

# A new treatment for focal dystonias: incobotulinumtoxinA (Xeomin<sup>®</sup>), a botulinum neurotoxin type A free from complexing proteins

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**Abstract:** Dystonia is a movement disorder of uncertain pathogenesis that is characterized by involuntary and inappropriate muscle contractions which cause sustained abnormal postures and movements of multiple or single (focal) body regions. The most common focal dystonias are cervical dystonia (CD) and blepharospasm (BSP). The first-line recommended treatment for CD and BSP is injection with botulinum toxin (BoNT), of which two serotypes are available: BoNT type A (BoNT/A) and BoNT type B (BoNT/B). Conventional BoNT formulations include inactive complexing proteins, which may increase the risk for antigenicity, possibly leading to treatment failure. IncobotulinumtoxinA (Xeomin<sup>®</sup>; Merz Pharmaceuticals GmbH, Frankfurt, Germany) is a BoNT/A agent that has been recently Food and Drug Administration-approved for the treatment of adults with CD and adults with BSP previously treated with onabotulinumtoxinA (Botox<sup>®</sup>; Allergan, Inc, Irvine, CA) – a conventional BoNT/A. IncobotulinumtoxinA is the only BoNT product that is free of complexing proteins. The necessity of complexing proteins for the effectiveness of botulinum toxin treatment has been challenged by preclinical and clinical studies with incobotulinumtoxinA. These studies have also suggested that incobotulinumtoxinA is associated with a lower risk for stimulating antibody formation than onabotulinumtoxinA. In phase 3 noninferiority trials, incobotulinumtoxinA demonstrated significant improvements in CD and BSP symptoms in both primary and secondary measures, compared with baseline, and met criteria for noninferiority versus onabotulinumtoxinA. In placebo-controlled trials, incobotulinumtoxinA also significantly improved the symptoms of CD and BSP, with robust outcomes in both primary and secondary measures. The use of incobotulinumtoxinA has been well tolerated in all trials, with an adverse event profile similar to that of onabotulinumtoxinA. Based on these data, incobotulinumtoxinA is a safe and effective BoNT/A for the treatment of CD and BSP, and may pose a lower risk for immunogenicity leading to treatment failure compared with other available BoNT agents. This paper reviews the treatment of focal dystonias with BoNTs, in particular, incobotulinumtoxinA. Controlled trials from the existing incobotulinumtoxinA literature are summarized.

**Keywords:** blepharospasm, botulinum toxin, cervical dystonia, complexing proteins, dystonia, incobotulinumtoxinA (Xeomin<sup>®</sup>)

## Introduction

Dystonia is a movement disorder characterized by sustained, involuntary muscle contractions that lead to repetitive twisting movements and abnormal postures in affected areas, including the head, neck, face, trunk, or arms and legs.<sup>1,2</sup> These symptoms result from concomitant contraction of agonist and antagonist muscles, with overflow into adjacent muscles.<sup>1</sup> The pathophysiology of dystonia is still unclear, but it is believed to involve a deficiency of cortical motor inhibition, possibly stemming from a motor

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network dysfunction associated with abnormalities in the sensorimotor cortex, basal ganglia, and cerebellum.<sup>1,3-5</sup> Dystonia is variously classified according to whether it is primary (idiopathic) or secondary to other neurologic conditions, injuries, abnormalities, or drug effects; childhood- or adult-onset; and the body area(s) affected.<sup>1</sup> When described based on body distribution, classifications of dystonia include: (1) focal, in which one region, such as craniofacial, neck, limb, or axial (shoulders, trunk), is involved, (2) segmental, which includes  $\geq 2$  adjacent regions, (3) multifocal, in which  $\geq 2$  nonadjacent regions are involved, (4) generalized, which may include one or both legs, the trunk, and/or other regions, and (5) hemidystonia, in which the ipsilateral arms and legs are affected.<sup>1,6</sup> Focal dystonias occur most frequently in adults, whereas generalized dystonias often begin in childhood.<sup>2</sup> Primary, adult-onset, focal dystonia is by far the most common type of dystonia.<sup>1,7-9</sup>

The two most common types of focal dystonias, in order of prevalence, are cervical dystonia (CD; also known as spasmodic torticollis) and blepharospasm (BSP),<sup>7,10</sup> although BSP has been reported to be more common than CD in specific populations.<sup>11-13</sup> The estimated prevalence of these disorders has been reported to be between six and nine per 100,000 for CD and about three per 100,000 for BSP.<sup>7,10,14</sup> Both CD and BSP are associated with multiple adverse effects on quality of life, including social and occupational dysfunction and disability, embarrassment, anxiety, and depression.<sup>15-19</sup>

CD is characterized by involuntary contractions of cervical muscles that cause abnormal head movements and postures, and may feature jerking or twisting movements, transient spasms, shoulder elevation, stiffness/tightness, and an irregular jerky head tremor.<sup>15,20</sup> Individuals with BSP, on the other hand, exhibit involuntary, repetitive, spasmodic, and sustained eyelid closure.<sup>21,22</sup> A hallmark of both BSP and CD, as well as other forms of dystonia, is the presence of a sensory trick, or “geste antagoniste,” that may assist a patient in maintaining a normal posture.<sup>23</sup> Mean age at onset is about 41 years for persons with CD and 56 years for those with BSP.<sup>24</sup> Despite these differences in clinical features, CD and BSP may share etiologic and pathophysiologic mechanisms.<sup>25</sup> In patients with CD, BSP occurs concomitantly in approximately 10% of those affected,<sup>20,26</sup> and about 30% of patients with BSP experience spread of dystonic symptoms to the neck.<sup>27</sup> Both CD and BSP appear to be associated with a bilateral reduction in striatal postsynaptic dopamine D2 receptor binding, as indicated by functional imaging studies,<sup>28-30</sup> although recent findings suggest that the defect in focal dystonia may be in D3, rather

than D2, receptor expression.<sup>31</sup> In addition, both CD and BSP have been associated with enhancement of the blink reflex, which suggests hyperexcitability of brainstem pathways,<sup>32-34</sup> impaired recognition of facial expression of disgust, which involves basal ganglia activation,<sup>35</sup> and bilateral impaired sensory spatial discrimination, which suggests abnormal sensory processing within the somatosensory cortex.<sup>36</sup> A brain voxel-based morphometry study also revealed similar alterations in gray matter structures related to sensorimotor processing in patients with BSP and CD.<sup>37</sup>

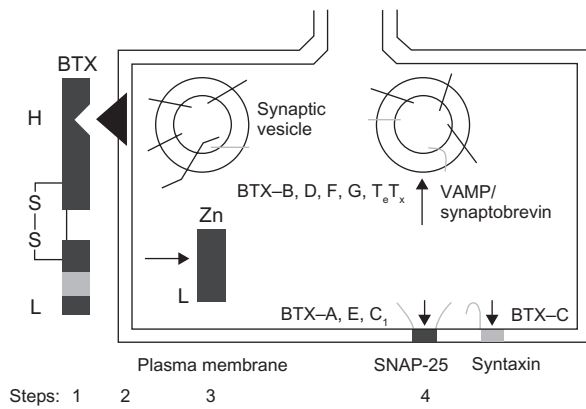
The aim of this review is to familiarize the clinician with the differing biological and physical properties of botulinum toxins (BoNTs) used for treatment of focal dystonias and to summarize the clinical profile of incobotulinumtoxinA (Xeomin®; Merz Pharmaceuticals GmbH, Frankfurt, Germany), the most recently Food and Drug Administration (FDA)-approved BoNT.

## Methods

The clinical data for incobotulinumtoxinA summarized in this review were obtained by performing a PubMed search using the terms “Xeomin,” “NT201,” “NT 201,” and “incobotulinumtoxinA.” Two phase 1 trials in healthy volunteers were identified in the search and were included in the review. All clinical trials in focal dystonia (CD or BSP) identified via this search were also included; additionally, pooled analyses and subanalyses generated from these trials and presented at society conferences were included. All the clinical trials in focal dystonia were well-controlled, double-blind trials with the exception of an open-label immunogenicity trial in CD that reported an objective outcome measure: presence of neutralizing antibodies.

