.* 2017;88(14):1321-1328.
53. Kanovský P, Slawek J, Denes Z, et al. Efficacy and safety of treatment with incobotulinum toxin A (botulinum neurotoxin type A free from complexing proteins; NT 201) in post-stroke upper limb spasticity. *J Rehabil Med.* 2011;43(6):486-492.
54. Kanovský P, Slawek J, Denes Z, et al. Efficacy and safety of botulinum neurotoxin NT 201 in poststroke upper limb spasticity. *Clin Neuropharmacol.* 2009;32(5):259-265.
55. Truong DD, Gollomp SM, Jankovic J, et al. Sustained efficacy and safety of repeated incobotulinumtoxinA (Xeomin®) injections in blepharospasm. *J Neural Transm (Vienna).* 2013;120(9):1345-1353.
56. Dressler D, Adib SF, Bigalke H. IncobotulinumtoxinA (Xeomin) can produce antibody-induced therapy failure in a patient pretreated with abobotulinumtoxinA (Dysport). *J Neural Transm.* 2014;121(7):769-771.
57. Dressler D, Pan L, Adib Saberi F. Antibody-induced failure of botulinum toxin therapy: re-start with low-antigenicity drugs offers a new treatment opportunity. *J Neural Transm (Vienna).* 2018;125(10):1481-1486.
58. Hefter H, Hartmann C, Kahlen U, Moll M, Bigalke H. Prospective analysis of neutralising antibody titres in secondary non-responders under continuous treatment with a botulinumtoxin type A preparation free of complexing proteins-a single cohort 4-year follow-up study. *BMJ Open.* 2012;2(4).
59. Carruthers J, Solish N, Humphrey S, et al. Injectable daxibotulinumtoxina for the treatment of glabellar lines: a phase 2, randomized, dose-ranging, double-blind, multicenter comparison with onabotulinumtoxina and placebo. *Dermatol Surg.* 2017;43(11):1321-1331.
60. Jankovic J, Truong D, Patel AT, et al. Injectable daxibotulinumtoxinA in cervical dystonia: a phase 2 dose-escalation multicenter study. *Mov Disord Clin Pract.* 2018;5(3):273-282.
61. Allergan announces results of higher dose Botox® cosmetic (onabotulinumtoxinA) for the treatment of moderate to severe glabellar lines. PRNewswire, 14 September 2018. Available from: https://www.prnewswire.com/news-releases/allergan-announces-results-of-higher-dose-botox-cosmetic-onabotulinumtoxina-for-the-treatment-of-moderate-to-severe-glabellar-lines-300713102.html.
62. Maas C. Safety and efficacy of escalating doses of incobotulinumtoxinA for extended treatment of glabellar frown lines: a randomized, double-blind study. *J Am Acad Dermatol.* 2018;79(3):AB250.
63. Thistle F. Greenhouse gas emissions and botulinum neurotoxin packaging. *Hospital Pharmacy Europe.* 2012; 64:27-30.

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