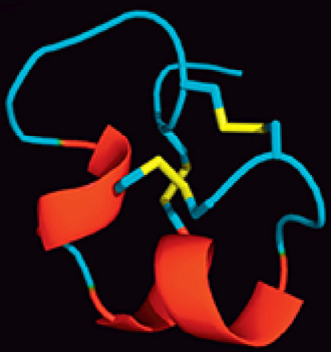
Toxicon

An interdisciplinary journal on the toxins derived from animals, plants and microorganisms



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SUPPLEMENT ISSUE:
TOXINS 2021 VIRTUAL CONFERENCE

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TOXINS 2021 Virtual Conference

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The purpose of this society is to advance knowledge on the properties of toxins and antitoxins and to bring together scholars interested in these substances through a common society. Membership consists of those who have conducted and published meritorious original investigations in toxinology, while persons who do not qualify for membership but are interested in the field of toxinology are eligible for associate membership.

Further information concerning the Society and applications for membership may be obtained from the Secretary-Treasurer.

Editorial: TOXINS 2021Virtual Conference

This special supplement of *Toxicon* contains the abstracts accepted for TOXINS 2021Virtual Conference: "Basic Science and Clinical Aspects of Botulinum and Other Neurotoxins", scheduled for January 16-17, 2021.

There continues to be rapid advancements in the understanding of the biology of botulinum neurotoxins (BoNTs) and the optimal methods for their use. The contents of this supplement constitute the latest information on the basic science of BoNTs, as well as current best practices and techniques in their administration, in a broad array of disorders, to facilitate optimal patient outcomes.

Topics that are addressed in the abstracts appearing in this supplement, presented by many of the foremost experts in each category, include:

- Latest insights into the structure, pharmacology, and activity of BoNTs
- Discovery and engineering of novel BoNT molecules and formulations
- Updates on emerging study data and clinical experience with the use of BoNTs for treating spasticity, dystonias and other movement disorders, headache and other pain syndromes, aesthetic and dermatologic indications, hypersecretory disorders, urologic disorders, and in investigational applications
- New information regarding optimizing BoNT therapy (ie, injection protocols, dosing paradigms, and methods of localization) and evaluating treatment outcomes

This supplement should prove helpful in your research or clinical use of BoNTs.

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TOXINS 2021 Virtual Conference

DAXIBOTULINUMTOXINA FOR INJECTION DEMONSTRATES CONSISTENT EFFICACY, DURATION, AND SAFETY IN FEMALES INDEPENDENT OF AGE: SUBGROUP ANALYSIS FROM A LARGE, PHASE 3 PROGRAM (SAKURA)

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Introduction: It is generally accepted that as age increases, the efficacy of botulinum toxin decreases. This has been demonstrated in multiple studies with aesthetic botulinum toxins. DaxibotulinumtoxinA for Injection (DAXI) is a novel botulinum toxin type A in development for treatment of glabellar lines. The Phase 3 SAKURA program enrolled ~3000 subjects and provided sufficient data to examine the efficacy, duration, and safety of DAXI among females according to age.

Methods: Two pivotal trials (n=609) and an open-label safety study (n=2691) enrolled adults with moderate or severe glabellar lines who received 40 U DAXI in a standardized 5-point injection pattern into the corrugator and procerus muscles. Glabellar line severity was assessed by investigators and subjects using validated 4-point rating scales. This analysis focused on the first DAXI treatment among females who were stratified into 3 cohorts: 18-45, >45-55, and >55 years. Further analyses were performed on females <65 and ≥65 years.

Results: In the Phase 3 program, 87.4% of subjects were female (34.2% were 18-45, 34.8% >45-55, 31.1% >55, and 8.6% ≥65 years). Baseline demographics were broadly similar between cohorts. Peak efficacy of DAXI was similar between cohorts. The proportion of subjects who achieved none or mild glabellar lines (per investigator) at week 4 was 97.2% (18-45), 96.3% (>45-55), 96.9% (>55), and 96.7% (≥65). Duration was consistent between cohorts. Median time to loss of none or mild glabellar lines (per investigator and subject) was 24.0 weeks for 18-45, >45-55, >55, and 24.4 weeks for ≥65 years. Treatment-related adverse events (AEs) were generally transient and mild. Incidence of AEs was similar between cohorts; however, the proportion of females with eyelid ptosis increased with age.

Conclusions: Data from this analysis demonstrate that the efficacy and duration of DAXI is similarly high in females independent of age. Notably, response rates were the same or higher in females ≥65 years.

Keywords: Aesthetics; Botulinum toxin; Facial rejuvenation; Glabellar lines; Injectable

SUSTAINED EFFICACY AND TOLERABILITY OF ONABOTULINUMTOXINA IN NAIVE AND NON-NAIVE PATIENTS WITH CERVICAL DYSTONIA: PRELIMINARY COMPLETER ANALYSIS FROM CD-PROBE

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Introduction: Cervical dystonia (CD) is the most common form of adult-onset focal dystonia. Botulinum toxin is the standard of care in this patient population. This study evaluated the sustained effectiveness and tolerability of onabotulinumtoxinA in patients with CD naïve and non-naïve to botulinum toxin.

Methods: CD-PROBE (Cervical Dystonia Patient Registry for Observation of BOTOX® Efficacy) was a multi-center, prospective, observational study of 3 onabotulinumtoxinA treatments for CD. Patients were stratified by prior exposure to botulinum toxin (naïve/non-naïve). Patients completing 3 treatments with accompanying data were included. Assessments: shift in severity between injections, Cervical Dystonia Impact Profile (CDIP-58), total Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) scores, interval between injections, and total dose. Adverse events (AEs) were recorded.

Results: Overall, 350 patients were included: 212 naïve and 138 non-naïve to botulinum toxin at baseline. Shifts in severity after each onabotulinumtoxinA treatment were generally similar in naïve and non-naïve patients (Table 1). Most patients with mild/moderate symptoms maintained or improved their severity scores. Of those with the highest severity scores, 30.0%-66.7% shifted to a lower severity score across the 3 injection cycles. Sustained improvements were seen in all CDIP-58 subscales and were generally similar between naïve and non-naïve patients. In both naïve and non-naïve patients, total TWSTRS scores improved regardless of CD severity and the median time interval between injections and the most common AEs (dysphagia, muscular weakness, headache, and neck pain) were similar. OnabotulinumtoxinA doses tended to be lower in naïve than non-naïve patients.

Conclusions: In this preliminary completer analysis, repeat onabotulinumtoxinA treatments at consistent intervals attenuated disease severity and were well tolerated regardless of prior botulinum toxin exposure.

Funding: Allergan plc

Keywords: Cervical dystonia; Effectiveness; OnabotulinumtoxinA

Table 1.
Shift in CD Severity by Treatment Cycle.

Botulinum Toxin Naïve n/n (%)

		Injection 2 CD Severity		
	Total (n=212)	Mild (n=105)	Moderate (n=97)	Severe (n=10)
Injection 1 CD Severity				
Mild	79/212 (37.3)	62/79 (78.5)	17/79 (21.5)	0/79 (0)
Moderate	111/212 (52.4)	38/111 (34.2)	71/111 (64.0)	2/111 (1.8)
Severe	22/212 (10.4)	5/22 (22.7)	9/22 (40.9)	8/22 (36.4)

		Injection 3 CD Severity		
	Total (n=212)	Mild (n=110)	Moderate (n=89)	Severe (n=13)
Injection 2 CD Severity				
Mild	105/212 (49.5)	85/105 (81.0)	20/105 (19.0)	0/105 (0)
Moderate	97/212 (45.8)	24/97 (24.7)	67/97 (69.1)	6/97 (6.2)
Severe	10/212 (4.7)	1/10 (10.0)	2/10 (20.0)	7/10 (70.0)

		Peak Effect Office Visit 3/Exit CD Severity		
	Total (n=212)	Mild (n=131)	Moderate (n=71)	Severe (n=10)
Injection 3 CD Severity				
Mild	110/212 (51.9)	97/110 (88.2)	12/110 (10.9)	1/110 (0.9)
	89/212 (42.0)	34/89 (38.2)	54/89 (60.7)	1/89 (1.1)
Severe	13/212 (6.1)	0/13 (0)	5/13 (38.5)	8/13 (61.5)

Botulinum Toxin Non-naïve n/n (%)

		Injection 2 CD Severity		
	Total (n=138)	Mild (n=49)	Moderate (n=75)	Severe (n=14)
Injection 1 CD Severity				
Mild	35/138 (25.4)	29/35 (82.9)	4/35 (11.4)	2/35 (5.7)
Moderate	79/138 (57.2)	20/79 (25.3)	55/79 (69.6)	4/79 (5.1)
Severe	24/138 (17.4)	0/24 (0)	16/24 (66.7)	8/24 (33.3)

		Injection 3 CD Severity		
	Total (n=138)	Mild (n=58)	Moderate (n=66)	Severe (n=14)
Injection 2 CD Severity				
Mild	49/138 (35.5)	42/49 (85.7)	7/49 (14.3)	0/49 (0)
Moderate	75/138 (54.3)	16/75 (21.3)	52/75 (69.3)	7/75 (9.3)
Severe	14/138 (10.1)	0/14 (0)	7/14 (50.0)	7/14 (50.0)

		Peak Effect Office Visit 3/Exit CD Severity		
	Total (n=138)	Mild (n=81)	Moderate (n=48)	Severe (n=9)
Injection 3 CD Severity				
Mild	58/138 (42.0)	54/58 (93.1)	4/58 (6.9)	0/58 (0)
Moderate	66/138 (47.8)	25/66 (37.9)	40/66 (60.6)	1/66 (1.5)
Severe	14/138 (10.1)	2/14 (14.3)	4/14 (28.6)	8/14 (57.1)

PROLONGED BOTULINUM TOXIN THERAPY IN PATIENTS WITH HEMIFACIAL SPASM

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Introduction. There are currently two types of treatment of primary hemifacial spasm (HFS) available: botulinum toxin type A (BTA) and vascular decompression. Surgical treatment is routinely offered to patients referred to the Center for Neurosurgery, whereas BTA treatment is an option chosen by patients, who are fearful of surgical treatment.
Aim: The aim of the study was to evaluate the efficacy of BTA in patients with HFS who had declined surgery.
Methods: The study included thirty-two patients (24 (75.0%) women and 8 (25.0%) men) aged 34.2±6.9 years with primary HFS who had declined surgical treatment (vascular decompression). BTA (incobotulinumtoxinA) was injected to restore facial symmetry on both sides with a total dose of 45-80 IU at 1.5- to 5-month intervals. Depending on the degree of patient satisfaction with treatment, the number of treatment sessions ranged from 3 to 12. HFS severity was assessed using the Rating scale of intensity of facial and orbicularis muscle spasm and the HFS-30 questionnaire. Treatment efficacy was assessed using the clinical global impression (CGI) scale. The mean follow-up period was 24±6 months (range: 6 to 48 months).
Results: Neurovascular conflict was observed on the left side in 20 (62.5%) and on the right side in 12 (37.5%) patients. In 25 (78.1%) patients, spasm intensity on the rating scale of intensity of facial and orbicularis muscle spasm was 2 points, and in the other 7 (21.9%) patients, it was 3 points. HFS severity (based on responses to the HFS-30 questionnaire) was 3 points in 20 (62.5%) patients and 2 points in the other 12 (37.5%) patients. After 3 BTA therapy sessions, 5 (15.6%) patients stopped treatment because of unsatisfactory results and agreed to undergo surgery. These patients reported minimal improvement on the CGI scale. The other 27 patients continued BTA treatment of HFS symptoms. After one year of regular treatment sessions, 19 (59.4%) patients remained on BTA treatment, were followed up for 3 years, and still continue the treatment. Sixteen (84.2%) of 19 patients reported significant improvement and 3 (15.8%) moderate improvement on the CGI scale. During the study period, reversible adverse events were observed, which did not require BTA therapy cessation: moderate facial asymmetry (n=9; 28.1%); mild lagophthalmos (n=4; 12.5%) and xerophthalmus (n=7; 21,9%).
Conclusion: In cases where HFS patients refuse surgical treatment, BTA injections should be recommended at 1.5- to 5-month intervals, depending on the intensity of HFS symptoms, to improve patient quality of life.
Funding: Merz Pharmaceuticals
Keywords: Hemifacial spasm; Botulinum toxin type A; IncobotulinumtoxinA

QUANTITY OF PRABOTULINUMTOXINA IN 100-U VIALS

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Several botulinum toxin type A (BoNT-A) products are marketed for therapeutic and aesthetic use.
Well-established products such as abobotulinumtoxinA (aboBoNT-A), onabotulinumtoxinA (onaBoNT-A), and incobotulinumtoxinA (incoBoNT-A) have recently been joined by other BoNT-A products, such as prabotulinumtoxinA (praBoNT-A), marketed in South Korea. The potency units of each BoNT-A product are not interchangeable. Previous publications have measured the relative quantity and activity of 150 kDa neurotoxin (responsible for neuronal silencing) in aboBoNT-A, onaBoNT-A, and incoBoNT-A vials.[1] This study expands on prior work to include the quantity of 150 kDa BoNT-A in praBoNT-A vials.
A sandwich enzyme-linked immunosorbent assay with antibodies specific to 150 kDa BoNT-A was used to capture, detect, and quantitate BoNT-A in the presence of albumin and other clostridial proteins within product vials. A calibration curve was generated using recombinant, pure 150 kDa BoNT-A, against which praBoNT-A mass was quantitated (in ng).
The amount of 150 kDa BoNT-A within a praBoNT-A 100-U vial was 0.57 ng, equating to 5.7 pg/U. The previously generated quantity per unit of commercial products is shown in the Table.
The quantity of 150 kDa BoNT-A per unit of praBoNT-A is within the range of other commercially produced toxins, and close to that of aboBoNT-A. When dosed at total recommended dosages, aboBoNT-A contains more active neurotoxin than onaBoNT-A, incoBoNT-A, and praBoNT-A (Table). The amount of active neurotoxin in the approved aboBoNT-A dose has been proposed as a possible reason for its extended duration of action compared with onaBoNT-A and incoBoNT-A.[2] Further research is required

Table
Total quantity of active 150 kDa BoNT-A in maximum recommended doses of BoNT-A products.