## BoNT treatment of focal dystonia: overview

BoNT is the first-line recommended treatment for most types of focal dystonia, including both CD and BSP.<sup>38,39</sup> BoNT acts primarily by binding with high specificity and affinity to presynaptic cholinergic axon terminals and blocking the release of acetylcholine into the neuromuscular junction, thereby causing temporary denervation and muscle weakness for periods typically lasting 3–4 months.<sup>40,41</sup> A total of seven antigenically distinct serotypes (types A–G) of naturally occurring toxin have been isolated from unique strains of *Clostridium botulinum*. These serotypes vary by their mechanism of blocking fusion of the acetylcholine-containing synaptic vesicle with the cell membrane, thereby preventing neurotransmitter release into the neuromuscular



**Figure 1** The mechanism of action of botulinum toxins involves a four-step process: (1) activation by proteolytic cleavage of the polypeptide chain into a 100 kDa heavy chain (H) and a 50 kDa light chain (L), linked by a disulfide bond (S–S), and binding of the H to the presynaptic membrane of the motor axon terminal, (2) internalization of the toxin complex by energy-dependent endocytosis, (3) release of the L into the cytoplasm, and (4) cleaving by the L at various sites (vertical arrows), depending on the serotype, thus preventing fusion of the acetylcholine-containing synaptic vesicle with the cell membrane at the neuromuscular junction.<sup>40</sup> Reprinted with permission.<sup>40</sup>  
**Abbreviations:** BTX, botulinum toxin; SNAP-25, synaptosomal-associated protein 25; VAMP, vesicle-associated membrane protein; Zn, zinc.

junction and achieving denervation (Figure 1).<sup>40,41</sup> BoNT type A (BoNT/A) cleaves the target protein synaptosomal-associated protein 25 and BoNT type B (BoNT/B) cleaves synaptobrevin-2 (Figure 1); these serotypes are used in clinical practice.<sup>40,41</sup>

The most commonly used BoNT treatments include three BoNT/A products and one BoNT/B product, each of which has a unique generic name designated by the FDA for use in the United States (Table 1).<sup>42,43</sup> All of these agents have demonstrated efficacy in clinical trials, but they have varying pharmacologic properties including potency, dosing,

constituents and excipients, storage requirements, and tolerability profiles (Table 1).<sup>38,39,41,44–46</sup> However, only a modest number of randomized, controlled clinical trials comparing BoNT agents in patients with focal dystonia have been reported in the literature.<sup>44,45,47–51</sup>

Currently-marketed BoNT agents may vary with respect to their risk for stimulating antibody formation, leading to immunoresistance and the potential for treatment failure.<sup>41,52,53</sup> In patients being treated for CD, de novo treatment with BoNT/B has been associated with a high rate of immunoresistance, contributing to treatment failure in up to 44% of individuals.<sup>54</sup> The rate of immunoresistance in type A agents appears to range from <1% to 5%.<sup>2,53,55</sup> Possible risk factors for immunoresistance include higher doses, the total amount of clostridial protein administered, and increased duration and frequency of treatment.<sup>52,53</sup> Clinical data also suggest that patients may vary in their immune reactivity to similar doses of BoNT.<sup>53</sup> The issue of immunoresistance is of particular importance in patients with dystonia, because limited options are available for the management of BoNT/A failure. For example, a study of ten patients with CD who had experienced complete therapeutic failure with BoNT/A found that alternative BoNT/B treatment induced a stable therapeutic response in three of the participants, but only a temporary response followed by treatment failure in the remainder of the individuals.<sup>56</sup>

The presence of nontoxic hemagglutinating and non-hemagglutinating complexing proteins in several of the available BoNT agents (Table 1) may also lead to immunoresistance.<sup>53,57</sup> The neurotoxin present in all serotypes of BoNT (types A–G) is noncovalently associated with complexing

**Table 1** Marketed brands of botulinum toxins<sup>42,43</sup>

Generic name	OnabotulinumtoxinA	RimabotulinumtoxinB	AbobotulinumtoxinA	IncobotulinumtoxinA
Brandname	Botox	Myobloc/Neurobloc <sup>a</sup>	Dysport	Xeomin
Manufacturer	Allergan, Inc. (United States)	Solstice Neurosciences (United States)	Ipsen (France)	Merz Pharmaceuticals GmbH (Germany)
Serotype	A	B	A	A
Specific activity, U/ng	20	75–125	40	167
Packaging, units/ vial	100	2500, 5000, or 10000	500	100
Constituents and excipients	Hemagglutinin and nonhemagglutinin proteins, human albumin, saccharose, sodium chloride	Hemagglutinin and nonhemagglutinin proteins, human albumin solution 0.05%, sodium chloride, sodium succinate	Hemagglutinin and nonhemagglutinin proteins, human albumin solution 20%, lactose	Human albumin, saccharose
pH	7.4	5.6	7.4	7.4
Molecular weight, kDa	900	700	500–900	150
Preparation	Vacuum-dried	Solution (5000 U/mL)	Lyophilized	Lyophilized
Storage of packaged product	2°C to 8°C	2°C to 8°C	2°C to 8°C	Room temperature
Storage once reconstituted	2°C to 8°C for 24 hours	Does not require reconstitution	2°C to 8°C for 4 hours	2°C to 8°C for 24 hours

**Notes:** <sup>a</sup>Myobloc is the brand name in Canada, the United States, and Korea; Neurobloc is the brand name in the European Union, Norway, and Iceland.

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proteins to form toxin complexes, which are encoded in two gene clusters<sup>57</sup> and are present in the natural state. The first cluster encodes the actual neurotoxin and a nontoxic, nonhemagglutinin protein, and the second cluster encodes three hemagglutinin proteins (HA1, HA2, and HA3).<sup>58,59</sup> Two different complexes are produced by *C. botulinum* (serotypes A–D and G): a complex containing the toxin and the nontoxic, nonhemagglutinin protein (300 kDa), and a larger complex containing the toxin and HA1–3 (500–600 kDa). Serotype A also forms a third complex with an even higher molecular weight. This complex contains the toxin and nontoxic, nonhemagglutinin protein in addition to varying numbers of other hemagglutinin proteins (880–1000 kDa in total).<sup>57</sup> Based on experimental studies, the natural functions of the complexing proteins appear to include protecting the neurotoxin from low pH and proteases, stabilizing the neurotoxin's biologic activity, and facilitating adherence of the neurotoxin to muscle tissues,<sup>57,60–62</sup> suggesting a role in preventing degradation of the toxin within the gastrointestinal tract and increasing the likelihood of absorption – hence, a biologic effect. Hypothetically, by increasing the size (molecular weight) of the toxin complex, complexing proteins may also limit diffusion of the neurotoxin out of the target tissue, potentially lowering the risk for such diffusion-related adverse events (AEs) as dysphagia in patients with CD.<sup>62,63</sup> Experimental studies suggest, however, that BoNT complexing proteins are not essential for the clinical activity of the neurotoxin in humans, because at increasing pH levels, the complexes quickly dissociate at an increasing rate.<sup>64</sup> At physiologic pH in humans, this process occurs in <1 minute,<sup>65</sup> whereas the clinical effect is known to become augmented over days. Experimental and clinical studies have also shown that complexing proteins do not appear to modify the diffusion of BoNT from target tissues.<sup>64–68</sup>

In addition, assay studies have found that complexing proteins have significantly greater immunogenicity than does the purified neurotoxin alone, with antibody formation up to 60 times greater in reaction to the BoNT complex and up to 35 times greater in reaction to the complexing hemagglutinins, compared with the neurotoxin alone.<sup>69–71</sup> Although the precise relationship between antibody formation and treatment failure is unclear, almost half of all secondary nonresponders to BoNT therapy for focal dystonia screen positive for antibody formation.<sup>72</sup> It has been speculated that the immune activity generated by the presence of complexing proteins can induce a greater likelihood of an antigenic response against the neurotoxin itself – that is, a neutralizing antibody.

## Review of incobotulinumtoxinA

### History of development

IncobotulinumtoxinA (Xeomin) is a highly purified BoNT/A agent and the only BoNT product that is free of any complexing clostridial proteins (Table 1).<sup>65</sup> IncobotulinumtoxinA is FDA-approved for the treatment of adults with CD in both BoNT-naïve individuals and previously-treated patients, and for the treatment of BSP in adults previously treated with onabotulinumtoxinA (Botox®; Allergan, Inc, Irvine, CA).<sup>73</sup> Prior to the development of incobotulinumtoxinA, the manufacturing process of BoNT agents was hampered by a massive degradation of about 90% of the neurotoxin, with this proportion inactive and behaving as a toxoid.<sup>65</sup> A high level of inactive clostridial protein in a BoNT formulation is clinically important, because it increases the total amount of clostridial protein that must be administered to achieve a therapeutic effect, which, as noted, may increase the risk for an immune reaction.<sup>65,74</sup> In addition, both the diffusion of BoNT and the incidence of BoNT-related AEs have been observed to be dose-dependent.<sup>75–77</sup>

In view of these factors, a manufacturing process for incobotulinumtoxinA was devised that involves a series of steps to separate and purify the neurotoxin complex, eliminate the complexing proteins, minimize degradation, and prevent loss of biologic activity during dilution, formulation, and lyophilization.<sup>65</sup> As a result, incobotulinumtoxinA contains only the pure 150 kDa neurotoxin and contains 0.6 ng of protein per every 100 U vial.<sup>65</sup> By contrast, there is about 55 ng of protein in a vial of rimabotulinumtoxinB (Myobloc®/ Neurobloc®; Solstice Neurosciences, Malvern, PA), 5 ng in a vial of onabotulinumtoxinA, and 4.35 ng in a vial of abobotulinumtoxinA (Dysport®; Ipsen, Paris, France).<sup>65,66</sup> Complexing proteins add to the molecular weight of the injected solution and may hypothetically enhance the stability of the product and limit its diffusion to adjacent tissues. Given that incobotulinumtoxinA lacks the complexing proteins of other BoNT agents, it was evaluated for these pharmacologic properties, as well as for safety, tolerability, and efficacy.

### Pharmacologic profile

Stability studies conducted in accordance with the FDA guidelines for stability testing of drug products<sup>78</sup> revealed that incobotulinumtoxinA remained stable and highly potent when stored for 4 years at room temperature, thereby demonstrating that complexing proteins are not necessary for stabilization of a BoNT formulation prior to injection.<sup>79</sup> To address concerns about the greater risk for toxin spread, a randomized, controlled, double-blind, 52-week trial in 32 male volunteers<sup>68</sup>

was conducted to study diffusion into adjacent muscles of incobotulinumtoxinA compared with the higher molecular weight onabotulinumtoxinA. All subjects were injected with one agent in the extensor digitorum brevis (EDB) muscle of one foot and the other agent in the EDB of the contralateral foot, in equal doses (2, 4, 16, or 32 U). Surface electromyography was used to measure whether the amplitude of the compound muscle action potential (CMAP) in the adjacent muscles had been reduced with either neurotoxin. The study found that all incobotulinumtoxinA and onabotulinumtoxinA doses significantly reduced the CMAP M-wave amplitudes in the target EDB muscles in a dose-dependent fashion, with similar reductions in CMAP M-wave amplitudes in muscles adjacent to the EDB (abductor digiti quinti and abductor hallucis).<sup>68</sup> In fact, the CMAP M-wave amplitudes remained above the predefined threshold of effect, indicating that no clinically relevant diffusion had occurred. Hence, the absence of complexing proteins in BoNT formulations does not appear to increase the risk for diffusion of toxin.

Preclinical animal studies were also conducted to evaluate the immunogenicity of incobotulinumtoxinA. In a comparison study, female New Zealand white rabbits (n = 20 per group) received intracutaneous administration of either incobotulinumtoxinA or onabotulinumtoxinA at 16 lethal dose units per animal (approximately 5.34 lethal dose units/kg) for eight administrations at 2- to 8-week intervals, with a booster injection of 25 lethal dose units per animal at 10 weeks following the eighth injection.<sup>80</sup> Sera from both groups were initially screened for BoNT/A antibodies using an enzyme-linked immunosorbent assay, and antibody-positive sera were then tested for their ability to neutralize the paralytic effects of BoNT/A in a mouse hemidiaphragm assay. At week 36 – 3 weeks after the final (booster) injection – the enzyme-linked immunosorbent assay showed that seven of the 20 rabbits in the onabotulinumtoxinA group screened positive for BoNT/A antibodies, with four of these rabbits displaying BoNT/A-neutralizing activity in the hemidiaphragm assay. In contrast, one rabbit in the incobotulinumtoxinA group tested positive by enzyme-linked immunosorbent assay, but no neutralizing activity was detected in the hemidiaphragm assay. Considering the high doses and short injection intervals used in this study, these results suggest that incobotulinumtoxinA, without complexing proteins, poses a lower risk for immunogenicity leading to treatment failure than does the conventionally prepared BoNT/A agent that contains such proteins.<sup>80</sup>

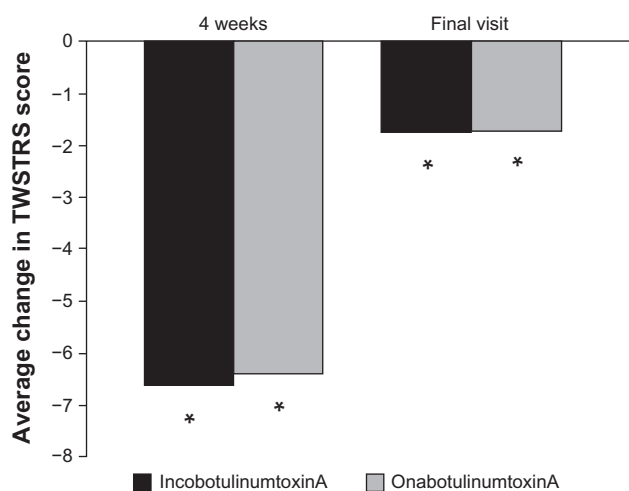
Other preclinical animal studies have demonstrated a similar pharmacologic profile of incobotulinumtoxinA and

onabotulinumtoxinA with respect to pharmacodynamic action, effects on cardiovascular function and toxicity following single or repeated dose administrations.<sup>80</sup> In addition, two phase 1 clinical studies in healthy volunteers showed that treatment with either incobotulinumtoxinA or onabotulinumtoxinA was associated with similar times to onset and duration of effect, as measured by surface electromyography of the injected EDB muscle.<sup>68,81</sup> The degree of reduction in CMAP amplitudes at 3 months following the injection is identical between the two toxin products.<sup>81</sup> Taken together, these studies indicate that the clinical effects of a BoNT/A product free of complexing proteins should be no different from those of a conventionally prepared BoNT/A formulation.

## Efficacy in patients with CD

The efficacy, safety, and tolerability of incobotulinumtoxinA have been evaluated in multiple clinical trials in patients with CD. The largest trial to date was a randomized, active-controlled, double-blind, phase 3 study designed to determine whether incobotulinumtoxinA was noninferior in efficacy to onabotulinumtoxinA in patients with CD.<sup>51</sup> The study, which was conducted at 51 centers in eleven European countries,<sup>51</sup> enrolled 463 patients with a documented stable therapeutic response to onabotulinumtoxinA over the prior two injection sessions, with the last onabotulinumtoxinA injection administered at least 10 weeks prior to randomization.<sup>51,82</sup> The patients were randomized to either incobotulinumtoxinA or onabotulinumtoxinA at the same doses they had received in the previous two prerandomization sessions with onabotulinumtoxinA. The dosage ranged from 70 U to 300 U, with a control visit conducted 4 weeks after injection and follow-up visits for up to 16 weeks. The primary efficacy variable was the change from baseline in the Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS)<sup>83</sup> severity score (0–35) at 28 ± 7 days postinjection. At baseline, patients in both the incobotulinumtoxinA group (n = 209) and the onabotulinumtoxinA group (n = 205) had a median TWSTRS severity score of 18, indicating moderate severity.

In both groups, these scores improved to a median of eleven points at day 28, with an average change of –6.6 points in the incobotulinumtoxinA group and –6.4 points in the onabotulinumtoxinA group ( $P < 0.0001$ , analysis of covariance, both agents; Figure 2). The median dose injected was 120 U in the incobotulinumtoxinA group and 122.5 U in the onabotulinumtoxinA group. In the noninferiority assessment, the least-squares mean difference between the groups was –0.33 points (favoring incobotulinumtoxinA) and the upper limit of the corresponding 95% confidence



**Figure 2** Average changes in Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) severity score following treatment with either incobotulinumtoxinA or onabotulinumtoxinA in adults with cervical dystonia.

**Note:** \* $P < 0.0001$  versus baseline (analysis of covariance).<sup>51,82</sup>

interval was lower than the predefined difference of 1.3 points in all analysis of covariance models, thereby demonstrating the noninferiority of incobotulinumtoxinA to onabotulinumtoxinA for the treatment of CD. In addition, no relevant differences in any secondary variables were reported between the two groups, including the TWSTRS severity score at the final visit, the TWSTRS pain subscore at the control and final visits, and the visual analog scale

pain score at the control and final visits (Table 2). Both treatments were also very similar in terms of time to onset of effect, time to waning of effect, and total duration of effect (Table 2). AEs were reported by similar percentages of patients in the incobotulinumtoxinA (28.1%) and onabotulinumtoxinA (24.1%) groups (Table 3), and serious AEs (SAEs) occurred in four incobotulinumtoxinA-treated patients and five onabotulinumtoxinA-treated patients.<sup>51</sup> All SAEs were judged either unrelated or unlikely to be related to treatment.<sup>51</sup> The results of this study suggest that incobotulinumtoxinA, when administered at the same doses as prior successful onabotulinumtoxinA treatments, is noninferior in clinical efficacy to onabotulinumtoxinA for the treatment of CD and has a similar side effect profile.