	A - Total Recommended Dosage (Glabellar Lines),[a] Product Units	B - Amount of Neurotoxin Per Product Unit, pg	C - Total Amount of Active BoNT-A (ng) Injected at the Recommended Dose, C = A × B
AboBoNT-A	50	5.4	0.27
OnaBoNT-A*	20	9.0	0.18
IncoBoNT-A	20	4.0	0.08
PraBoNT-A	20	5.7	0.11

* Botox Cosmetic®.
[a] According to prescribing information.

to determine if this is also true in relation to praBoNT-A.
Funding: Ipsen.
Keywords: Dose; Duration; Quantity; Units

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COMPARISON OF TWO INCOBOTULINUMTOXINA INJECTION TECHNIQUES FOR TREATING AXILLARY HYPERHIDROSIS

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Introduction and Objectives: The conventional strategy for applying botulinum toxin A to treat axillary hyperhidrosis (AH) uses multiple injection punctures.[1,2] However, an alternative technique of subcutaneous injection in a radial manner through two sites might reduce procedure duration (Dr. Rosa Flores, Mexico, personal communication based on Odderson[3]). This study compared the efficacy and safety of the two injection techniques for AH.
Methods: This was a randomized, evaluator-blinded trial. Each patient received 50 U of incobotulinumtoxinA injected intradermally using multiple punctures in one axilla and the same amount injected subcutaneously by radial approach in the other axilla. Outcomes assessed at follow-up visits (30, 120, 180, and 270 days) included procedure duration, pain, gravimetry, Minor's starch-iodine test, and safety.
Results: A total of 24 patients (67% female, mean age 34.7 years) with severe hyperhidrosis were included. Seven had a Hyperhidrosis Disease Severity Scale grade of 3 (sweating barely tolerable and frequently interferes with daily activities) and 17 had a grade of 4 (sweating intolerable and always interferes with daily activities). Radial injection was administered faster ($P<0.001$) but with higher pain scores ($P=0.001$) compared with multiple injection punctures. Both techniques yielded significant sweat reduction, with 95% of responders (50% or greater sweat reduction from baseline) after 30 days. Most patients injected with either technique had an excellent response (90%-100% sweat reduction) after 30 days that was sustained until at least day 270. The multiple-puncture group had a higher reduction in gravimetric measures at days 30 and 180 and Minor's test at day 270. Both injection techniques were well tolerated.
Conclusions: Multiple-puncture injection and radial injection of incobotulinumtoxinA are effective and safe approaches for treating AH, but these results indicate that the multiple-puncture injection technique improves outcomes at certain time points and is less painful than the radial injection technique.
Funding/Disclosures: This study was supported by Merz Pharmaceuticals GmbH, Frankfurt am Main, Germany. Ada Regina Trindade de Almeida is a speaker, consultant, and researcher for Merz and Allergan.
Keywords: Axillary hyperhidrosis; Botulinum toxin type A; IncobotulinumtoxinA; Multiple puncture; Radial

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THE ADMINISTRATIVE PRACTICES ADOPTED TO DELIVER BOTULINUM TOXIN IN ITALY: A MULTICENTER STUDY OF THE ITALIAN BOTULINUM TOXIN NETWORK

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Introduction: Thirty years have passed since botulinum toxin became a medical treatment provided by the Italian National Health System for the therapy of movement disorders and later for spasticity, migraine and vegetative disorders. However, the administrative procedures for dispensing the drug in the various Italian regions are little known. The purpose of this study by the Italian Botulinum Toxin Network was to learn about the administrative practices adopted to deliver botulinum toxin in Italy.
Methods: The study was carried out using a survey questionnaire submitted to 140 medical centers in all Italian regions. The questions submitted to the individual centers were:

- Type and number of prescriptions used for each treatment
- The prescribing doctor (neurologist, other)
- Where the treatment was carried out: outpatient clinic, day hospital, or hospital
- The authorized health facilities (hospitals, accredited clinics)
- Possibility of providing treatment in a private environment
- Methods of reimbursement of expenses of the national health system
- Distribution of the drug through hospital pharmacies
- How was informed consent obtained from the patient?

Results: Responses were classified according to region of origin in order to obtain a complete national evaluation of the results obtained. About one hundred centers replied to the questionnaire. The results show extreme variability in the type and number of prescriptions used within the same region and also within the same center in different outpatient clinics (eg, for migraine and movement disorders), while the use in hospital outpatient clinics showed a strong consistency.
Conclusions: Our data show a wide variability and substantial difference in the administrative methods of dispensing, supplying, and paying for botulinum toxin in hospital centers for botulinum toxin therapy in the different Italian regions. This fact causes considerable problems for patients and often makes it difficult to access effective therapy in the treatment of various neurological pathologies.
Keywords: Administrative procedures; Botulinum toxin; Italian Botulinum Toxin Network; Rete Italiana Tossina Botulinica

Appendix
Italian Botulinum Toxin Network Administrative Project participants: Albani G.; Altavista M.C.; Ardolino G.; Assetta M.; Autunno M.; Babbini M.T.; Baldacci F.; Balestrieri F.; Barbieri S.; Bargellesi S.; Bentivoglio A.; Bertolasi L.; Bono F.; Borsato C.; Calabrese R.; Callegarini C.; Campiglio L.; Capone L.; Carmillo L.; Casellato C.; Cassano D.; Cassetta E.; Castagna A.; Castronovo G.; Ceravolo R.; Cesaretti C.; Cevoli S.; Cherchi A.; Coletti Moja

M.; Contardi S.; Danni M.C.; Aurizio C.; Avenia L.; De Bartolo M.; De Blasiis P.; Del Colle R.; Dimanico U.; Doretti A.; Eleopra R.; Fabbrini G.; Foresti C.; Frasson E.; Frontoni M.; Garau E.; Giovannelli M.; Girlanda P.; Gori S.; Guccione A.; Ialongo T.; Iannacchero R.; La Spada S.; Lanfranchi S.; Leggio U.; Lelli S.; Lettieri C.; Liotti V.; Lispi L.; Lori S.; Maderna L.; Maggi L.; Magistrelli L.; Mailland E.; Mampreso E.; Manzo L.; Marchese R.; Marinelli L.; Marra M.; Masi G.; Mazzucchi S.; Mercenaro M.; Milano E.; Millevolte M.; Misceo S.; Moccia M.; Onesta M.G.; Oppo V.; Osio M.; Pascarella A.; Pastorelli F.; Petracca M.; Piano C.; Picelli A.; Pierangeli G.; Plasmati R.; Polidori L.; Pozzolante R.; Pratesi L.; Priori A.; Prudenzano M.P.; Quatrale R.; Reggiani M.; Romano M.; Rossi V.; Sacco S.; Sances G.; Santoro A.; Scaglione Cesa L.M.; Serra G.; Sidoti V.; Soliveri P.; Squintani G.; Tambasco N.; Terranova C.; Torelli P.; Treviso M.; Trompetto C.; Tugnoli V.; Ungaro D.; Valzania F.

MANAGEMENT OF LOCALIZED SCLERODERMA WITH BOTULINUM TOXIN

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Localized scleroderma is a rare disease that occasionally presents with neurologic involvement, especially hemimasticatory spasm. This can occur due to the disease of the deeper skin layers affecting the muscles, cartilage, and trigeminal nerve. We report the case of a 47-year-old woman with overlap syndrome (localized scleroderma and rheumatoid arthritis) with hemimasticatory spasm on the left side of the face and intense pain secondary to morphea. The spasm and intense pain were refractory to multiple treatments (eg, immunosupressants, anticonvulsants, and antidepressants). In this case, we describe the clinical presentation, the challenges faced in the management of the patient, and botulinum toxin as a new therapeutic option in morphea with neurologic involvement. Treatment with botulinum toxin was undertaken with an improvement in pain and in the involuntary movement.

Keywords: Botulinum toxin; Hemimasticatory spasm; Neurologic involvement; Refractory; Scleroderma

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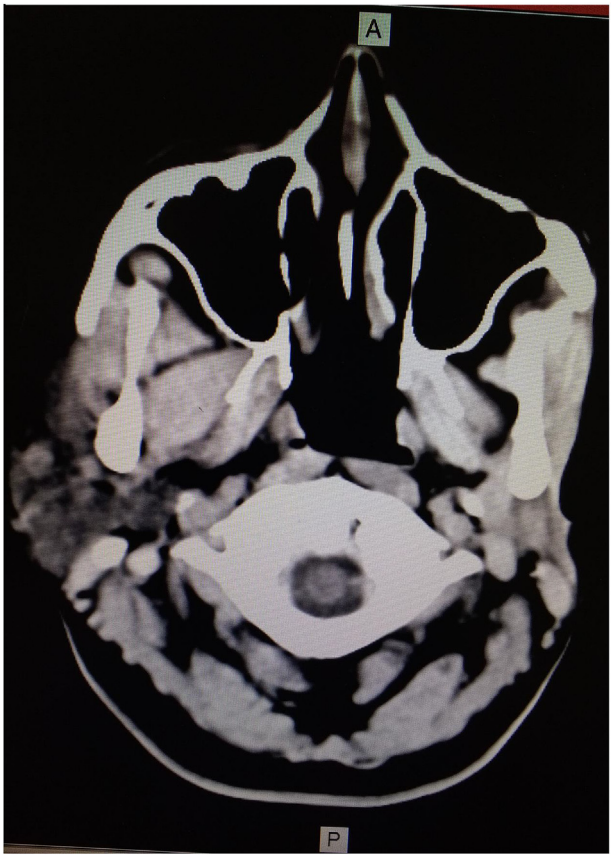

Fig. Image showing a CT scan of the head where decreased muscle mass and atrophy can be observed in the masseter muscles on the left side of the face.

MINIMAL E-CADHERIN INHIBITOR PROTEIN DERIVED FROM HEMAGGLUTININ, A NON-TOXIC COMPONENT OF BOTULINUM TOXIN COMPLEX

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Introduction and Objectives: Hemagglutinin (HA) is one of the non-toxic components of the botulinum toxin complex. HA binds to E-cadherin (E-cad) via direct protein-protein interactions, but not to N-cadherin and VE-cadherin. HA reversely blocks the function of E-cad by binding, and does not cleave E-cad. Thus, HA can be used as an E-cad modulator controlling the cell-cell adhesion. HA is the 470-kDa large protein complex composed of 12 subcomponents: six HA1, three HA2, and three HA3. In this study, we developed a new E-cad modulator derived from HA, which can be purified easily and inexpensively, for use in tissue engineering and cell biology tools.

Methods: Based on structural and biochemical information, the various kinds of mutations were rationally designed, and introduced to HA of type

B1 Okra. Recombinant HA subcomponents were expressed in *Escherichia coli* and purified. HAs were reconstituted in vitro. The barrier- disrupting activities of HAs were assessed by TER assay using Caco-2 cells.
Results: HAΔ1 (HA2+HA3) and mini-HA (HA1+HA2+HA3mini) disrupted the epithelial barrier, whereas mini-HAΔ1 failed to disrupt. According to the crystal structure of the HA-E-cad complex (Lee, et al, 2014) and the above results, it is assumed that mini-HAΔ1 is enough to bind to E-cad. Then, we created various mini-HAΔ1 mutants, and assessed them. As a result of these screenings, single-chain mini-HAΔ1 with three-point mutations and the domain-swapping of HA2 (named Nano-HA) bound to E-cad specifically, and disrupted the epithelial barrier effectively.
Conclusions: We developed a new E-cad modulator, Nano-HA derived from *Clostridium botulinum* HA. The Nano-HA is a 47-kDa protein, which is one-tenth of the HA wild-type, and can be prepared by simple one-step purification. As with the HA wild-type, Nano-HA specifically binds to E-cadherin, and disrupts the epithelial barrier effectively. The Nano-HA provides the foundation to develop much more effective proteins and fusion proteins targeting E-cadherin.
Keywords: Botulinum toxin complex; E-cadherin (E-cad); Hemagglutinin (HA); Molecular engineering

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CHEMODENERVATION OF OROMANDIBULAR DYSTONIA WITH BOTULINUM TOXINS: FIVE-YEAR EXPERIENCE