Another study investigated the safety and efficacy of incobotulinumtoxinA versus placebo in 233 patients with CD, including BoNT-naïve patients (39% of the population) and nonnaïve individuals (previously treated with BoNT/A or BoNT/B), at a low (120 U) and high (240 U) dose.<sup>84</sup> The dosing design of this study was based on the median dose used in the noninferiority trial of incobotulinumtoxinA and onabotulinumtoxinA (120 U),<sup>51</sup> and the typical dose used in other trials of BoNT/A agents for the treatment of CD (240 U).<sup>85</sup> This randomized, placebo-controlled trial, conducted at 37 study centers in the United States,

**Table 2** Secondary efficacy variables in noninferiority trial of IncobotulinumtoxinA vs OnabotulinumtoxinA<sup>51</sup>

Variable	IncobotulinumtoxinA		OnabotulinumtoxinA		Treatment comparison P value
	Mean (SD)	P value, compared with baseline	Mean (SD)	P value, compared with baseline	
TWSTRS severity score at final visit	-1.8 (3.4) (n = 209)	<0.0001 <sup>a</sup>	-1.8 (3.8) (n = 205)	<0.0001 <sup>a</sup>	0.74 <sup>a</sup>
TWSTRS pain subscore at control visit	-0.4 (0.8) (n = 213)	<0.0001 <sup>b</sup>	-0.6 (1.0) (n = 207)	<0.0001 <sup>b</sup>	0.41 <sup>b</sup>
TWSTRS pain subscore at final visit	-0.1 (0.9) (n = 210)	0.12 <sup>b</sup>	-0.2 (1.0) (n = 205)	0.003 <sup>b</sup>	0.09 <sup>b</sup>
VAS pain score at control visit	-8.8 (18.5) (n = 210)	<0.0001 <sup>b</sup>	-11.8 (19.4) (n = 206)	<0.0001 <sup>b</sup>	0.3 <sup>b</sup>
VAS pain score at final visit	-1.4 (22.0) (n = 207)	0.5 <sup>b</sup>	-4.5 (20.2) (n = 204)	0.002 <sup>b</sup>	0.06 <sup>b</sup>
<b>Time to event, mean (SD)</b>					<b>Relative risk P value<sup>c</sup></b>
Onset of effect, days	7.3 (4.3) (n = 189)		7.2 (4.1) (n = 182)		0.96
Waning of effect, weeks	9.9 (3.8) (n = 181)		10.0 (3.9) (n = 167)		0.88
Duration of effect, days	95.9 (30.0) (n = 192)		94.3 (31.4) (n = 180)		0.91

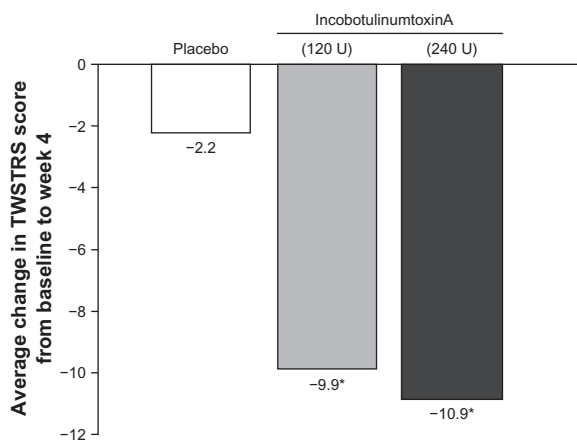
**Notes:** <sup>a</sup>Analysis of covariance; <sup>b</sup>Wilcoxon; <sup>c</sup>Cox regression.

**Abbreviations:** SD, standard deviation; TWSTRS, Toronto Western Spasmodic Torticollis Rating Scale; VAS, visual analog scale.

**Table 3** Adverse events following a single injection of either incobotulinumtoxinA or onabotulinumtoxinA in 463 patients with cervical dystonia<sup>51</sup>

Adverse event, n (%)	IncobotulinumtoxinA (n = 231)	OnabotulinumtoxinA (n = 232)
All	65 (28.1)	56 (24.1)
Dysphagia	25 (10.8)	19 (8.2)
Skeletal pain	8 (3.5)	5 (2.2)
Back pain	5 (2.2)	2 (0.9)
Muscle weakness	4 (1.7)	1 (0.4)
Headache	2 (0.9)	3 (1.3)
Vomiting	2 (0.9)	2 (0.9)
Erythematous rash	2 (0.9)	2 (0.9)
Diarrhea	1 (0.4)	3 (1.3)
Fatigue	1 (0.4)	3 (1.3)
Arthralgia	3 (1.3)	0 (0.0)
Asthenia	2 (0.9)	1 (0.4)
Myalgia	2 (0.9)	1 (0.4)
Mouth dryness	1 (0.4)	2 (0.9)
Dizziness	2 (0.4)	2 (0.9)

found that the changes from baseline to week four in total TWSTRS score were  $-9.9 \pm 10.4$  points with incobotulinumtoxinA 120 U and  $-10.9 \pm 11.7$  points with incobotulinumtoxinA 240 U, compared with  $-2.2 \pm 7.3$  points with placebo ( $P < 0.001$  versus placebo for both incobotulinumtoxinA groups) (Figure 3). AEs occurred in 55.1% of patients in the incobotulinumtoxinA 120-U group, 56.8% in the incobotulinumtoxinA 240-U group, and 45.9% in the placebo group. The most frequently reported AEs – dysphagia, neck pain, and muscular weakness – were similar to those observed in the other incobotulinumtoxinA 4-week trials.<sup>84</sup>



**Figure 3** Mean change from baseline at week four in Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) total score in 233 botulinum toxin type B-naïve and nonnaïve patients with cervical dystonia treated with incobotulinumtoxinA 120 U, incobotulinumtoxinA 240 U, or placebo.

**Note:** \* $P < 0.001$  versus placebo.<sup>85</sup>

Subanalyses of the data from this placebo-controlled trial were also conducted in the subgroups of BoNT-naïve and nonnaïve patients. In the toxin-naïve patients ( $n = 90$ ), the changes from baseline to week four in total TWSTRS score with incobotulinumtoxinA 120 U and incobotulinumtoxinA 240 U were  $-11.9 \pm 11.1$  points and  $-10.0 \pm 9.2$  points, respectively, versus  $-2.0 \pm 6.0$  with placebo ( $P < 0.001$  for both doses).<sup>86</sup> Changes in the TWSTRS severity score from baseline to week four in the incobotulinumtoxinA 120-U group and the incobotulinumtoxinA 240-U group were  $-4.1 \pm 4.3$  points and  $-5.4 \pm 5.5$  points, respectively, versus  $-1.9 \pm 4.5$  points in the placebo group. Compared with placebo, incobotulinumtoxinA was well tolerated, with dysphagia, muscular weakness, and neck pain the most frequently reported AEs with active treatment, which is similar to that with other toxins. The subanalysis in the patients previously treated with another BoNT product ( $n = 143$ ) showed that the mean changes in total TWSTRS score from baseline to week four with incobotulinumtoxinA 120 U and incobotulinumtoxinA 240 U were  $-8.5 \pm 9.7$  points and  $-11.4 \pm 13.1$  points, respectively, compared with  $-2.4 \pm 9.1$  points with placebo ( $P < 0.002$  for both doses).<sup>87</sup> The improvements in TWSTRS severity score from baseline to week four with incobotulinumtoxinA 120 U and incobotulinumtoxinA 240 U were  $-3.7 \pm 4.4$  points and  $-5.6 \pm 6.4$  points, respectively, versus  $-1.9 \pm 3.7$  points with placebo. AEs occurred in 55.3% of patients in the incobotulinumtoxinA 120-U group, 46.0% in the incobotulinumtoxinA 240-U group, and 34.8% in the placebo group. The most common AEs were dysphagia, neck pain, and injection-site pain, which was similar to those reported in the trial of BoNT-naïve patients.<sup>86</sup>

Taken together, the placebo-controlled study in patients with CD and subanalyses of the data showed that incobotulinumtoxinA generally has similar efficacy and tolerability at doses of 120 U and 240 U, and across BoNT-naïve and nonnaïve patient subgroups.

## Long-term safety and tolerability in patients with CD

The first long-term safety and tolerability evaluation of incobotulinumtoxinA<sup>88</sup> was conducted as an extension of the above-described efficacy study in 233 patients with CD, including both BoNT-naïve patients and those who had been previously treated with BoNT (type A or B).<sup>84</sup> Patients who completed the  $\leq 20$ -week randomized, placebo-controlled study were eligible to enter the extension phase and were treated with incobotulinumtoxinA

(120 U or 240 U;  $\leq 5$  injections) over 1 year (48-week treatment and 20-week follow-up). A total of 217 patients entered the extension phase, with 153 of them participating in the long-term safety analysis. The mean duration of time prior to reinjection was 10.0–14.5 weeks. During the extension period, 118 of the 153 patients (77.1%) experienced at least one AE (70.7% in the 120-U group; 83.3% in the 240-U group). The most frequently reported AEs were dysphagia, neck pain, and sinusitis. No SAEs were judged to be related to the incobotulinumtoxinA treatment, and the total incidence of AEs decreased with each injection interval, thus indicating no cumulative effect from repeated doses.<sup>88</sup>

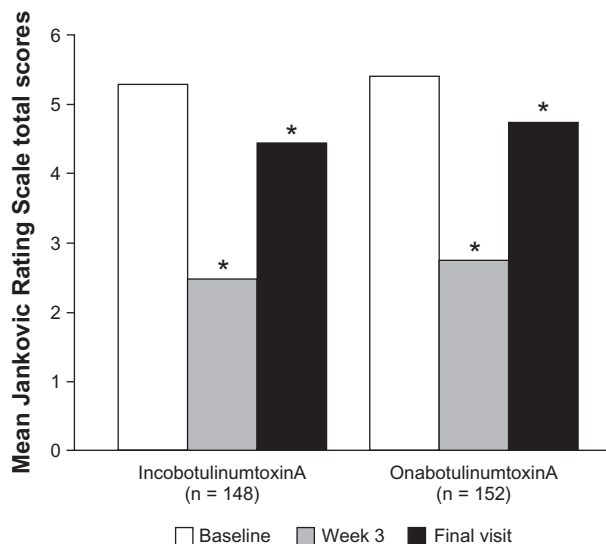
### Immunogenicity in patients with CD

The immunogenicity of incobotulinumtoxinA in persons with CD is being evaluated in an ongoing, open-label study of 100 patients.<sup>82</sup> In this study, 50 consecutive de novo patients and an additional 50 patients who had been previously treated with one of the three BoNT products now available in the United States (Table 1) were switched to incobotulinumtoxinA treatment at a dose relationship of 1:1 for onabotulinumtoxinA and 1:4 for abobotulinumtoxinA. Patients previously treated with rimabotulinumtoxinB had been switched from either onabotulinumtoxinA or abobotulinumtoxinA because of nonresponse related to immunoresistance. Antibody testing performed after 1 year and 2 years of continuous treatment with incobotulinumtoxinA demonstrated that no patient had developed secondary nonresponsiveness or antiBoNT/A neutralizing antibodies, including 100 patients who had been treated for >1 year and 34 patients who were treated continuously for >2 years. However, six patients who had experienced secondary nonresponsiveness as a result of antibody formation during their prior treatment with onabotulinumtoxinA or abobotulinumtoxinA also failed to achieve clinical benefit with incobotulinumtoxinA, underscoring the importance of minimizing the risk for immunoresistance from treatment onset.<sup>82</sup>

### Efficacy in patients with BSP

A phase 3 randomized, active-controlled, double-blind, noninferiority study comparing the efficacy of incobotulinumtoxinA with that of onabotulinumtoxinA in patients with BSP was conducted at 42 study centers in Europe and Israel.<sup>89</sup> In this trial, 300 patients with BSP who had received at least two prior injections with onabotulinumtoxinA that yielded a stable response were randomized to either incobotulinumtoxinA (n = 148) or

onabotulinumtoxinA (n = 152), with a maximum dose of 35 U per eye, and followed for 16 weeks with a control visit at 3 weeks. The primary efficacy variable was change from baseline in the sum score of the Jankovic Rating Scale (JRS) at the control visit (21  $\pm$  1 days postinjection).<sup>89</sup> Each of the treatments resulted in similar reductions in JRS scores of  $-2.90$  in the incobotulinumtoxinA group and  $-2.67$  in the onabotulinumtoxinA group, both of which were significant compared with baseline ( $P < 0.0001$ , analysis of covariance, for both; Figure 4). The difference between the two adjusted group means was  $-0.23$ , with the upper limit of the 95% confidence interval amounting to 0.22.<sup>89</sup> This was below the predefined limit for noninferiority (0.8), thus demonstrating that incobotulinumtoxinA was noninferior to onabotulinumtoxinA for the treatment of patients with BSP. The noninferiority of incobotulinumtoxinA to onabotulinumtoxinA was also supported by the results for secondary variables, including mean change from baseline at the control visit in scores on the Blepharospasm Disability Index (BSDI),<sup>17</sup> the Patient Evaluation of Global Response,<sup>90</sup> and the Global Assessment Scale.<sup>91</sup> Both agents significantly reduced mean BSDI scores and Patient Evaluation of Global Response scores from baseline to the control visit and the final visit ( $P < 0.0001$  for all changes), with no significant differences between incobotulinumtoxinA and onabotulinumtoxinA observed. For the Global Assessment Scale measure, investigators rated the efficacy of the medication as “very good” in a slightly higher percentage of patients



**Figure 4** Mean change from baseline in Jankovic Rating Scale total score at week three and at final visit (up to 16 weeks) after a single injection of either incobotulinumtoxinA or onabotulinumtoxinA in 300 patients with blepharospasm. **Note:** \* $P < 0.0001$  versus baseline (analysis of covariance).<sup>90</sup>



**Table 4** Adverse events following a single injection of either incobotulinumtoxinA or onabotulinumtoxinA in 300 patients with blepharospasm<sup>90</sup>

Adverse event, n (%)	IncobotulinumtoxinA (n = 148)	OnabotulinumtoxinA (n = 152)
Ptosis	9 (6.1)	7 (4.6)
Abnormal vision	2 (1.4)	5 (3.3)
Back pain	2 (1.4)	4 (2.6)
Rash	1 (0.7)	2 (1.3)
Upper respiratory tract infection	1 (0.7)	2 (1.3)
Face edema	1 (0.7)	2 (1.3)
Xerophthalmia	3 (2.0)	0 (0.0)
Arthralgia	1 (0.7)	1 (0.7)
Dizziness	2 (1.4)	0 (0.0)
Headache	1 (0.7)	1 (0.7)
Paresthesia	1 (0.7)	1 (0.7)
Depression	0 (0.0)	2 (1.3)
Palpitation	0 (0.0)	2 (1.3)
Photophobia	2 (1.4)	0 (0.0)
Dyspnea	2 (1.4)	0 (0.0)
Urinary tract infection	2 (1.4)	0 (0.0)

in the incobotulinumtoxinA group (34.9%) than in the onabotulinumtoxinA group (28.4%); however, this difference was not significant.<sup>89</sup>

Both study medications were well tolerated, with slightly fewer total AEs reported in the incobotulinumtoxinA group (56 events) than in the onabotulinumtoxinA group (62 events). The most commonly occurring AE with both agents was eyelid ptosis, which was reported in 6.1% of incobotulinumtoxinA-treated patients versus 4.6% of onabotulinumtoxinA-treated individuals (Table 4).<sup>89</sup>

IncobotulinumtoxinA was also compared with onabotulinumtoxinA in a smaller randomized, double-blind, parallel-group, pilot trial.<sup>92</sup> Patients with BSP previously treated with onabotulinumtoxinA ( $\geq 20$  U per eye) and scores  $>2$  on the JRS (n = 65) received 20–45 U per eye of incobotulinumtoxinA (n = 33) or onabotulinumtoxinA (n = 31) during a single treatment session. Patients were evaluated at 4, 8, and 14 weeks postinjection. The primary outcome variable was change in BSDI at week four. BSDI decreased from baseline in both groups at week four (1.3 for incobotulinumtoxinA and 2.8 for onabotulinumtoxinA) and at week eight (0.8 for incobotulinumtoxinA and 1.3 for onabotulinumtoxinA). JRS score decreased by 1.5 (both eyes) at week four and 1.3 (both eyes) at week eight for incobotulinumtoxinA. JRS score decreased by 2.3 (OS) and 2.2 (OD) at week four and 1.9 (both eyes) at week eight with onabotulinumtoxinA treatment. There were no significant differences between BoNT/A products in these outcome variables or

any predefined outcomes. AE profiles were similar with periorbital hematoma reported most frequently (27% for incobotulinumtoxinA and 23% for onabotulinumtoxinA), followed by headache (21% for incobotulinumtoxinA and 23% for onabotulinumtoxinA).<sup>92</sup>

The safety and efficacy of incobotulinumtoxinA versus placebo was further evaluated in a larger, randomized, double-blind study in patients with BSP with documented satisfactory response to two previous treatments with onabotulinumtoxinA and JRS severity subscores  $\geq 2$ .<sup>93</sup> A total of 109 patients were randomized in a 2:1 ratio to an individual dose of incobotulinumtoxinA, at up to 50 U per eye, or placebo, and were followed for up to 20 weeks. The primary efficacy measure was change from baseline at 6 weeks postinjection in the JRS severity subscore, as assessed by a blinded independent investigator. At 6 weeks, the JRS severity subscore had improved significantly more in the incobotulinumtoxinA group, by  $-0.83$  points, compared with a  $0.21$  increase (worsening) with placebo, resulting in a difference of  $1.0$  favoring incobotulinumtoxinA ( $P < 0.001$ ).<sup>93</sup> Functional impairment as indicated by BSDI scores improved by  $0.5$  points with incobotulinumtoxinA compared with placebo ( $P = 0.002$ ). AEs were reported in 70.3% of patients in the incobotulinumtoxinA group and 58.8% in the placebo group. The most commonly reported AEs (incobotulinumtoxinA versus placebo) were eyelid ptosis (18.9% versus 5.9%), dry eye (18.9% versus 11.8%), and dry mouth (14.9% versus 2.9%). Tolerability was rated as good/very good by 91.9% patients in the incobotulinumtoxinA group compared with 85.2% of placebo patients.