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Objective: To review the experience with the use of botulinum neurotoxin (BoNT) injections for management of oromandibular dystonia (OMD).
Background: OMD causes sustained or repetitive, forceful contractions of the muscles of the jaw and tongue, causing jaw opening, jaw closing, lingual, and mixed dystonias. When oral pharmacologic agents are inadequate, BoNT is the standard of care.
Method: A single-center, retrospective chart review (July 2013-June 2018) of BoNT injections for OMD was performed. Wastage was defined as leftover drug from the vial, that was prepared but not injected. A patient who had not received injections within the last 6 months was considered a "dropout".
Results: We had 43 patients (21 females), median age 58 years (range 21-88 years) with 231 encounters (average 5.4/patient, range 1-16). The most commonly used toxin was onabotulinumtoxinA (onaBoNT-A, 88%); very few patients were treated with incobotulinumtoxinA (incoBoNT-A, n=3) and rimabotulinumtoxinB (RimaBoNT-B, n=2).
Jaw-closing dystonia was more common (70%; 60% females) than jaw-opening (21%) and lingual dystonia (21%), with some overlap.
Isolated lingual dystonia injections were rare (11%) and commonly associated with jaw opening (56%), while jaw opening injections were never solitary. Isolated jaw closing injections were also uncommon (23%) and frequently associated with cervical (40%) and facial (40%) injections.
The average total dose (in units, with range) of onaBoNT-A was 66 (10-180) for jaw closing; 54 (30-100) for jaw opening, and 16 (5-30) for lingual dystonia. RimaBoNT-B (1000-2000) and incoBoNT-A (45-50) were only used for jaw-closing dystonia.
The average wastage of onaBoNT-A was 25 units/patient. The dropout rate over 5 years was 37% after the average of 4 sessions (range 1-11). The most common reason was lost to follow up (22%) and deceased (22%), while lack of benefits (17%) and side effects (dysphagia 17% and weakness 11%) were uncommon reasons.
Conclusion: In our series, jaw-closing dystonia was the most frequent condition, and most OMD disorders were segmental in nature. Although side effects and lack of benefits were infrequently reported, dropouts were common.
Funding: This work did not receive any grant or funding from private, public, or non-profit organizations.
Keywords: Oromandibular dystonia; Chemodenervation; BoNT; Movement disorders; Dystonia.
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Dr. Ameer has nothing to disclose.
Dr. Syed has nothing to disclose.
Dr. Bertoni has received personal compensation for consulting, serving on a scientific advisory board, speaking, or other activities for Kyowa.
Dr. Hellman has received personal compensation for consulting, serving on a scientific advisory board, speaking, or other activities for Teva Pharmaceuticals.
Dr. Torres-Russotto has received honoraria for consulting or speaking for AbbVie, Acadia Pharmaceuticals, Acorda Therapeutics, Adamas Pharmaceuticals, Allergan, GK Pharmaceuticals, Ipsen, and Sunovion.
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DOUBLE-BINDING BOTULINUM MOLECULE WITH REDUCED MUSCLE PARALYSIS: EVALUATION IN *IN VITRO* AND *IN VIVO* MODELS OF MIGRAINE

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With a prevalence of 15%, migraine is the most common neurological disorder and among the most disabling diseases considering the years lived with disability. Current oral medications for migraine show variable effects and are frequently associated with intolerable side effects, leading to the dissatisfaction of both patients and doctors. Injectable therapeutics, which include CGRP-targeting monoclonal antibodies and botulinum neurotoxin type A (BoNT/A), provide a new paradigm for treatment of chronic migraine, but are effective only in approximately 50% of subjects. Here, we investigated a novel engineered botulinum molecule, with markedly reduced muscle-paralysing properties, which could be beneficial for the treatment of migraine. This stapled botulinum molecule with duplicated binding domain – BiTox/AA – cleaves SNAP-25 with a similar efficacy to BoNT/A in neurons; however, the paralysing effect of BiTox/AA was 100 times less when compared to native BoNT/A following intramuscular injection. The performance of BiTox/AA was evaluated in cellular and animal models of migraine. BiTox/AA inhibited electrical nerve fiber activity in rat meningeal preparations while, in the trigeminovascular model, BiTox/AA raised electrical and mechanical stimulation thresholds in Aδ- and C-fiber nociceptors. In the rat glycerine-trinitrate (GTN) model, BiTox/AA proved effective in inhibiting GTN-induced hyperalgesia in the orofacial formalin test. We conclude that the engineered botulinum molecule provides a useful prototype for designing advanced future therapeutics with improved efficacy in the treatment of migraine.

ONABOTULINUMTOXINA CHANGES CORTICAL EXCITABILITY IN CHRONIC MIGRAINE PATIENTS AFTER PREVENTIVE TREATMENT: OBSERVATIONAL STUDY OVER 12 MONTHS

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Introduction: Chronic migraine (CM) is a common and disabling form of migraine that requires long-term preventive therapy for one year. OnabotulinumtoxinA (OnabotA) has regulatory approval as an effective medication for CM prophylactic treatment. In our previous study, we showed that even a single OnabotA injection in CM patients following the PREEMPT injection paradigm resulted in a change of cortical excitability parameters,[1] assessed by transcranial magnetic stimulation (TMS)[2]. However, it is still unknown whether these changes persist after repeated injections.
Study aim: To assess the impact of preventive treatment of CM with OnabotA on cortical excitability after 12 months using TMS.
Methods: Twenty-five patients with CM (median age: 44 years; 97% women, diagnosis according to the International Classification of Headache Disorders, 3rd Edition [ICHD-3], 2018) were included in this observational study. OnabotA injections were performed (following the PREEMPT paradigm) every 3 months for one year. We assessed motor cortex thresholds (MT, right/left [r/l]; % of maximal stimulator output), cortical silent period duration (CSP r/l, milliseconds [ms]) by motor cortex stimulation with recording of responses from abductor digiti minimi muscles (r/l), and phosphene threshold (PT, % of maximal stimulator output) by visual cortex stimulation, using TMS before, 3, and 12 months after treatment.
Results: MT r/l and CSP r/l increased significantly after 3 and 12 months' OnabotA treatment, compared to baseline, but PT did not change (Table).

Table.
TMS Parameters Before, 3, and 12 Months After OnabotA (n=25) CM Preventive Treatment.

Parameter		Before	3 Months	12 Months
MT, % Median (min-max)(25%;75%)	Right	40 (26-60)(37; 49)	45 (32-63)(38; 52)*	46 (31-62)(39; 52)**
	Left	42 (26-61)(38;51)	45 (33-62)(37;52)*	44 (31-62)(38;50)**
CSP duration, ms Median (min-max) (25%;75%)	Right	96 (47-161)(78;114)	117 (73-167)(96;138)*	116 (72-169)(95;137)**
	Left	96 (47-170)(72;120)	117 (75-170)(94;141)*	118 (73-168)(92;140)**
PT	% Median (min-max)(25%;75%)	63 (40-95)(52;74)	63 (46-93) (52;77)	62 (44-91)(50;73)

*Statistically significant difference between parameters before and after 3 months treatment (P <0.05).
**Statistically significant difference between parameters before and after 12 months treatment (P <0.05).

Conclusion: The results of our study showed that cortical excitability changes in CM patients after the first preventive OnabotA injections, and these changes persist after repeat injections.
Keywords: Chronic migraine; Cortical excitability; OnabotulinumtoxinA (OnabotA); Prophylactic treatment; Transcranial magnetic stimulation (TMS)

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EFFICACY AND SAFETY OF THREE ACTIVE DOSES OF ONABOTULINUMTOXINA FOR THE TREATMENT OF NEUROGENIC DETRUSOR OVERACTIVITY IN CHILDREN: RESULTS OF A RANDOMIZED CONTROLLED CLINICAL TRIAL

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Introduction: Neurogenic detrusor overactivity (NDO) negatively affects bladder health and quality of life. OnabotulinumtoxinA (onabotA) 200 U is an approved treatment for NDO in adults.
Methods: This multicenter, randomized, 48-week, double-blind study evaluated the efficacy and safety of onabotA in children (5-17 years) with NDO and UI not adequately managed with anticholinergics. Patients were randomized to one cystoscopic onabotA treatment (50 U [n=38], 100 U [n=45], or 200 U [n=30]; not to exceed 6 U/kg) delivered as 20 bladder wall injections of 0.5 mL, excluding the trigone. Use of placebo was not considered medically justified due to the renal risk in this NDO population, procedure invasiveness, and likely need for anesthesia; thus a low-dose comparator (50 U) was used for statistical testing.
Results: Adjusted mean reductions in UI episodes/day from baseline (BL) at week 6 (primary endpoint) were: 1.30 for 50 U and 100 U and 1.34 for 200 U (BL: 50 U, 2.81; 100 U, 2.99; 200 U, 3.68). At BL, 0%, 13.3%, and 3.6% of patients in the 50 U, 100 U, and 200 U arms were incontinence-free at night, increasing to 30.6%, 32.6%, and 28.6% at week 6. Mean change from BL at week 6 in urine volume at first morning clean intermittent catheterization (CIC) was 21.9, 34.9, and 87.5 mL in the 50 U, 100 U, and 200 U arms (BL: 203.5, 164.2, 187.7 mL); P=.0055 for 200 U vs 50 U. There was a dose-dependent mean reduction from BL in maximum detrusor pressure during the storage phase (Pdetmax) of 12.9, 20.1, and 27.3 cmH_2O with 50 U, 100 U, and 200 U (BL: 58.2, 56.5, 56.7 cmH_2O); P=.0157 for 200 U vs 50 U. The safety profile was similar across doses; urinary tract infection was the most commonly reported adverse event.
Conclusion: Each dose of onabotA was well tolerated and demonstrated clinical efficacy for UI treatment in children with NDO. However, the 200 U dose showed clinically and statistically greater improvements vs 50 U in the important efficacy measures of Pdetmax and volume at first morning CIC.
Funding: Allergan, an AbbVie company.
Keywords: Neurogenic detrusor overactivity; OnabotulinumtoxinA; Pediatric; Urinary incontinence

SAFETY OF INCOBOTULINUMTOXINA IN MULTIPATTERN TREATMENT OF UPPER- AND LOWER-LIMB SPASTICITY IN CHILDREN/ ADOLESCENTS WITH CEREBRAL PALSY: POOLED ANALYSIS OF 3 LARGE PHASE 3 STUDIES

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Introduction: This analysis assessed the safety and tolerability of repeated incobotulinumtoxinA treatment for lower-limb (LL), upper-limb (UL), or combined LL/UL spasticity in ambulant and non-ambulant children/adolescents with cerebral palsy (CP) using pooled data from 3 large Phase 3 studies.
Methods: Pediatric patients with spasticity (2-17 years of age; uni- or bilateral CP; Gross Motor Function Classification System [GMFCS] level I-V; Ashworth Scale [AS] score ≥2 in clinical patterns for treatment; clinical need for treatment) were enrolled. Patients received total body incobotulinumtoxinA doses of 16 U/kg body weight (BW, ≤400 U) for LL spasticity in 2 injection cycles (ICs) in TIM (NCT01893411). In TIMO (NCT01905683), TIM completers and new recruits received 4 ICs with 16-20 U/kg (≤400-500 U) for LL or combined LL/UL treatment. In XARA (NCT02002884), patients received 4 ICs with 16-20 U/kg (≤400-500 U) for UL or combined LL/UL treatment. Adverse events (AEs) were assessed in the pooled population.
Results: In total, 907 patients (59.6% male, mean [SD] age 6.7 [4.2] years, BW 23.3 [13.9] kg) received multipattern treatment; 753 patients (83.0%) completed the studies and received up to 6 ICs. Across all ICs, 363 (40.0%) experienced an AE; 33 (3.6%) had ≥1 treatment-related AE. The most common AEs were nasopharyngitis, bronchitis, and upper-respiratory tract infection. Serious AEs (SAEs) and AEs of special interest (AESIs) were reported for 49 (5.4%) and 18 (2.0%) patients, respectively. AESIs reported in >1 patient were muscular weakness (6 patients, 0.7%), dyspnea, constipation, and dysphagia (3 patients, 0.3% each). There was no increased incidence of AEs, SAEs, or AESIs with repeated dose. No deaths were reported in these studies.
Conclusions: IncobotulinumtoxinA was safe and well tolerated for LL, UL, or combined multipattern treatment over up to 6 ICs in a comprehensive population of ambulant and non-ambulant pediatric patients with spasticity (GMFCS levels I-V).
Funding: Merz Pharmaceuticals GmbH, Frankfurt am Main, Germany
Keywords: Cerebral palsy; IncobotulinumtoxinA; Multilevel; Multipattern; Pediatric; Safety

COLLABORATIVE STUDY FOR BONT ACTIVITY DETERMINATION IN VITRO USING THE BINACLE (BINDING AND CLEAVAGE) ASSAY

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The botulinum neurotoxin (BoNT) serotypes A and B are used to treat a wide variety of disorders associated with muscle overactivity. Each batch of these toxin products has to be subjected to an exact activity determination to avoid unwanted side effects. Originally, these potency measurements were based on toxicity testing in mice. Although several alternative methods have been developed, no generally accepted in vitro method exists to date that is applicable to all relevant BoNT products and freely available for all potential users.
The BINACLE (binding and cleavage) assay measures the activity of BoNT/A and BoNT/B in vitro based on their specific receptor-binding and proteolytic properties.[1,2]
In-house studies showed that this method is very sensitive, specific, and applicable to all approved BoNT products. Moreover, a transferability study demonstrated that the assay protocol can be transferred directly to other laboratories.[3] Accordingly, this in vitro assay may represent a suitable and widely applicable alternative to the mouse toxicity tests. An international collaborative trial is currently being conducted to promote acceptance of the BINACLE assay by BoNT manufacturers and regulatory authorities.
Funding: German Federal Ministry of Education and Research (BMBF project no. 031L0148) and the German Research Foundation (DFG).
Keywords: Botulinum neurotoxins; BINACLE (binding and cleavage) assay; In vitro activity test; Potency determination; Collaborative study

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EFFICACY OF INCOBOTULINUMTOXINA IN THE TREATMENT OF 6- TO 17-YEAR-OLD CHILDREN AND ADOLESCENTS WITH CHRONIC SIALORRHEA ASSOCIATED WITH NEUROLOGICAL DISORDERS AND/OR INTELLECTUAL DISABILITY

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Introduction and Objectives: The SIPEXI study investigated efficacy and safety of repeated intraglandular incobotulinumtoxinA (incoBoNT/A) injections in children/adolescents with sialorrhea associated with neurological disorders.
Methods: SIPEXI was a prospective, multicenter, phase III study (NCT02270736) with a randomized, double-blind, parallel-group, placebo-controlled main period (MP, 1 injection cycle [IC]) and an open-label extension period (OLEX, 3 ICs). Children/adolescents with chronic sialorrhea associated with neurological disorders and/or intellectual disability, and severe drooling were enrolled. Patients aged 6-17 years (yrs) were randomized to receive a body weight–dependent dose of incoBoNT/A according to weight classes of around 2 U/kg (total dose of 75 U for patients ≥30 kg) or placebo in the MP. In the OLEX, all received up to 3 further incoBoNT/A ICs. The follow-up period was 16 weeks for each IC. Primary endpoints were the change in unstimulated salivary flow rate (uSFR) from baseline to MP week 4, and the Carers' Global Impression of Change Scale