## Pooled data analyses

Pooled analyses of data from multiple clinical trials were conducted to evaluate the overall efficacy, safety, and tolerability of incobotulinumtoxinA across both CD and BSP patient populations. In one such analysis, efficacy data were pooled from two pivotal clinical trials in patients with CD and BSP,<sup>51,89</sup> which included a total of 343 incobotulinumtoxinA-treated patients and 340 onabotulinumtoxinA-treated patients, as well as one trial for the treatment of spasticity in poststroke patients, which included 73 incobotulinumtoxinA-treated patients and 75 placebo-treated individuals.<sup>94</sup> For the evaluation of safety and tolerability, this analysis also pooled data from six clinical trials in patients with BSP, CD, and upper limb spasticity, including 539 incobotulinumtoxinA-treated patients, 442 onabotulinumtoxinA-treated patients, and 75 placebo-treated patients. The results of this analysis showed

that incobotulinumtoxinA and onabotulinumtoxinA have equivalent efficacy, with similar onset, waning, and duration of effect. These results were confirmed by similar ratings for both agents in the physician Global Impression of Efficacy, in which 70.6% of onabotulinumtoxinA-treated patients and 71.8% of incobotulinumtoxinA-treated patients were rated as “good” or “very good.” No clinically relevant differences were detected between active treatment groups in the focal dystonia trials, or between incobotulinumtoxinA and placebo in the poststroke spasticity trials. All AEs were either already known and/or were judged by the investigator as unlikely to be related to incobotulinumtoxinA treatment. This analysis further estimated that as of 2009, >67,000 patients had been treated with incobotulinumtoxinA, with no new safety concerns having been reported.<sup>95</sup>

Another pooled analysis was conducted to evaluate the efficacy of incobotulinumtoxinA in CD and BSP populations.<sup>95</sup> This analysis included two active-controlled trials<sup>51,89</sup> and two placebo-controlled trials,<sup>84,93</sup> one each in the CD and BSP populations. Efficacy data were available for a total of 613 patients who had received incobotulinumtoxinA treatment. In the placebo-controlled studies, the mean percentage improvement with incobotulinumtoxinA versus placebo in the primary efficacy outcomes was similar across studies (23.2%–26.5%), and patient-evaluated global response to treatment was significantly superior compared with placebo ( $P < 0.001$ ); 53.4% of incobotulinumtoxinA-treated patients reported at least moderate symptomatic improvement compared with 12.0% of placebo-treated individuals. Across the four studies, the mean onset of treatment was 6.0–7.7 days, the mean waning of effect was 6.5–10.6 weeks, and the mean duration of effect was 10.6–14.0 weeks.<sup>95</sup>

In addition, a review of the AEs in the two noninferiority trials of incobotulinumtoxinA and onabotulinumtoxinA showed that most AEs associated with the use of either agent were of mild or moderate severity (Table 5).<sup>80</sup> The incidence of SAEs was also low across both studies, occurring at a slightly lower rate with incobotulinumtoxinA than with onabotulinumtoxinA; no deaths were reported during these trials (Table 5).<sup>80</sup> None of the SAEs was considered to be related to the study medication in either trial; no AEs led to treatment withdrawal in the incobotulinumtoxinA groups, whereas only one withdrawal due to an AE was reported with the use of onabotulinumtoxinA (Table 5).<sup>80</sup>

## Review of the data

Overall, a considerable amount of preclinical and clinical trial data on incobotulinumtoxinA has been collected, including individual studies and pooled analyses of these studies. The

preclinical data established that incobotulinumtoxinA will remain stable for up to 4 years at room temperature<sup>79</sup> and demonstrated a low risk for diffusion from target tissues following injection, which is similar to that with onabotulinumtoxinA.<sup>68</sup> In addition, preclinical data demonstrated that incobotulinumtoxinA has a low potential for immunogenicity leading to treatment failure, which was lower than that with onabotulinumtoxinA.<sup>80</sup> In an ongoing clinical trial in patients with CD, no cases of antibody formation were reported in 100 patients who had been treated for >1 year and in 34 patients treated continuously for >2 years.<sup>94</sup> In clinical studies in healthy volunteers, incobotulinumtoxinA also demonstrated a similar pharmacologic profile to onabotulinumtoxinA in terms of onset of effect, duration of effect, and overall efficacy,<sup>51,81</sup> as well as pharmacodynamic actions and adverse effects.<sup>80</sup>

Randomized, active-controlled, clinical studies have shown that incobotulinumtoxinA is noninferior in efficacy to onabotulinumtoxinA for the treatment of both CD and BSP.<sup>51,89</sup> A randomized, controlled study also determined that incobotulinumtoxinA significantly reduced CD symptoms compared with placebo in BoNT-naïve and nonnaïve patients.<sup>84,86,87</sup> In patients with BSP who had been previously treated with BoNT, incobotulinumtoxinA significantly reduced BSP symptoms compared with placebo.<sup>93</sup> Across the clinical CD trials, both the primary efficacy measures (which were either the TWSTRS total score or the TWSTRS severity score in all trials) and the secondary outcomes (including both patient- and physician-rated scales) demonstrated the noninferiority of incobotulinumtoxinA to onabotulinumtoxinA and significant benefits, compared with placebo. In patients with BSP previously treated with BoNT, secondary measures, including the BSDI, Patient Evaluation of Global Response, and Global Assessment Scale, also supported the primary outcome result (JRS total score) in demonstrating the noninferiority of incobotulinumtoxinA to onabotulinumtoxinA and the superiority of incobotulinumtoxinA compared with placebo.<sup>17,89,93</sup>

With regard to safety and tolerability, incobotulinumtoxinA is well tolerated, and has demonstrated an AE profile similar to that of onabotulinumtoxinA in both CD and BSP patients (Tables 3 and 4).<sup>51,89</sup> Most AEs associated with either agent are of mild to moderate severity, and few have led to withdrawal from treatment (Table 5).<sup>80</sup> It also should be noted that incobotulinumtoxinA is contraindicated in people with known hypersensitivity to BoNT/A or to any of the excipients used in this product (Table 1), or with generalized disorders of muscle activity (eg, myasthenia gravis, Lambert–Eaton syndrome).<sup>73,80</sup> Patients treated with incobotulinumtoxinA should be closely observed when

**Table 5** Summary of adverse events occurring in noninferiority trials of IncobotulinumtoxinA and OnabotulinumtoxinA in patients with focal dystonia<sup>89</sup>

Measure, n (%)	Cervical dystonia trial (n = 463)		Blepharospasm trial (n = 300)	
	IncobotulinumtoxinA (n = 231)	OnabotulinumtoxinA (n = 232)	IncobotulinumtoxinA (n = 148)	OnabotulinumtoxinA (n = 152)
Total number of patients with AEs	65 (28.1)	57 (24.6)	40 (27.0)	45 (29.6)
Severity				
Mild	46 (19.9)	43 (18.5)	22 (14.9)	29 (19.1)
Moderate	27 (11.7)	20 (8.6)	22 (14.9)	18 (11.6)
Severe	3 (1.3)	3 (1.3)	3 (2.0)	1 (0.7)
Patients with SAEs	4 (1.7)	5 (2.2)	3 (2.0)	5 (3.3)
Total number of SAEs	6	6	3	6
AEs leading to withdrawal	0	1	0	0
AEs leading to death	0	0	0	0

**Abbreviations:** AEs, adverse events; SAEs, serious adverse events.

there is concomitant use of agents that may potentiate the effects of incobotulinumtoxinA, including aminoglycoside antibiotics, spectinomycin, or other agents that interfere with neuromuscular transmission (eg, tubocurarine-like agents), or muscle relaxants.<sup>73,80</sup>

## Conclusion

IncobotulinumtoxinA is a safe and effective agent, compared with placebo, for the treatment of patients with CD or BSP and is noninferior to onabotulinumtoxinA. Based on the results of clinical trials, incobotulinumtoxinA is indicated in the United States for the treatment of adults with CD and for the treatment of BSP in adults previously treated with onabotulinumtoxinA. The absence of complexing proteins in this formulation of BoNT/A does not seem to confer any differences in preinjection stability, risk for diffusion outside of target muscles, or time course of response following injection. By contrast, the risk for immunogenicity and possible treatment failure may be lower than that with other formulations. However, additional long-term clinical data regarding immunogenicity are warranted.

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## References

1. Tarsy D, Simon DK. Dystonia. *N Engl J Med*. 2006;355(8):818–829.
2. Tanabe LM, Kim CE, Alagem N, Dauer WT. Primary dystonia: molecules and mechanisms. *Nat Rev Neurol*. 2009;5(11):598–609.

3. Neychev VK, Fan X, Mitev VI, Hess EJ, Jinna HA. The basal ganglia and cerebellum interact in the expression of dystonic movement. *Brain*. 2008;131(Pt 9):2499–2509.
4. Zoons E, Booi J, Nederveen AJ, Dijk JM, Tijssen MA. Structural, functional and molecular imaging of the brain in primary focal dystonia-A review. *Neuroimage*. 2011;56(3):1011–1020.
5. Vidailhet M, Grabli D, Roze E. Pathophysiology of dystonia. *Curr Opin Neurol*. 2009;22(4):406–413.
6. Albanese A, Barnes MP, Bhatia KP, et al. A systematic review on the diagnosis and treatment of primary (idiopathic) dystonia and dystonia plus syndromes: report of an EFNS/MDS-ES Task Force. *Eur J Neurol*. 2006;13(5):433–444.
7. Nutt JG, Muentner MD, Aronson A, Kurland LT, Melton LJ 3rd. Epidemiology of focal and generalized dystonia in Rochester, Minnesota. *Mov Disord*. 1988;3(3):188–194.
8. Duffey PO, Butler AG, Hawthorne MT, Barnes MP. The epidemiology of the primary dystonias in the north of England. *Adv Neurol*. 1998;78:121–125.
9. Greene P, Kang UJ, Fahn S. Spread of symptoms in idiopathic torsion dystonia. *Mov Disord*. 1995;10(2):143–152.
10. Epidemiological Study of Dystonia in Europe (ESDE) Collaborative Group. A prevalence study of primary dystonia in eight European countries. *J Neurol*. 2000;247(10):787–792.
11. Papantoniou AM, Beghi E, Fogli D, et al. Prevalence of primary focal or segmental dystonia in adults in the district of Foggia, southern Italy: a service-based study. *Neuroepidemiology*. 2009;33(2):117–123.
12. Matsumoto S, Nishimura M, Shibasaki H, Kaji R. Epidemiology of primary dystonias in Japan: comparison with Western countries. *Mov Disord*. 2003;18(10):1196–1198.
13. Sugawara M, Watanabe S, Toyoshima I. Prevalence of dystonia in Akita Prefecture in Northern Japan. *Mov Disord*. 2006;21(7):1047–1049.
14. Cossu G, Mereu A, Deriu M, et al. Prevalence of primary blepharospasm in Sardinia, Italy: a service-based survey. *Mov Disord*. 2006;21(11):2005–2008.
15. Chan J, Brin MF, Fahn S. Idiopathic cervical dystonia: clinical characteristics. *Mov Disord*. 1991;6(2):119–126.
16. Dauer WT, Burke RE, Greene P, Fahn S. Current concepts on the clinical features, aetiology and management of idiopathic cervical dystonia. *Brain*. 1998;121(Pt 4):547–560.
17. Jankovic J, Kenney C, Grafe S, Goertelmeyer R, Comes G. Relationship between various clinical outcome assessments in patients with blepharospasm. *Mov Disord*. 2009;24(3):407–413.
18. Müller J, Kemmler G, Wissel J, et al; Austrian Botulinum Toxin and Dystonia Study Group. The impact of blepharospasm and cervical dystonia on health-related quality of life and depression. *J Neurol*. 2002;249(7):842–846.

19. Lim VK. Health related quality of life in patients with dystonia and their caregivers in New Zealand and Australia. *Mov Disord.* 2007;22(7):998–1103.
20. Jankovic J, Leder S, Warner D, Schwartz K. Cervical dystonia: clinical findings and associated movement disorders. *Neurology.* 1991;41(7):1088–1091.
21. Grandas F, Elston J, Quinn N, Marsden CD. Blepharospasm: a review of 264 patients. *J Neurol Neurosurg Psychiatry.* 1988;51(6):767–772.
22. Jankovic J, Havins WE, Wilkins RB. Blinking and blepharospasm. Mechanism, diagnosis, and management. *JAMA.* 1982;248(23):3160–3164.
23. Martino D, Liuzzi D, Macerollo A, Aniello MS, Livrea P, Defazio G. The phenomenology of the geste antagoniste in primary blepharospasm and cervical dystonia. *Mov Disord.* 2010;25(4):407–412.
24. O’Riordan S, Raymond D, Lynch T, et al. Age at onset as a factor in determining the phenotype of primary torsion dystonia. *Neurology.* 2004;63(8):1423–1426.
25. Defazio G, Berardelli A, Hallett M. Do primary adult-onset focal dystonias share aetiological factors? *Brain.* 2007;130(Pt 5):1183–1193.
26. Haussermann P, Marzoch S, Klinger C, Landgrebe M, Conrad B, Ceballos-Bauman A. Long-term follow-up of cervical dystonia patients treated with botulinum toxin A. *Mov Disord.* 2004;19(3):303–308.
27. Weiss EM, Hershey T, Karimi M, et al. Relative risk of spread of symptoms among the focal onset primary dystonias. *Mov Disord.* 2006;21(8):1175–1181.
28. Hierholzer J, Cordes M, Schelosky L, et al. Dopamine D2 receptor imaging with iodine-123-iodobenzamide SPECT in idiopathic rotational torticollis. *J Nucl Med.* 1994;35(12):1921–1927.
29. Perlmutter JS, Stambuk MK, Markham J, et al. Decreased [18F] spiperone binding in putamen in idiopathic focal dystonia. *J Neurosci.* 1997;17(2):843–850.
30. Naumann M, Pirker W, Reiners K, Lange KW, Becker G, Brücke T. Imaging the pre- and postsynaptic side of striatal dopaminergic synapses in idiopathic cervical dystonia: a SPECT study using [123I] epidepride and [123I] beta-CIT. *Mov Disord.* 1998;13(2):319–323.
31. Karimi M, Moerlein SM, Videen TO, et al. Decreased striatal dopamine receptor binding in primary focal dystonia: a D2 or D3 defect? *Mov Disord.* 2011;26(1):100–106.
32. Pauletti G, Berardelli A, Cruccu G, Agostino R, Manfredi M. Blink reflex and the masseter inhibitory reflex in patients with dystonia. *Mov Disord.* 1993;8(4):495–500.
33. Tolosa E, Montserrat L, Bayes A. Blink reflex studies in focal dystonias: enhanced excitability of brainstem interneurons in cranial dystonia and spasmodic torticollis. *Mov Disord.* 1988;3(1):61–69.
34. Berardelli A, Rothwell JC, Day BL, Marsden CD. Pathophysiology of blepharospasm and oromandibular dystonia. *Brain.* 1985;108(Pt 3):593–608.
35. Rinnerthaler M, Benecke C, Bartha L, Entner T, Poewe W, Mueller J. Facial recognition in primary focal dystonia. *Mov Disord.* 2006;21(1):78–82.
36. Molloy FM, Carr TD, Zeuner KE, Dambrosia JM, Hallett M. Abnormalities of spatial discrimination in focal and generalized dystonia. *Brain.* 2003;126(Pt 10):2175–2182.
37. Obermann M, Yaldizli O, De Greiff A, et al. Morphometric changes of sensorimotor structures in focal dystonia. *Mov Disord.* 2007;22(8):1117–1123.
38. Albanese A, Asmus F, Bhatia KP, et al. EFNS guidelines on diagnosis and treatment of primary dystonias. *Eur J Neurol.* 2011;18(1):5–18.
39. Simpson DM, Blitzer A, Brashear A, et al; Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. Assessment: botulinum neurotoxin for the treatment of movement disorders (an evidence-based review): report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. *Neurology.* 2008;70(19):1699–1706.
40. Jankovic J. Botulinum toxin in clinical practice. *J Neurol Neurosurg Psychiatry.* 2004;75(7):951–957.
41. Adam OR, Jankovic J. Treatment of dystonia. *Parkinsonism Relat Disord.* 2007;13(Suppl 3):S362–S368.
42. Albanese A. Terminology for preparations of botulinum toxins: what a difference a name makes. *JAMA.* 2011;305(1):89–90.
43. Wenzel R, Jones D, Borrego JA. Comparing two botulinum toxin type A formulations using manufacturers’ product summaries. *J Clin Pharm Ther.* 2007;32(4):387–402.
44. Comella C, Jankovic J, Shannon KM, et al; Dystonia Study Group. Comparison of botulinum toxin serotypes A and B for the treatment of cervical dystonia. *Neurology.* 2005;65(9):1423–1429.
45. Pappert EJ, Germanson T; Myobloc/Neurobloc European Cervical Dystonia Study Group. Botulinum toxin type B vs type A in toxin-naïve patients with cervical dystonia: randomized, double-blind, noninferiority trial. *Mov Disord.* 2008;23(4):510–517.
46. Hallett M, Benecke R, Blitzer A, Comella CL. Treatment of focal dystonias with botulinum neurotoxin. *Toxicon.* 2009;54(5):628–633.
47. Sampaio C, Ferreira JJ, Simões F, et al. DYSBOT: a single-blind, randomized parallel study to determine whether any differences can be detected in the efficacy and tolerability of two formulations of botulinum toxin type A – Dysport and Botox – assuming a ratio of 4:1. *Mov Disord.* 1997;12(6):1013–1018.
48. Odegren T, Hjaltason H, Kaakkola S, et al. A double-blind, randomised, parallel group study to investigate the dose equivalence of Dysport® and Botox® in the treatment of cervical dystonia. *J Neurol Neurosurg Psychiatry.* 1998;64(1):6–12.
49. Ranoux D, Gury C, Fondarai J, Mas JL, Zuber M. Respective potencies of Botox and Dysport: a double-blind, randomised, crossover study in cervical dystonia. *J Neurol Neurosurg Psychiatry.* 2002;72(4):459–462.
50. Nüssgens Z, Roggenkämper P. Comparison of two botulinum-toxin preparations in the treatment of essential blepharospasm. *Graefes Arch Clin Exp Ophthalmol.* 1997;235(4):197–199.
51. Benecke R, Jost WH, Kanovsky P, Ruzicka E, Comes G, Grafe S. A new botulinum toxin type A free of complexing proteins for treatment of cervical dystonia. *Neurology.* 2005;64(11):1949–1951.
52. Jankovic J, Vuong KD, Ahsan J. Comparison of efficacy and immunogenicity of original versus current botulinum toxin in cervical dystonia. *Neurology.* 2003;60(7):1186–1188.
53. Dressler D, Hallett M. Immunological aspects of Botox®, Dysport® and Myobloc™/Neurobloc®. *Eur J Neurol.* 2006;13 Suppl 1:11–15.
54. Dressler D, Bigalke H. Botulinum toxin type B de novo therapy of cervical dystonia: frequency of antibody induced therapy failure. *J Neurol.* 2005;252(8):904–907.
55. Brin MF, Comella CL, Jankovic J, Lai F, Naumann M; CD-017 BoNTA Study Group. Long-term treatment with botulinum toxin type A in cervical dystonia has low immunogenicity by mouse protection assay. *Mov Disord.* 2008;23(10):1353–1360.
56. Dressler D, Bigalke H, Benecke R. Botulinum toxin type B in antibody-induced botulinum toxin type A therapy failure. *J Neurol.* 2003;250(8):967–969.
57. Frevert J, Dressler D. Complexing proteins in botulinum toxin type A drugs: a help or a hindrance? *Biologics.* 2010;4:325–332.
58. Hauser D, Eklund MW, Boquet P, Popoff MR. Organization of the botulinum neurotoxin C1 gene and its associated non-toxic protein genes in *Clostridium botulinum* C 468. *Mol Gen Genet.* 1994;243(6):631–640.
59. Minton NP. Molecular genetics of clostridial neurotoxins. *Curr Top Microbiol Immunol.* 1995;195:161–194.
60. Ohishi I, Sugii S, Sakaguchi G. Oral toxicities of *Clostridium botulinum* toxins in response to molecular size. *Infect Immun.* 1977;16(1):107–109.
61. Ohishi I, Sakaguchi G. Oral toxicities of *Clostridium botulinum* type C and D toxins of different molecular sizes. *Infect Immun.* 1980;28(2):303–309.
62. Aoki KR, Ranoux D, Wissel J. Using translational medicine to understand clinical differences between botulinum toxin formulations. *Eur J Neurol.* 2006;13 Suppl 4:10–19.
63. Zhou Y, Foss S, Lindo P, Sarkar H, Singh BR. Hemagglutinin-33 of type A botulinum neurotoxin complex binds with synaptotagmin II. *FEBS J.* 2005;272(11):2717–2726.
64. Eisele KH, Fink K, Vey M, Taylor HV. Studies on the dissociation of botulinum toxin type A complexes. *Toxicon.* 2011;57(4):555–565.