(GICS) rating at MP week 4. Further endpoints included changes in these parameters at later visits. Adverse events were recorded.
Results: We present the efficacy results for 6- to 17-year-old patients (mean age 10 yrs; approximately 60% with cerebral palsy; >85% with intellectual disability). In the MP, 148 patients received incoBoNT/A and 72 received placebo. Two hundred sixteen patients completed the MP. Of these, 214 patients entered and 189 patients completed the OLEX. At MP week 4, significantly larger improvements were seen for incoBoNT/A compared to placebo in uSFR (P=0.0012) and carers' GICS ratings (P=0.032) (Figure). Other endpoints consistently supported these results. During the OLEX, prolonged and sustained treatment effects were seen. No unexpected safety concerns arose (see safety results in separate abstract).
Conclusions: IncoBoNT/A is effective for the treatment of chronic sialorrhea in children and adolescents.
Funding: Merz Pharmaceuticals GmbH, Frankfurt am Main, Germany
Keywords: Botulinum neurotoxin type A; Chronic sialorrhea; IncobotulinumtoxinA; Pediatric

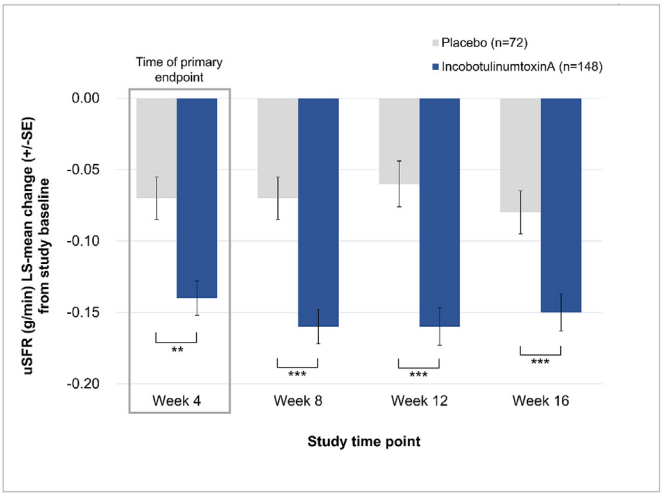

Fig. Unstimulated salivary flow rate (uSFR), mean changes from study baseline to main period visits. Comparison of incobotulinumtoxinA vs placebo, with ** $P < 0.01$, *** $P \leq 0.001$.

SAFETY OF INCOBOTULINUMTOXIN/A IN THE TREATMENT OF 6- TO 17-YEAR-OLD CHILDREN AND ADOLESCENTS WITH CHRONIC SIALORRHEA ASSOCIATED WITH NEUROLOGICAL DISORDERS AND/OR INTELLECTUAL DISABILITY

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Introduction and Objectives: The SIPEXI study investigated efficacy and safety of repeated intraglandular incobotulinumtoxinA (incoBoNT/A) injections in children/adolescents with sialorrhea associated with neurological disorders.
Methods: SIPEXI was a prospective, multicenter, phase III study (NCT02270736) with a randomized, double-blind, parallel-group, placebo-controlled main period (MP, 1 injection cycle [IC]) and an open-label extension period (OLEX, 3 ICs). Children with chronic sialorrhea were enrolled in a stepwise manner: older patients first, with safety review before recruitment of younger cohorts to give early warning of unexpected safety issues. Patients aged 6-17 years (yrs) were randomized to receive a body weight–dependent dose of incoBoNT/A according to weight classes of around 2 U/kg (total dose 75 U for patients ≥30 kg) or placebo in the MP. Injections were performed under ultrasound guidance. In the OLEX, all received up to 3 further incoBoNT/A ICs. Safety was assessed by analyzing adverse events (AEs). AEs of special interest (AESIs, including dysphagia, aspiration, and pneumonia aspiration) were questioned. A dentist assessed dental/periodontal AEs.
Results: We present the safety results for 6- to 17-year-old patients (mean age 10 yrs; approximately 60% with cerebral palsy; >85% with intellectual disability). In the MP, 148 patients received incoBoNT/A and 72 placebo. Two hundred sixteen patients completed the MP. Of these, 214 patients entered and 189 patients completed the OLEX. AE rates during the MP were similar for incoBoNT/A (18.2%) and placebo (15.3%) (Table). Rates of serious AEs, related AEs, and AESIs were low, with only 1 AESI (dysphagia). In the OLEX, 43.4% of patients had AEs. The rate did not increase with repeated ICs. Respiratory infections were the most common AEs; dental AEs were rare. No unexpected safety concerns arose.
Conclusions: IncoBoNT/A is safe and well tolerated for treatment of sialorrhea in children/adolescents at body weight–dependent doses up to 75 U per IC.
Funding: Merz Pharmaceuticals GmbH, Frankfurt am Main, Germany
Keywords: Botulinum neurotoxin type A; IncobotulinumtoxinA; Chronic sialorrhea; Pediatric

Table.
Summary of Adverse Events (AEs) During Main Period (MP) and Open-Label Extension Period (OLEX).

	Main period		OLEX (cumulative over 3 cycles)
Number of patients (%) with	Placebo (n=72)	IncoBoNT/A (n=148)	IncoBoNT/A (n=145)
AE(s)	11 (15.3)	27 (18.2)	63 (43.4)
AE(s) related to treatment	0 (0.0)	2 (1.4)	8 (5.5)
Adverse event(s) of special interest (AESI(s))	0 (0.0)	1 (0.7)	4 (2.8)
AESI(s) related to treatment	0 (0.0)	1 (0.7)	4 (2.8)
Serious AE(s) (SAE(s))	1 (1.4)	0 (0.0)	8 (5.5)
SAE(s) related to treatment	0 (0.0)	0 (0.0)	0 (0.0)
AE(s) leading to discontinuation	1 (1.4)	1 (0.7)	4 (2.8)
AE(s) leading to discontinuation related to treatment	0 (0.0)	0 (0.0)	1 (0.7)
Fatal AE(s)	0 (0.0)	0 (0.0)	0 (0.0)

OLEX data from patients who had received incoBoNT/A already in the MP. AEs during MP were defined as treatment-emergent with onset or worsening at or after first injection of incoBoNT/A or placebo up to and before first injection of OLEX or, in case of discontinuation before OLEX, up to and including 16 weeks after first injection or last study visit, whichever was later.

EFFICACY AND SAFETY OF INCOBOTULINUMTOXIN/A IN THE TREATMENT OF 2- TO 5-YEAR-OLD CHILDREN WITH CHRONIC SIALORRHEA ASSOCIATED WITH NEUROLOGICAL DISORDERS AND/OR INTELLECTUAL DISABILITY

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Introduction and Objectives: The SIPEXI study investigated the efficacy and safety of repeated intraglandular incobotulinumtoxinA (incoBoNT/A) injections for sialorrhea associated with neurological disorders, also in young children aged 2-5 years (yrs).
Methods: SIPEXI was a prospective, multicenter, phase III study (NCT02270736) enrolling children with chronic sialorrhea associated with neurological disorders and/or intellectual disability. The younger cohort of 2- to 5-year-olds (N=35) was recruited after older children had been enrolled and assessed for safety. The 2- to 5-year-olds received up to 4 injection cycles (ICs) of incoBoNT/A with body weight–dependent doses according to weight classes of around 2 U/kg. The follow-up period was 16 weeks per IC. Efficacy outcomes included Carers' Global Impression of Change Scale (GICS) ratings (scale from -3 [very much worse] to +3 [very much improved]). Adverse events (AEs) were recorded. AEs of special interest (AESIs, including dysphagia, aspiration, and pneumonia aspiration) were questioned.
Results: We present the results for 2- to 5-year-old patients (mean age 4 yrs; 57% with cerebral palsy; >94% with intellectual disability). Thirty-five patients were treated with incoBoNT/A, 33 out of 35 completed all 4 ICs. Good treatment effects were seen, although results were descriptive only (small sample size) (Figure). GICS ratings showed consistent improvements at all visits, with mean ratings around +1.1. Other endpoints supported the results. A sustained effect of incoBoNT/A was seen after repeated ICs, with notable improvements over time. The AE rate varied between the ICs (1st: 14.3%; 2nd: 21.2%; 3rd: 15.2%; 4th: 33.3%). Few related AEs and serious AEs (non-related) and no AESIs occurred. Most AEs were respiratory infections. No unexpected safety concerns arose.
Conclusions: Treatment of chronic sialorrhea with body weight–dependent doses of incoBoNT/A showed clinically relevant improvements and few and minor side effects in children aged 2-5 years.
Funding: Merz Pharmaceuticals GmbH, Frankfurt am Main, Germany
Keywords: Botulinum neurotoxin type A; Chronic sialorrhea; IncobotulinumtoxinA; Pediatric

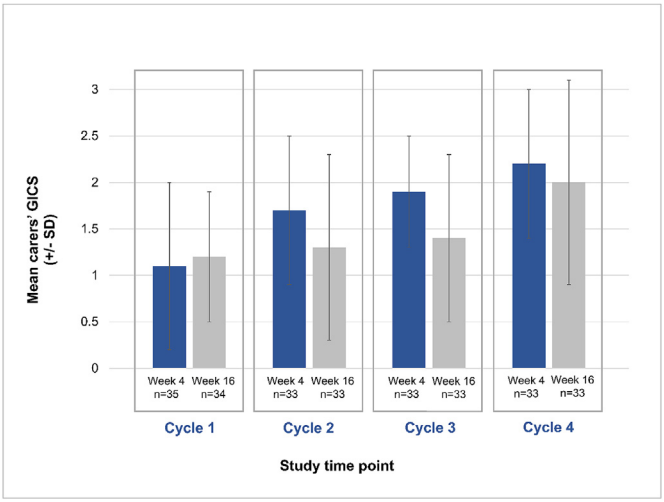

Fig 1. Carers' Global Impression of Change Scale (GICS) mean ratings for 2- to 5-year-olds. GICS 7-point scale from -3 (very much worse) to +3 (very much improved).

SAFETY RECOMMENDATIONS FOR TREATMENT WITH BOTULINUM TOXIN DURING THE COVID-19 PANDEMIC PREPARED BY THE ITALIAN BOTULINUM TOXIN NETWORK IN COLLABORATION WITH THE ACCADEMIA LIMPE-DISMOV, SISC, AND ANIRCEF ASSOCIATIONS

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Overview: In order to minimize the risk of virus transmission in the era of COVID-19, the Italian Botulinum Toxin Network, in collaboration with Accademia LIMPE-DisMov, ANIRCEF, and SISC, propose recommendations for good practice in performing botulinum toxin injections.
Recommendations to prevent SARS-CoV-2 infection:
Structural recommendations:

- Daily sanitization of areas to be used for botulinum toxin injections
- Take the patient's body temperature at the entrance to the facility.
- Maintain appropriate social distancing in the waiting room.
- Provide healthcare personnel with personal protective equipment (PPE), such as surgical or FFP2 masks, footwear, headgear, disposable gowns, gloves and visors/glasses, and sanitizing agents.[1]
- Provide patients with disposable materials, hand disinfectant, and surgical masks.

Patients should be instructed as follows:

- Come to the outpatient clinic with a protective surgical mask and nitrile gloves. (this also applies to persons accompanying patients)
- The person accompanying the patient must wear the protective surgical mask and, after delivering the patient, must wait outside the clinic or hospital during botulinum toxin treatment.

Related to the Procedure:

- The patient must disinfect the hands with hydroalcoholic or chlorine gel before entering the clinic. It is mandatory for the patient to wear non-sterile gloves and a surgical mask.
- Doctor and staff must remove all jewelry and personal items. Wash hands for at least 20 seconds with soap and water or alcohol solution. Put on the first pair of gloves. Put on footwear and headgear, then the disposable gown over the uniform, then a FFP2 mask; put on goggles or visor and the second pair of gloves.[2]
- When finished with a patient and moving on to the next, follow the sequence indicated. Remove the second pair of gloves together with the disposable gown; remove the goggles or visor and sanitize them. Practice hand hygiene using soap and water or alcohol solution. In addition, if the botulinum toxin injection procedure involves the craniocervical area or near the oral cavity, it is mandatory that healthcare professionals change the FFP2 mask after each injection session, unless a protective visor is used.
- When finished with a patient and moving on to the next, clean all

surfaces with which the patient has come into contact (eg, bed, chair) with hydroalcoholic disinfectants (70% ethyl alcohol) or chlorine-based disinfectants (0.1% sodium hypochlorite solutions). The most frequently handled surfaces should be protected with disposable barriers, which should be disposed of in special waste containers at the end of the session.
- Frequently exchange the air in the clinic between patients.[3]

Conclusions: These recommendations indicate the minimum procedures necessary for safe botulinum toxin treatment in individuals who do not suffer from symptoms related to SARS-CoV-2 infection. New knowledge about SARS-COV-2 infection may lead to changes in the recommendations for good practice.
Keywords: Botulinum toxin; COVID-19 pandemic; Italian Botulinum Toxin Network; Rete Italiana Tossina Botulinica; Safety Recommendations; SARS-CoV-2 infection

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Appendix: Italian Botulinum Toxin Network Safe Infiltration Project participants:
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SPASTICITY TREATMENT WITH BOTULINUM TOXIN DURING THE COVID-19 PANDEMIC