65. Frevert J. Xeomin is free from complexing proteins. *Toxicon*. 2009; 54(5):697–701.
66. Panjwani N, O'Keefe R, Pickett A. Biochemical, functional and potency characteristics of type A botulinum toxin in clinical use. *Botulinum J*. 2008;1(1):153–166.
67. Dodd SL, Rowell BA, Vrabas IS, Arrowsmith RJ, Weatherill PJ. A comparison of the spread of three formulations of botulinum neurotoxin A as determined by effects on muscle function. *Eur J Neurol*. 1998;5(2): 181–186.
68. Wohlfarth K, Müller C, Sassin I, Comes G, Grafe S. Neurophysiological double-blind trial of a botulinum neurotoxin type A free of complexing proteins. *Clin Neuropharmacol*. 2007;30(2):86–94.
69. Kukreja R, Chang TW, Cal 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.
70. Singh BR, Lopez T, Silvia MA. Immunological characterization of type A botulinum neurotoxin in its purified and complexed forms. *Toxicon*. 1996;34(2):267–275.
71. Sharma SK, Singh BR. Immunological properties of Hn-33 purified from type A *Clostridium botulinum*. *J Nat Toxins*. 2000;9(4): 357–362.
72. Lange O, Bigalke H, Dengler R, Wegner F, deGroot M, Wohlfarth K. Neutralizing antibodies and secondary therapy failure after treatment with botulinum toxin type A: much ado about nothing? *Clin Neuropharmacol*. 2009;32(4):213–218.
73. Xeomin (incobotulinumtoxinA). Prescribing information. 2010. Available from: [http://www.xeomin.com/files/Xeomin\\_PI.pdf](http://www.xeomin.com/files/Xeomin_PI.pdf). Accessed May 2, 2011.
74. Dressler D, Benecke R. Pharmacology of therapeutic botulinum toxin preparations. *Disabil Rehabil*. 2007;29(23):1761–1768.
75. Borodic GE, Ferrante R, Pearce LB, Smith K. Histologic assessment of dose-related diffusion and muscle fiber response after therapeutic botulinum A toxin injection. *Mov Disord*. 1994;9(1):31–39.
76. Poewe W, Deuschl G, Nebe A, et al; German Dystonia Study Group. What is the optimal dose of botulinum toxin A in the treatment of cervical dystonia? Results of a double blind, placebo controlled, dose ranging study using Dysport®. *J Neurol Neurosurg Psychiatry*. 1998;64(1): 13–17.
77. Brashear A, Lew MF, Dykstra DD, et al. Safety and efficacy of NeuroBloc (botulinum toxin type B) in type A-responsive cervical dystonia. *Neurology*. 1999;53(7):1439–1446.
78. ICH Q1A(R2) Guideline. Stability testing of new drug substances and products. 2003. Available from: <http://www.fda.gov/downloads/regulatoryinformation/guidances/ucm128204.pdf>. Accessed Nov 3, 2011.
79. Grein S, Fink K. Complexing proteins are not required for stability of Botulinum neurotoxin type A preparations. Poster presented at: 71st Annual Assembly of the American Academy of Physical Medicine and Rehabilitation (AAPM&R); November 4–7, 2010; Seattle, WA. Poster P106.
80. Jost WH, Blümel J, Grafe S. Botulinum neurotoxin type A free of complexing proteins (XEOMIN®) in focal dystonia. *Drugs*. 2007;67(5): 669–683.
81. Jost WH, Kohl A, Brinkmann S, Comes G. Efficacy and tolerability of a botulinum toxin type A free of complexing proteins (NT 201) compared with commercially available botulinum toxin type A (Botox®) in healthy volunteers. *J Neural Transm*. 2005;112(7):905–913.
82. Benecke R. Xeomin in the treatment of cervical dystonia. *Eur J Neurol*. 2009;16 Suppl 2:S6–S10.
83. Consky ES, Lang AE. Clinical assessments of patients with cervical dystonia. In: Jankovic J, Hallett M, editors. *Therapy with Botulinum Toxin*. New York, NY: Marcel Dekker; 1994:211–237.
84. Comella CL, Jankovic J, Truong DD, Hanschmann A, Grafe S; on behalf of the US XEOMIN Cervical Dystonia Study Group. Efficacy and safety of incobotulinumtoxinA (NT 201, XEOMIN®, botulinum neurotoxin type A, without accessory proteins) in patients with cervical dystonia. *J Neurol Sci*. 2011;308(1–2):103–109.
85. Botox (onabotulinumtoxinA). Prescribing information. 2010. Available from: [http://www.allergan.com/assets/pdf/botox\\_pi.pdf](http://www.allergan.com/assets/pdf/botox_pi.pdf). Accessed May 2, 2011.
86. Grafe S, Comella C, Jankovic J, Truong D, Hanschmann A. Efficacy and safety of NT 201 (botulinum neurotoxin type A free from complexing proteins) in treatment-naïve cervical dystonia patients [abstract]. *Mov Disord*. 2009;24(Suppl 1):S92–S93:Tu-401.
87. Grafe S, Comella C, Jankovic J, Truong D, Hanschmann A. Efficacy and safety of NT 201 (botulinum neurotoxin type A free from complexing proteins) in pre-treated cervical dystonia patients [abstract]. *Mov Disord*. 2009;24(Suppl 1):S92:Tu-400.
88. Grafe S, Hanschmann A. Safety and efficacy of repeated NT 201 (botulinum neurotoxin type A free from complexing proteins) injections of patients with cervical dystonia: a first long-term safety analysis [abstract]. *Neurology*. 2010;74(9 Suppl 2):A88:P01.270.
89. Roggenkämper P, Jost WH, Bihari K, Comes G, Grafe S; NT 201 Blepharospasm Study Team. Efficacy and safety of a new Botulinum Toxin Type A free of complexing proteins in the treatment of blepharospasm. *J Neural Transm*. 2006;113(3):303–312.
90. Wissel J, Müller J, Dressnandt J, et al. Management of spasticity associated pain with botulinum toxin A. *J Pain Symptom Manage*. 2000;20(1):44–49.
91. Endicott J, Spitzer RL, Fleiss JL, Cohen J. The global assessment scale. A procedure for measuring overall severity of psychiatric disturbance. *Arch Gen Psychiatry*. 1976;33(6):766–771.
92. Wabbels B, Reichel G, Fulford-Smith A, Wright N, Roggenkämper P. Double-blind, randomised, parallel group pilot study comparing two botulinum toxin type A products for the treatment of blepharospasm. *J Neural Transm*. 2011;118(2):233–239.
93. Jankovic J, Comella C, Hanschmann A, Grafe S. Efficacy and safety of incobotulinumtoxinA (NT 201, Xeomin) in the treatment of blepharospasm-A randomized trial. *Mov Disord*. 2011;26(8):1521–1528.
94. Benecke R, Grafe S, Sassin I, Comes G. Overall clinical efficacy and overall tolerability of NT 201; botulinum neurotoxin free from complexing proteins [abstract]. *Mov Disord*. 2009;24(Suppl S1):S84:Mo-403.
95. Grafe S, Hanschmann A. Safety and efficacy of repeated NT 201 (botulinum neurotoxin type A free from complexing proteins) injections of patients with focal dystonia [abstract]. *Neurology*. 2010; 74(9 Suppl 2):A87:P01.268.

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