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Introduction and Objectives: Intramuscular botulinum toxin type A (BoNT-A) is an effective treatment for focal spasticity. Since the coronavirus disease 2019 (COVID-19) outbreak, all deferrable clinical activities have been suspended or postponed for several months worldwide. This monocentric, observational, cross-sectional study aims to investigate the impact of the COVID-19 pandemic on treatment of spasticity with BoNT-A in adult patients.
Methods: This study examined the BoNT-A spasticity treatments scheduled at a central hospital in the first six months of the COVID-19 outbreak in Portugal. We analyzed how many appointments were suspended and the extent of the delay of those that were rescheduled. Also, we compared the number of treatments that took place in that period with the number during the previous year.
Results: In the first three months of the COVID-19 outbreak, all scheduled treatments were either suspended or rescheduled. After that, there was a progressive return of the treatments, starting with the rescheduled ones (n=23) that took place with 2 to 6 months of delay. There were also some patients who missed their appointments (n=7) or that were discharged due to clinical reasons (n=12). By the end of the first semester, the total number of treatments, although with no immediate side effects noted, was about 50% less than the previous year.
Conclusions: The reorganization of non-urgent clinical activities that occurred due to the COVID-19 pandemic led to the interruption or delay of spasticity treatments. In the present study, the first three months had more repercussions, with a complete suspension of treatments, that were partially recovered in the second trimester. All the treatments that took place in the first semester had no immediate complications recorded, which is a good preliminary indicator of the safety of BoNT-A treatment during the COVID-19 outbreak, but further studies are needed.
Keywords: Botulinum toxin; COVID-19; Pandemic; Spasticity

PREGNANCY OUTCOMES FOLLOWING EXPOSURE TO ONABOTULINUMTOXINA UPDATE: 29 YEARS OF SAFETY OBSERVATION

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Introduction and Objectives: The safety of onabotulinumtoxinA during pregnancy remains an important topic for healthcare providers and their patients. This analysis evaluated pregnancy outcomes following onabotulinumtoxinA exposure to provide a cumulative 29-year update.
Methods: The Allergan Global Safety Database contains reports of onabotulinumtoxinA administration before/during pregnancy, including prospective (reported before outcome known) and retrospective (outcome known when reported) cases. The database was searched (1/1/90-12/31/18) for eligible cases where treatment occurred during pregnancy or ≤3 months prior to conception. To minimize reporting bias, prevalence rates for overall and major birth defects were estimated from prospective cases of live births only.
Results: Of 913 pregnancies, 397 (43.5%) were eligible with known outcomes. Maternal age was known in 215 cases, with 45.6% of mothers ≥35 years. Indication was known in 340 cases, with the most frequent being cosmetic (35.3%), migraine/headache (30.3%), and movement disorders (12.1%). OnabotulinumtoxinA dose information was known in 242 cases: 32.2% <50 U, 11.2% 50 U to <100 U, 40.1% 100 U to <200 U, and 16.5% ≥200 U. Of 195 prospective pregnancy cases with 197 fetuses, there were 152 (77.2%) live births and 45 (22.8%) fetal losses (32 spontaneous abortions, 13 elective abortions). Of the 152 live births, 148 (97.4%) had normal outcomes and 4 had abnormal outcomes. Among the 4 abnormal outcomes, there were 1 major birth defect, 2 minor fetal defects, and 1 birth complication. The prevalence rate for overall fetal defects was 2.6% (4/152, 95% CI: 1.0-6.6%) and 0.7% (1/152, 95% CI: 0.1-3.6%) for major fetal defects (3%-6% in the general population).
Conclusions: This 29-year retrospective analysis of safety data in onabotulinumtoxinA-exposed mothers demonstrated that the prevalence rate of major fetal defects was consistent with the rates reported in the general population. No new safety signals were identified.
Funding: Allergan, prior to acquisition by AbbVie.

Keywords: Birth defects; Fetal defects; OnabotulinumtoxinA; Pregnancy outcomes; Safety

WRITER'S CRAMP: A SINGLE-CENTER EXPERIENCE

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Background: Task-specific dystonia is characterized by abnormal muscle contraction, triggered by a specific action. Writer's cramp is the most recognized form of task-specific dystonia. Botulinum neurotoxin (BoNT) is the first option for treatment of writer's cramp.
Method: In this study, we describe the demographic and clinical features of 54 patients diagnosed with writer's cramp at the Neurology Department of Istanbul University, Istanbul Faculty of Medicine and the response to treatment with BoNT.
Results: Forty-one patients were male. Mean age at onset was 35.8 ± 10.7 years (range: 15-59 years) and mean age at admission was 40.7 ± 10.9 years (range: 22-69 years). The most common professions in our cohort were teachers (10 patients) and clerks (8 patients). The dominant hand was the left hand in 4 patients. Flexor type was the most common subtype, affecting 37 patients, followed by extensor (8 patients), and mixed types (7 patients). Nineteen patients complained of pain along with the abnormal contractions. Injections were performed with either electromyography (EMG) or ultrasound guidance. Flexor digitorum profundus, flexor pollicis longus and flexor digitorum superficialis were the most frequently injected muscles. Proximal muscles were involved in 19 patients. Treatment benefit was satisfactory in 29 patients and moderate in 12 patients. However, 12 patients reported no benefit.
Conclusion: Our patients showed similar clinical characteristics and responses to BoNT treatment as patients in previous reports. EMG or ultrasound guidance and careful muscle selection are the most important factors in attaining good clinical outcome.
Funding: None.
Keywords: Dystonia; Task-specific; Botulinum neurotoxin; BoNT injection

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SPARC: A NEW APPROACH TO QUANTIFYING MOVEMENT SMOOTHNESS IN PATIENTS WITH IDIOPATHIC CERVICAL DYSTONIA AND EVALUATING THE EFFICACY OF BOTULINUM NEUROTOXIN

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Introduction: Botulinum neurotoxin (BoNT) injections are considered the gold standard treatment for patients affected by idiopathic cervical dystonia (ICD),[1] in reducing muscle spasms, abnormal postures, and pain, and increasing cervical range of motion. Recent studies in Parkinson's disease patients have demonstrated the utility of measuring movement smoothness with the SPectral ARC length measure (SPARC).[2-5]. However, ICD patients' movement smoothness has not yet been quantified in comparison with healthy controls (HC) and in relationship to BoNT treatments.
Methods: Fifteen consecutive ICD patients (8 males, 7 females; mean age: 52.2 years) mainly affected by dystonic head rotation were recruited in the BoNT clinic. They underwent a kinematic movement analysis with an optoelectronic system (Smart, BTS), which measured SPARC calculated from head angular velocity signals, in movements toward the direction of the dystonic rotation and away from it. Fifteen healthy subjects were tested during repetitive right and left head rotation at a self-selected velocity. Four ICD patients were assessed at baseline (T0), 6 weeks (T1), and 12 weeks (T2) after standard BoNT treatment. The patients were assessed using the Toronto Western Spasmodic Torticollis Rating (TWSTRS) Scale. Statistical analysis was performed using linear mixed effects models.
Results: Statistical analysis showed a significant difference between SPARC in ICD patients and healthy controls at T0 in the direction toward dystonic rotation (P=0.0061) and away from it (P=0.0025). The timeline (Fig. 1) showed in the ICD patients, a significant improvement in SPARC at T1 as well as the median TWSTRS scores at T1 compared with T0 and T2.
Conclusions: SPARC has been demonstrated to be a valid measure of movement smoothness to differentiate HC and ICD patients and a possible synthetic index to quantitatively evaluate BoNT efficacy. Further studies are mandatory and the sample size needs to be enlarged.
Keywords: BoNT; Botulinum neurotoxin; Cervical dystonia; Smoothness; SPARC

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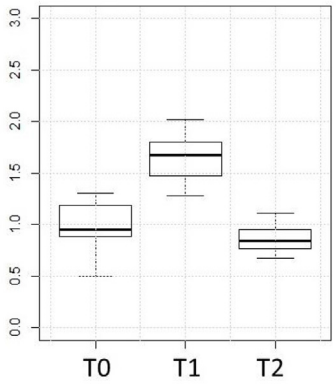

Fig. 1. SPARC (Spectral ARC length measure) at T0-T1-T2.

3D RECONSTRUCTION AND ANALYSIS OF NEUROMUSCULAR JUNCTION DISTRIBUTION IN WHOLE SKELETAL MUSCLES IN THE RAT USING LIGHT-SHEET MICROSCOPY

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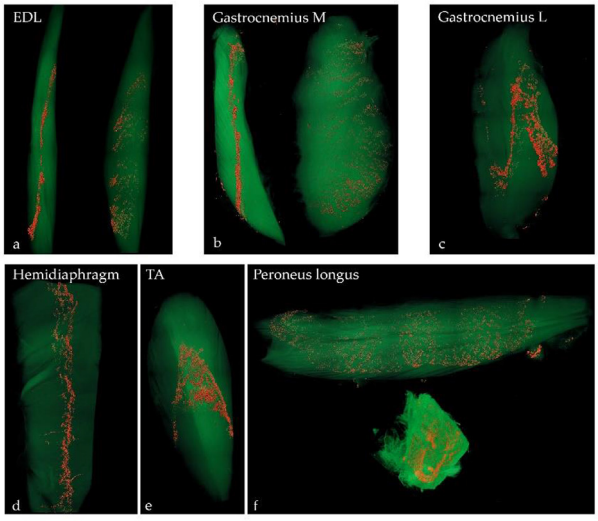
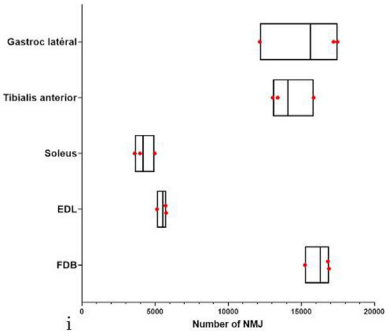
Introduction: Botulinum neurotoxins (BoNTs) are injected in different muscles to treat spasticity. Depending on muscle size, one or multiple

injections are used according to label recommendations in order to target most neuromuscular junctions (NMJ). Information about NMJ distribution and number in muscles is scarce, although it would help improve BoNT administration. A new approach was set up to quantitatively assess NMJ in entire rat muscles.
Methods: Fluorescent α-bungarotoxin, which binds specifically to post-synaptic nicotinic acetylcholine receptors, was intravenously injected into anesthetized rats before sacrifice. Eight types of muscles (n=3) were formalin-fixed, transparized and scanned using a light-sheet microscope. For each muscle, 3D acquisition tiles were fused to reconstruct the organ. An automated image-processing methodology, including 3D image denoising and segmentation, was developed to count NMJ.
Results: The distribution and density of NMJ were muscle specific (Figure 1). In some muscles, NMJ formed a plane at a ±20° angle all along the muscle (extensor digitorum longus [EDL]) or perpendicular across muscle fibers (diaphragm) or even formed a cone-shaped structure in the muscle's center (e.g., tibialis). The mean number of NMJ ranged from 4,179 (soleus) to 16,318 (flexor digitorum brevis [FDB]) and was not correlated with muscle size. In the EDL, 5,527 NMJ were observed, matching the number of myofibers counted on histological cross-sections. In the FDB (the smallest muscle analyzed) NMJ were about 2 times smaller than in the gastrocnemius.
Conclusions: Light-sheet microscopy allows the acquisition of high-quality qualitative and quantitative NMJ phenotyping data. Although translation to humans is difficult, this work highlights the specific distribution of NMJ in muscles, and might enable observation of denervation (fragmentation, swelling) and reinnervation (sprouting) processes, and the study of BoNT diffusion after intramuscular administration.
Keywords: α-bungarotoxin; Light sheet microscopy (LSM); Neuromuscular junction (NMJ)

Figure 1: Distribution of NMJ (in red) in different rat muscles (in green)
EDL: Extensor digitorum longus; TA: Tibialis anterior; FDB: Flexor digitorum brevis



g: video FDB, 1X h: video EDL, 10X



SAFETY AND EFFICACY OF ONABOTULINUMTOXINA FOR TREATMENT OF MASSETER MUSCLE HYPERTROPHY: RESULTS FROM A PHASE 2 DOSE-ESCALATION STUDY

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Introduction: Benign bilateral masseter muscle hypertrophy (MMH), characterized by mandibular angle prominence, negatively impacts facial aesthetics. In this study, we assessed the safety and efficacy of onabotulinumtoxinA (onabotA) vs placebo for MMH treatment.
Methods: In this 12-month, multicenter, randomized (4:1), double-blind study, participants received dose-escalated onabotA (24, 48, 72, or 96 U) or placebo and were followed up monthly for 1 year. Primary and secondary endpoints were reduction in lower facial volume measured by using VECTRA M3 photography and change in MMH severity assessed by using the Masseter Muscle Prominence Scale (MMPS) at day 90. Participant perspective was captured by using the Lower Facial Shape Questionnaire (LFSQ). Safety assessment included evaluation of adverse events (AEs), computed tomography (CT) scans of the mandible, and dental exams.
Results: One hundred eighty-seven participants (mean age, 35 years; female, 82%; Asian, 80%) were assigned to 4 onabotA treatment groups (24 U, n=37; 48 U, n=37; 72 U, n=38; 96 U, n=38) or placebo (n=37). At day 90, lower facial volume was significantly reduced in all groups vs placebo (P<0.001). Significant reduction (P<0.001) was maintained with 48, 72, or 96 U for 6 months after 1 treatment and for 6 more months after retreatment. At day 90, the number of responders achieving MMPS grade ≤3 or a >2-grade change was significant in all groups (P≤0.008 vs placebo), which was maintained through day 180 in the 48, 72, and 96 U groups (P<0.028). LFSQ items indicated reduced symptoms in all treatment groups. The most frequent treatment-related AE was mastication disorder (onabotA, 6.0%; placebo, 2.7%). CT evaluation confirmed the primary endpoint results. No safety signals emerged in CT scans or dental exams.
Conclusions: OnabotA significantly reduced masseter volume and MMH severity at day 90 in all treatment groups. No clear safety patterns were identified with dose escalation, although local facial paresis was reported with 96 U only.
Funding: Allergan Aesthetics, an AbbVie Company.
Keywords: Aesthetic; Computed tomography; Masseter muscle hypertrophy; OnabotulinumtoxinA; Photography

ABSENCE OF NEUTRALIZING ANTIBODY FORMATION DURING INCOBOTULINUMTOXINA TREATMENT OF SPASTICITY IN BOTULINUM TOXIN-NAÏVE CHILDREN WITH CEREBRAL PALSY: POOLED ANALYSIS OF THREE PHASE 3 STUDIES

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Introduction and Objectives: Neutralizing antibodies (NAbs) have been linked to secondary non-response to botulinum neurotoxin type A (BoNT-A) injections; this still controversial issue is of special concern when treating conditions like pediatric spasticity. We investigated NAb formation in three large Phase 3 studies with incobotulinumtoxinA, a BoNT-A with no complexing proteins, in children/adolescents with cerebral palsy (CP) who received multipattern spasticity treatment.
Methods: Pediatric patients with lower-limb (LL), upper-limb (UL), or combined LL/UL spasticity (2-17 years; uni- or bilateral CP; Ashworth Scale score ≥2 in clinical patterns for treatment) were enrolled. Patients received total body incobotulinumtoxinA doses of ≤16-20 U/kg (max. 400-500 U) depending on the study (TIM: NCT01893411; TIMO: NCT01905683; XARA: NCT02002884) and Gross Motor Function Classification System level I–V, for up to six injection cycles (ICs). Occurrence of NAbs against BoNT-A was investigated in those ≥21 kg at screening and end of study. Blood samples were analyzed using a fluorescence immunoassay (FIA) for antibodies, and positive samples were then tested for NAbs using a hemidiaphragm assay.
Results: Nine hundred seven patients (59.6% male, mean [SD] age 6.7 [4.2] years and body weight 23.3 [13.9] kg) received treatment. In total, 386/403 (95.8%) and 318/422 (75.4%) patients with bodyweight ≥21 kg were tested using FIA at screening and end of study, respectively, with 150/403 (37.2%) and 167/422 (39.6%) being toxin-naïve (Table). Eleven individual patients tested positive for NAbs at screening and/or end of study, all of whom had previously been treated with other BoNT-As (onabotulinumtoxinA/abobotulinumtoxinA). None developed a secondary non-response to incobotulinumtoxinA. No toxin-naïve patients developed NAbs after incobotulinumtoxinA treatment.
Conclusions: NAb formation was not observed in toxin-naïve children/adolescents with CP treated with up to six ICs of incobotulinumtoxinA.
Funding: Merz Pharmaceuticals GmbH, Frankfurt am Main, Germany
Keywords: Adolescents; Antibodies; Cerebral palsy; Children; IncobotulinumtoxinA

Table: FIA and HDA neutralizing antibody results for children and adolescents ≥21 kg with CP

Study	Visit	Test result	FIA n/N	HDA[a] n/N
TIM	Screening	Positive	19/127	3/127
		Negative	102/127	6/127
		Missing	6/127	10/127
		N/A[a]	–	108/127
	End of study[b]	Positive	6/90	0/90
		Negative	60/90	4/90
		Missing	24/90	2/90
		N/A[a]	–	84/90
TIMO	Screening[c]	Positive	22/120	2/120
		Negative	91/120	8/120
		Missing	7/120	12/120
		N/A[a]	–	98/120
	End of study[d]	Positive	19/147	4/147
		Negative	95/147	9/147
		Missing	33/147	7/147
		N/A[a]	–	127/147
XARA	Screening	Positive	19/156	3/156
		Negative	133/156	9/156
		Missing	4/156	7/156
		N/A[a]	–	137/156
	End of study	Positive	21/185	3/185
		Negative	117/185	4/185
		Missing	47/185	14/185
		N/A[a]	–	164/185
Total	Screening	Positive	60/403	8/403
		Negative	326/403	23/403
		Missing	17/403	29/403
		N/A[a]	–	343/403
	End of study	Positive	46/422	7/422
		Negative	272/422	17/422
		Missing	104/422	23/422
		N/A[a]	–	375/402

[a]Only positive tested blood samples in FIA were tested; otherwise, HDA testing was not applicable.
[b]Only patients not taking part in TIMO.
[c]Only patients newly recruited in TIMO.
[d]Patients of both studies TIM and TIMO and newly recruited subjects of TIMO.
CP, cerebral palsy; FIA, fluorescence immunoassay; HDA, hemidiaphragm assay; N/A, not applicable.

EFFICACY AND SAFETY OF BOTULINUM TOXIN THERAPY FOR LINGUAL DYSTONIA

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Introduction and Objectives: The aim of the study was to examine the efficacy and safety of botulinum toxin type A (BTA) treatment of lingual dystonia.
Methods: Of 16 patients (11 females and 5 males, mean age ± SD: 44.6±12.9 years) in this study, 4 (25.0 %) had an isolated form of lingual dystonia, and 9 (56.2 %) had dystonia of the muscles of the tongue as a symptom of oromandibular dystonia. For 3 (18.8%) patients, lingual dystonia was the predominant symptom of segmental dystonia. Second and third injections were administered to 14 and 12 patients, respectively. The change in dystonia was measured using the Burke-Fahn-Marsden Scale. Patients were treated with BTA injection into the muscles of the tongue and bottom of the mouth with intraoral and submandibular access.
Results: By type of lingual muscle contraction, 9 patients (56.1 %) had protrusion of the tongue, 3 (18.7 %) laterotrusion, 3 (18.7 %), curling, and 1 (6.5 %), retraction. All patients had speech impairment, and 6 (37.5 %) had difficulty swallowing. The average dose of abobotulinumtoxinA (Dysport®) used was 100 units ([U], range: 80-150 U). Fourteen (87.5 %) patients received repeat injections. If the effect from the first injection was insufficient, the BTA dose was increased. With repeated injections, the average BTA dose reached 120 U (range: 100-160 U). One month after injection, we observed an improvement in speech and swallowing for 12 of 16 patients. Symptoms of oromandibular dystonia were decreased on the Burke-Fahn-Marsden Scale from 6 (4-7) to 4 (2-6, $P<0.05$). The greatest effect was observed with protrusion and laterotrusion of the tongue.
The effectiveness of the treatment was short and lasted for 1.5-2 months. No serious side effects were identified; mild dysphagia was observed in 11.9% of patients and resolved after 2 weeks.
Conclusions: BTA injections were effective in different forms of lingual dystonia but the improvement lasted for only a short period of 1.5-2 months.
Keywords: Botulinum toxin type A; Lingual dystonia

FUNCTIONAL BENEFIT OF ULTRASOUND-GUIDED BOTULINUM TOXIN TREATMENT FOR ACTION TREMOR OF THE UPPER LIMB

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Introduction and Objectives: Current oral treatments for action tremor are often unsatisfactory, either because of limited benefit, contraindications to usage, or adverse events. Botulinum toxin treatment is recommended for treatment of essential tremor of the upper limb (Hallett, et al, 2013). Ultrasound-assisted injections increase the accuracy of muscle targeting while minimizing damage to adjacent structures. The authors aimed to investigate the functional benefit of ultrasound-guided treatment with botulinum toxin type A in patients with action tremor of the upper limb.
Methods: Thirteen patients with upper limb tremor, aged 27 to 91 (median age: 74) years, were referred for treatment with botulinum toxin, after failure of, or contraindications to, oral medications . Nine patients suffered from essential tremor, three from a cerebellar-type tremor, and one from Holmes tremor. Patients were treated with botulinum toxin type A injections to muscles of one of the upper limbs under ultrasound guidance. Muscles were selected according to the pattern of movement associated with tremor. Pre-and post-treatment evaluations were performed using Goal-Attainment Scaling (GAS) after 1 to 3 treatments (not less than 12 weeks apart).
Results: The average total dose was 119 U for abobotulinumtoxinA and 52 U for onabotulinumtoxinA (5 and 8 cases, respectively). Treatment resulted in functional benefit for patients in 11/13 cases (average GAS score change 13.8, range: 0-24.8). There was mild transient paresis of finger extension, with no significant functional impairment, in 2 cases. In one patient with essential tremor who suffered from previous paresis of the treated upper limb, treatment was effective with no added weakness.
Conclusions: Ultrasound-guided botulinum toxin treatment of action tremor may be an effective treatment for disabling action tremor of the upper limb. In carefully selected cases, this treatment may be useful even in the presence of previous weakness from other causes.
Keywords: Botulinum toxin; Action tremor; Upper limb; Goal-attainment scaling

Reference
Hallett M, Albanese A, Dressler D, et al. Evidence-based review and assessment of botulinum neurotoxin for the treatment of movement disorders. *Toxicon*. 2013;67:94-114.

SAFETY, PHARMACODYNAMIC RESPONSE, AND TREATMENT SATISFACTION WITH ONABOTULINUMTOXINA 40 U, 60 U, AND 80 U IN SUBJECTS WITH MODERATE-TO-SEVERE DYNAMIC GLABELLAR LINES

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Introduction: OnabotulinumtoxinA (onabotA) 20 U reduces glabellar line (GL) severity at maximum frown for up to 4 months. Small dose-ranging studies have suggested that >20 U doses may increase efficacy and duration of response for GLs.
Methods: A 48-week, double-blind, placebo-controlled study compared the safety, treatment satisfaction, and pharmacodynamic response of single onabotA 40, 60, and 80 U doses with the approved 20 U dose and placebo in adult females with investigator-assessed moderate or severe dynamic GLs on the Facial Wrinkle Scale (FWS). Key efficacy endpoints included the percentage of subjects with investigator-assessed ≥1-grade FWS improvement from baseline at maximum frown (responder rate) at week 24, median duration of response, and proportion of responders reporting "mostly" or "very satisfied" on the Facial Line Satisfaction Questionnaire (FLSQ).
Results: Of the 226 subjects in the modified intention-to-treat population, 88.9% were white, with a mean (SD) age of 48.0 (12.2) years. Utilizing a constant injection volume of 0.05 mL per site across all onabotA doses, responder rates for placebo and onabotA 20, 40, 60, and 80 U at 24 weeks were 0%, 16.0%, 32.0%, 30.6%, and 38.5%, respectively, with statistically significant ($P<.05$) between-group differences favoring onabotA 40 and 80 U vs 20 U. Median duration of response was also longer for all higher onabotA doses (≥24.0 weeks) vs 20 U (19.7 weeks). FLSQ item scores demonstrated high subject satisfaction. No onabotA dose-response effect on safety was seen. One subject each experienced mild eyelid ptosis (80 U group) and eyebrow ptosis (20 U group); both events resolved without sequela.
Conclusions: This pharmacodynamic dose-response study showed increased duration of treatment response and patient-reported satisfaction with escalating onabotA doses compared with 20 U for treatment of moderate-to-severe GLs.
Funding: Allergan Aesthetics, an AbbVie Company
Keywords: Aesthetic; Glabella; Duration; OnabotulinumtoxinA; Satisfaction

EFFICACY AND SAFETY OF INCOBOTULINUMTOXINA FOR UPPER- OR COMBINED UPPER- AND LOWER-LIMB SPASTICITY IN CHILDREN AND ADOLESCENTS WITH CEREBRAL PALSY: RESULTS OF THE PHASE 3 XARA STUDY

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Introduction: The objective of this study was to assess the efficacy and safety of incobotulinumtoxinA for upper- and combined upper- and lower-limb spasticity in ambulant and non-ambulant children and adolescents with cerebral palsy (CP).
Methods: XARA (NCT02002884) was a randomized, Phase 3 study with a double-blind main period (MP) and an open-label extension (OLEX) period. Patients 2-17 years of age with uni- or bilateral CP and Ashworth Scale (AS) scores ≥2 in main clinical target patterns, flexed elbow and/or flexed wrist, were enrolled. In the MP, patients were randomized (2:1:1) to 3 incobotulinumtoxinA dose groups (8, 6, 2 U/kg body weight [BW]; maximum 200, 150, 50 U/upper limb) with additional lower-limb injections (total body dose ≤16-20 U/kg BW [≤400-500 U], depending on Gross Motor Function Classification System [GMFCS] level). Patients received 3 further injection cycles (ICs) in the OLEX, with doses per the 8-U/kg BW group. Outcomes included AS, Global Impression of Change Scale (GICS), and adverse events (AEs).
Results: Three hundred fifty patients (62.9% male, mean [SD] age 7.3 [4.4] years old, BW 25.0 [15.0] kg, 30.9% GMFCS IV-V) were treated; 281 (80.3%) completed the study, receiving 4 incobotulinumtoxinA ICs. In the MP, AS scores for the upper-limb main clinical pattern improved significantly from baseline to Week 4 ($P<0.0001$, mixed model for repeated measures [MMRM]), with a significantly greater improvement in the 8-U/kg versus the 2-U/kg dose group ($P=0.017$, MMRM). Improvements were observed in all treated upper- and lower-limb clinical patterns and across all OLEX ICs.

GICS scores confirmed global improvements in upper- and lower-limb spasticity but did not differ between dose groups. There was no increased incidence of AEs with increasing dose or repeated treatment.
Conclusions: In this large pediatric study, data show the efficacy and safety of incobotulinumtoxinA for muscle tone reduction in the multi-level, multi-pattern treatment of spasticity in patients with CP (GMFCS I-V).
Funding: Merz Pharmaceuticals GmbH, Frankfurt am Main, Germany
Keywords: IncobotulinumtoxinA; Cerebral palsy; Lower-limb spasticity; Pediatric; Phase 3; Upper-limb spasticity

COST-EFFECTIVENESS OF BONT-A PRODUCTS FOR TREATMENT OF PEDIATRIC SPASTICITY IN THE UNITED KINGDOM

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Introduction: For children with impaired motor function, pain, or other functional difficulties attributable to focal spasticity, the UK National Institute for Health and Clinical Excellence guidelines recommend treatment with botulinum neurotoxin type A (BoNT-A). Two BoNT-A products are licensed for pediatric use in the United Kingdom: abobotulinumtoxinA (aboBoNT-A) and onabotulinumtoxinA (onaBoNT-A), but these treatments differ in terms of doses, costs, and injection frequencies. The objective of this analysis was to evaluate the average expenditure per response obtained with aboBoNT-A or onaBoNT-A for managing pediatric spasticity of the upper (ULS) and lower (LLS) limbs.
Methods: Cost-effectiveness analyses were conducted for BoNT-A treatment of ULS and LLS in the UK. Effectiveness was defined as response to therapy and was based on data from systematic literature reviews and meta-analyses of randomized controlled trials. BoNT-A dose was based on product label and the average weight of a pediatric (6-year-old) patient in the UK. Injection interval was based on clinical trial data, adjusted to consider differences in use observed in real-world studies of BoNT-A use in adult spasticity. Resource use for responders and non-responders was based on a survey of UK physicians treating pediatric LLS. Unit costs were informed by UK fee schedules.
Results: AboBoNT-A resulted in lower annual costs compared with onaBoNT-A for the management of ULS (£487 saved) and LLS (£429 saved). Results were driven by differences in injection intervals and a higher treatment response rate for people receiving aboBoNT-A compared with onaBoNT-A. Total cost per responder was lower for people receiving aboBoNT-A compared with onaBoNT-A (ULS: £37,880 vs. £50,100; LLS: £46,459 vs. £66,037). Results were robust to sensitivity analyses.
Conclusion: AboBoNT-A may be a cost-effective choice for treatment of spasticity in children. These findings could help deliver more effective and efficient healthcare in the UK.
Funding: Ipsen
Keywords: Botulinum neurotoxin type A; Costs; Cost-effectiveness analysis**;** Quality of life; Spasticity

WORLDWIDE CLINICIAN SURVEY ON PRACTICE PATTERNS AND PERCEPTIONS ON USE OF ADJUNCT THERAPIES FOLLOWING BOTULINUM TOXIN INJECTION FOR LIMB SPASTICITY

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Introduction and Objectives: Botulinum toxin (BoNT) injection is an effective treatment for focal spastic muscle overactivity. Adjunctive therapies are non-pharmacological treatments used in addition to BoNT injection in order to increase the efficacy of BoNT. Evidence suggests that casting, taping, electrical stimulation, and physiotherapy result in enhanced spasticity reduction.[1] A wide variety of adjunctive therapies exists, which are supported by expert consensus,[2] but the effects of their usage in real-world practice is poorly understood.[3] The aim of this worldwide survey was to determine current practice and perceptions of the use of adjunctive therapies following BoNT injections for treatment of limb spasticity.
Methods: A 22-item questionnaire related to clinical practice demographics, clinician self-reported use of adjunctive therapies, clinician opinions on barriers to the use of adjunctive therapies, and future research priorities was translated into 7 languages and distributed worldwide through national and international professional societies. Ethics approvals were obtained; the survey was hosted online on the UBC survey platform.
Results: Five hundred twenty-seven clinicians from 52 countries and 6 continents responded to the survey. The mean age of responders was 45 (range: 29-90 years) years with 18 (range:1-55) years of experience and 10 (range: 1-35) years of performing BoNT injections. The pathologies treated by responders using BoNTs were stroke, traumatic brain injury, spinal cord injury, multiple sclerosis, and cerebral palsy. The most frequently used physical interventions were home-based active exercise programs (57%), home stretching programs (57%), and splinting (50%), followed by immediate (within 30 minutes post-injection) active movement exercises (30%), and stretching (28%). A smaller proportion of clinicians utilized electrical stimulation, constraint-induced movement therapy, upper extremity casting, transcutaneous electrical nerve stimulation, and extracorporeal shockwave therapy.
The main barriers to provision of these interventions were time constraints, limited financial resources, and lack of evidence. The responders proposed to focus future research on immediate active movement exercises and passive stretching of injected muscles as a priority.
Conclusions: Worldwide, clinicians frequently use adjunctive therapies after BoNT injection to enhance spasticity reduction. The most frequently used physical interventions were home active exercise, home stretching programs, and splinting. Time and financial constraints were identified as barriers to implementation of adjunctive therapies. Future research should focus on developing the evidence for combined physical interventions and botulinum toxin treatment in the rehabilitation of individuals with spasticity.
Keywords: Botulinum toxin; Care survey; Muscle spasticity

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SUCCESSFUL TREATMENT OF HAND DYSTONIA WITH BOTULINUM TOXIN IN A DYT12 PATIENT

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Introduction and Objectives: Dystonia 12 (DYT12) or rapid-onset dystonia-parkinsonism is a rare neurological disease with onset in childhood or early adulthood. The responsible mutated gene is ATP1A3 (19q13.2).[1] In the classical phenotype, patients develop dystonia, combined with a non-tremulous parkinsonian syndrome, postural instability, dysarthria, and dysphagia.[1] The few published reports of ATP1A3-mutated patients who received pallidal surgery (deep brain stimulation or pallidotomy targeting the globus pallidus pars interna [GPi-DBS]) are disappointing as all implanted patients failed to improve.[2,3] In this situation, botulinum toxin is one of the remaining options to treat focal dystonia.
Methods: We report the case of a 16-year-old boy affected by DYT12 since he was 12 and presenting with asymmetric generalized dystonia, parkinsonian syndrome, and prominent bulbar involvement. Due to a severe, particularly painful, and invalidating dystonia of his left hand (Fig. 1A and 1B) he asked for botulinum toxin to relieve the symptoms.
We injected 200 units of incobotulinumtoxinA (Xeomin®) into the left flexor digitorum profundus, flexor digitorum superficialis, and flexor pollicis longus, under combined ultrasound and electromyographic guidance to increase the chances of successful treatment. The dilution employed was 2 mL into 100 units. It was decided to administer the injections under general anesthesia, because of the large number of injections needed and the boy's needle phobia.
Results: One month later, his left hand was free from dystonia (Fig. 1C), easy to mobilize, and much less painful. Also, he was able to pick up little objects. The effect of the botulinum toxin was sustained for at least three months.
Conclusions: As GPi-DBS is not currently demonstrated as effective in DYT12 treatment, botulinum toxin can be successfully used to selectively treat limb dystonia. Guidance techniques, such as electromyography, ultrasound, or both, should be used as the dystonic pattern may be complex.
Funding: None
Keywords: Rapid-onset dystonia-parkinsonism; DYT12; Hand dystonia; Botulinum toxin

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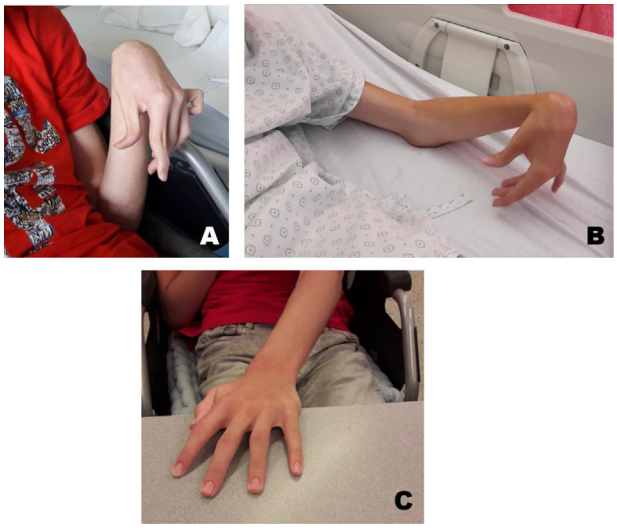

Fig. 1 A and B. Left forearm before treatment, with severe hand dystonia. **C**. Left hand one month after treatment (only the left hand has been injected).

ATHLETE WITH BRAIN DAMAGE: FROM SPASTICITY TO MYOFASCIAL PAIN

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Introduction and Objectives: Cerebral palsy is a non-progressive neurological disorder that occurs around the time of birth. This is the case of a patient who grew up with spastic paraparesis. He reached adulthood with autonomous gait after three surgeries in which multiple tenotomies were carried out in the lower limbs. In the periods between interventions, he required periodic injections with onabotulinumtoxinA. The patient was a member of the national adaptive swimming team. He came to the clinic with a complaint of mechanical pain in the left buttock and right lumbar and abdominal regions, which had caused him to stop swimming training.
Methods: On physical examination, trigger points typical of myofascial pain syndrome were found in the following muscles: left piriformis, right quadratus lumborum, and right rectus abdominis (related to osteitis pubis due to the kick made when swimming). There were no symptoms manifested in the adductors due to the surgical elongation procedure. Pubic enthesopathy was confirmed by ultrasound (Figure), which showed increased heterogeneity with hypoechoic foci and gross calcifications in the entheses of the pubic musculature with angiogenesis. Treatment of trigger points with intramuscular onabotulinumtoxinA injections was performed. A total of 150 units (U) was injected into: one point in the left piriformis (50 U), one in the right quadratus lumborum (50 U), and two in the right rectus abdominis (25 U each).
Results: In two weeks, the patient presented with pain relief shown by a reduction in score from 8 to 4 points on the visual analogue pain scale. After that he was able to do intense stretching exercises, and restarted swimming training four weeks after injections.
Conclusions: Treatment with botulinum toxin inhibits the hypercontractility of trigger points. The dose and location of the injections must be aligned with the therapeutic objectives: in this patient, the objectives evolved from improvement of the gait pattern to relief of myofascial pain syndrome related to a clinical history of spastic paraparesis and a desire to resume swimming practice.
Keywords: Adaptive sport; Botulinum toxin; Brain damage; Myofascial pain syndrome; Pain; Pubic enthesopathy

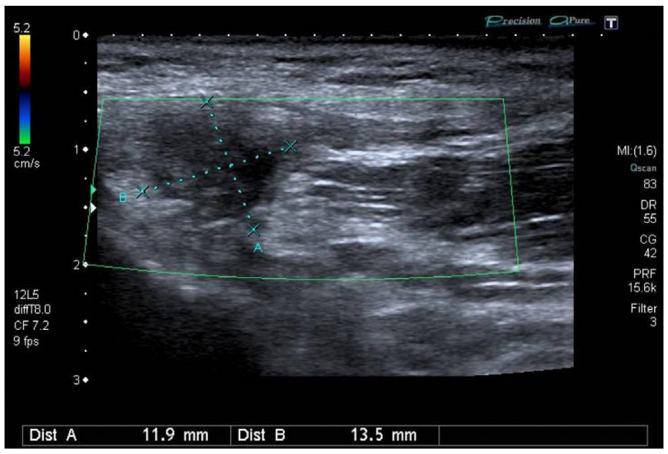

Fig. Ultrasound scan showing pubic enthesopathy.

A LONGITUDINAL GENOMIC AND FUNCTIONAL ANALYSIS OF *CLOSTRIDIUM BOTULINUM* ISOLATED FROM AN INFANT BOTULISM CASE

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Introduction and objectives: Infant botulism results from the germination of environmental spores of *Clostridium botulinum*, *C. butyricum* or *C. baratii* that subsequently colonize the infant intestine and produce botulinum neurotoxin. We previously reported a case of intestinal botulism in a 3-month-old infant, who was colonized by *C. botulinum* for a total of seven months (Derman et al, 2014). *C. botulinum* was isolated from the infant feces and from the household dust over time. The objectives of this work were to examine the genomic and phenotypic heterogeneity of the *C. botulinum* population established in the infant gut and to identify virulence factors or traits involved in *C. botulinum* pathogenesis.
Methods: *C. botulinum* isolates from the infant feces and from the infant household dust were initially genome sequenced. Bioinformatic analysis led to the identification of mutations associated with relevant phenotypic traits for bacterial pathogenesis. These were further investigated in vitro and validated using various microbiological and immunological methods, such as motility assay, sporulation assay, and toxin production quantification by enzyme-linked immunosorbent assay (ELISA).
Results: Genomic analysis of the different (fecal and dust) isolates revealed a total of 61 single-nucleotide polymorphisms or insertion/deletions that were associated with sporulation, toxinogenesis, motility, carbon metabolism, or quorum sensing. Each genotype could be associated to some extent to a distinct phenotype, illustrating how the *C. botulinum* population diversified within the infant gut over time.
Conclusions: The temporal genomic and phenotypic changes observed within the *C. botulinum* population present in the infant gut bring novel insights into the ecological fitness and adaptation of *C. botulinum* to the intestinal environment. This may be instrumental in establishing novel approaches for prevention and treatment of intestinal botulism.
Funding: This study was financially supported by the HiLife Fellows Program, the University of Helsinki, and the Academy of Finland (grant number 1299700). This project has also received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No. 683099).
Keywords: *Clostridium botulinum*; Genomics; Gut ecology; Infant botulism

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BOTULINUM TOXIN TREATMENT OF FOCAL DYSTONIA IN KERNICTERUS-RELATED MOVEMENT DISORDERS

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Introduction and Objectives: Kernicterus (chronic bilirubin encephalopathy) is a preventable, chronic, debilitating condition caused by severe neonatal hyperbilirubinemia.[1] Various neurologic sequelae have been described, including hearing loss, cognitive impairment, hyperkinetic movement disorders (dystonia, athetosis, chorea), spasticity, and oculomotor disturbances (especially impairment of upward gaze).[2,3] Classical lesions are especially found in the globus pallidus and subthalamic nucleus,[4,5] explaining the frequency of movement disorders in this condition. Cerebellum and brainstem nuclei are also vulnerable in this process.[3]
Management of patients with movement disorders due to kernicterus mainly focuses on physical therapies.[1] For the specific treatment of dystonia, trihexyphenidyl is usually not beneficial, but benzodiazepines may be used. Botulinum toxin has been proposed by some authors to relieve muscle tone,[6] as well as intrathecal baclofen pumps. Globus pallidus pars interna-deep brain stimulation (GPi-DBS) has been suggested in some cases, but a limited number of trials has demonstrated only minor or partial improvements in dystonic symptoms.[7]
Methods: We report the case of an 11-year-old boy affected by kernicterus. He was born in Iraq with a G6PD (glucose-6-phosphate dehydrogenase deficiency) mutation and presented with severe neonatal hyperbilirubinemia. Exchange transfusion could not be performed before 10 days of life. The child presented with athetoid cerebral palsy, associated with generalized dystonic features. The right arm was the most affected, as it was constantly elevated and flexed behind his head (Figure A and B).
As trihexyphenidyl is contraindicated with G6PD mutation and diazepam inefficient, the parents asked for a specific treatment to relieve the disabling dystonic posture of the right arm. Botulinum toxin was proposed as the parents refused DBS. IncobotulinumtoxinA (Xeomin®), which we used in this case, is not a treatment for kernicterus, but for related movement disorders. We injected 200 units of incobotulinumtoxinA in the deltoid, supraspinatus, biceps brachii, brachialis, and brachioradialis muscles of the right arm, under combined ultrasound and electromyographic guidance.
The dilution employed was 2 mL into 100 units and the injections were administered under general anesthesia.
Results: A few weeks later (Figure C and D), we observed a significant improvement of the right arm dystonic posture as the arm could now be kept close to the trunk. Daily care was made easier as well as passive mobilization by the physical therapist.
The effect of the botulinum toxin was sustained for at least three months, and similar Results have been observed with the next treatment sessions.
Conclusions: When anticholinergics or GPi-DBS cannot be used in the treatment of generalized dystonia—for whatever reason—botulinum toxin can be successfully employed to selectively treat limb dystonia as in this case of kernicterus. Toxin injections provide a focal addition to the standard treatment. Patients' needs and functional impairments should guide the injection schedule.
Keywords: Botulinum toxin; Focal dystonia treatment; Kernicterus; Upper limb dystonia

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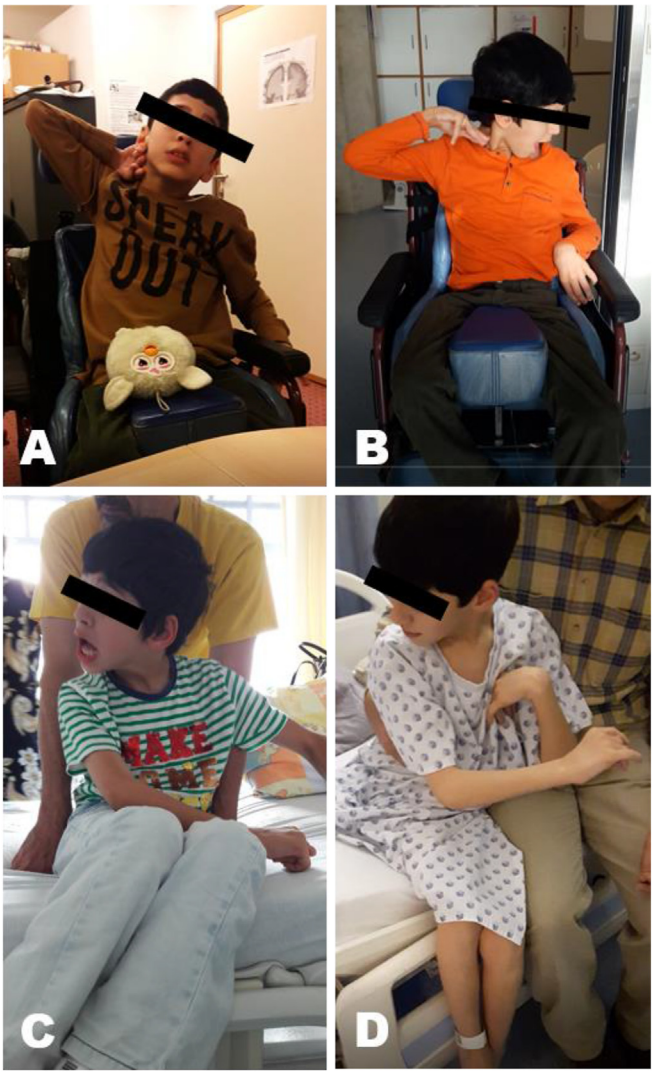

Fig A and B. Right upper limb before treatment, with severe dystonic posture bringing the arm towards the head. **C and D**. Right upper limb 3 weeks after treatment. Elevation and flexion of the arm have significantly decreased.

BOTULINUM TOXIN AS A TREATMENT FOR BLEPHAROSPASM

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Introduction and Objectives: Blepharospasm is a type of facial dystonia that causes eyelid fluttering, blinking, or even eye closure. Botulinum toxin type A (BoNT-A) is the treatment of choice for blepharospasm due to its capacity to block acetylcholine release at nerve terminals and thereby paralyze muscles. The aim of this study is to determine whether botulinum toxin type A in treatment of blepharospasm is safe and how effective it is.
Methods: To evaluate BoNT effectiveness, the Jankovic Rating Scale (JRS) was administered during an interview with blepharospasm patients who received a quarterly BoNT-A injection at Hospital de Clínicas da Universidade Federal do Paraná (HC-UFPR). The interviewer also asked the patients about the occurrence of adverse events (AEs) from their last injection. Patients included were those suffering from blepharospasm and receiving treatment with BoNT therapy at HC-UFPR who agreed to participate in this study. Exclusion criteria were use of other treatments for dystonia or presence of other types of dystonia.
Results: Forty-seven patients met the criteria for study enrolment. Their mean JRS score at baseline was 4.68. After BoNT-A injection, the average JRS score was 2.28, which indicates an improvement of 2.4 points (51.28%). Of the 87 injection procedures analyzed, AEs were reported in 4 (4.6%): unilateral ptosis in 3; and unilateral eyebrow lifting, causing facial asymmetry in 1; all adverse events lasted less than two weeks.
Conclusions: Botulinum toxin type A seems to be an effective therapeutic for blepharospasm, and patients receiving it reported significant improvement in quality of life. Adverse events were mild and of short duration, attesting to the safety of the treatment. Further studies to compare these results with outcomes of BoNT-A therapy in other types of dystonias may be useful.
Keywords: Blepharospasm; Botulinum toxin; Facial dystonia

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EFFECTS OF BOTULINUM TOXIN IN MEIGE'S SYNDROME

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Introduction and Objectives: Botulinum toxin type A (BoNT-A) is considered the treatment of choice for many dystonias due to its role in reducing muscle tone. Meige's syndrome is a facial dystonia characterized by blepharospasm and oromandibular spasms, possibly accompanied by cervical dystonia, and it is usually treated with BoNT. The aim of this study was to determine the safety and efficacy of BoNT therapy in Meige's syndrome patients.
Methods: Meige's syndrome patients were interviewed during their regular quarterly BoNT injection sessions at Hospital de Clínicas da

Universidade Federal do Paraná (HC-UFPR). They were evaluated using the Burke-Fahn-Marsden Scale (BFMS). In addition, they were asked if they had experienced any adverse events with BoNT therapy; whether they were trying any other treatments (eg, anticholinergic medications); and if they were willing to be a part of this study. Patients using other therapies and those who declined to participate in the study were excluded.
Results: Forty-three patients met the study inclusion criteria and agreed to participate. BFMS scores before and after BoNT-A injections differed by 4.2 points. Blepharospasm showed the most improvement (1.7 points on the BFMS), followed by cervical dystonia (1.58), oromandibular dystonia (1.4), and dysphagia/dysphonia (1.1). Ninety-six injection sessions were analyzed, and adverse events (AEs) were reported in 17 (17.7%). Unilateral ptosis was the most common AE (reported by 6 patients), followed by bilateral ptosis (4 patients), deviation of the mouth (3 patients), head drop (2 patients), bilateral eyebrow elevation (1 patient), and dysphonia (1 patient). AEs lasted less than a week in 14 patients, and less than a month in three.
Conclusions: These results suggest that botulinum toxin is an effective treatment for Meige's syndrome, producing significant improvement after its use. It also appears to be a safe therapy, associated with mild AEs of short duration.
Keywords: Botulinum toxin; Cervical dystonia; Facial dystonia; Meige's syndrome; Oromandibular dystonia

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IMPROVEMENT OF SPASTIC PARESIS AND CERVICAL DYSTONIA MANAGEMENT: ASSESSMENT OF SEVEN YEARS OF THE INNOVATIVE INTERNATIONAL EDUCATIONAL PROGRAM IXCELLENCE NETWORK®

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Introduction: Botulinum neurotoxin type A (BoNT-A) is a well-established treatment for spastic paresis (SP) and cervical dystonia (CD), but proper management requires specific training. The Ixcellence Network® is a high-level international educational program developed for physicians administering BoNT-A to patients with SP and CD. Here we assess its educational quality and impact on attendees' practice.
Methods: A Steering Committee of six experts designed the program to encourage innovative methods and approaches to patient management, including diagnosis, tailored treatment, and rehabilitation, and selected ten training centers with an expertise in these topics. Attendees' feedback on quality and impact of the program was collected at the end of each course (T0) and six months later by email (T6).
Results: Between September 2012 and December 2019, 929 physicians participated in training courses dedicated to adult SP (49%), pediatric SP (23%), and CD (28%). Among the 797 attendees who answered the T0 questionnaire, 77% reported an excellent general level of satisfaction, 94% stated that they were provided with new information, and 95% felt that the training achieved their personal objectives. Among the 338 physicians who provided feedback at T6 (40%), 92% confirmed that the training had changed their daily practice and 91% that it helped improve their self-confidence. Some of the attendees shared information learnt during the training with their colleagues (88% of the 263 respondents who attended courses between January 2015 and December 2018) (Figure). Moreover, of the 127 physicians who attended courses between January 2017 and December 2019, 83% would consider training their colleagues.
Conclusions: Ixcellence Network® provides an interactive way for physicians to develop their specialized skills and expertise to train their peers at a local level. Qualitative feedback from the attendees indicates that they are satisfied with the level of this focused training.
Funding: Ipsen
Keywords: Botulinum toxin; Cervical dystonia; Continuing medical education; Patient management; Spastic paresis

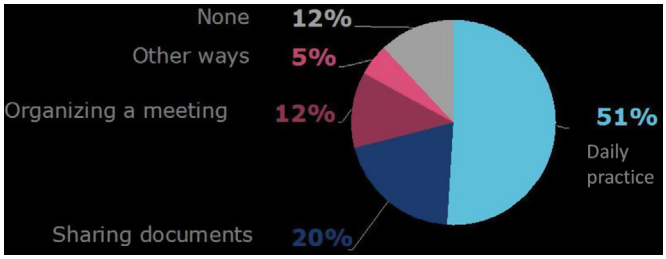

Fig. Sharing of information with their colleagues by attendees after course (263).

ENGINEERING SNAP23 SPECIFICITY INTO THE HIGHLY SELECTIVE *CLOSTRIDIUM BOTULINUM* PROTEASE

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Introduction: Botulinum neurotoxin serotype A (BoNT/A), which is used in a wide range of treatments, cuts a single peptide bond in SNAP25 (S25). Reengineering the extensive protein-protein interface between BoNT/A's protease domain (LC/A) and S25 could expand its therapeutic applications. Here we use directed evolution to retarget LC/A's substrate specificity towards the non-neuronal SNARE protein, SNAP23 (S23).
Methods: Libraries of LC/A variants were screened against fluorescence-polarization substrates featuring the C-terminal domains of S23 or S25.[1] Normalizing the initial rate of proteolysis for S23 to the analogous rate for S25 revealed the variant with the highest S23 specificity in each round.
Results: Eight rounds of directed evolution yielded omLC/A, featuring 1300-fold improved cleavage specificity and 120-fold improved catalytic efficiency targeting S23 versus S25 compared to a previously reported LC/A variant.[2] The BoNT/A holotoxin equipped with omLC/A (omBoNT/A) retains its S23 activity.

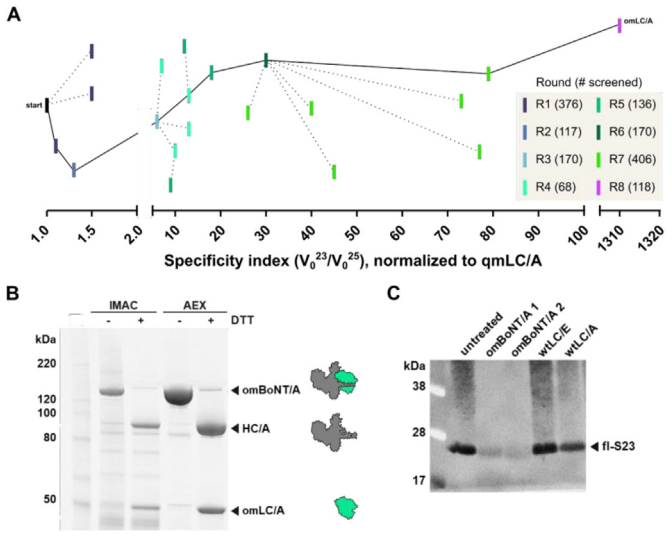

Fig. 1. Directed evolution of a SNAP23-specific LC/A. (A) Improvement in S23/S25 specificity (specificity index) over a previously reported variant (start) through eight rounds (R1 to R8) of directed evolution. (B) Recombinant omBoNT/A is ≈95% nicked upon DTT reduction. AEX, anion exchange. (C) In vitro cleavage of full-length S23 (fl-S23) TCEP-reduced omBoNT/A visualized with a C-terminal anti-S23 antibody. The untreated, wtLC/A, and wtLC/E lanes provide negative controls.

Conclusions: This report demonstrates the plasticity of LC/A's hyper-specific substrate preferences. The successful incorporation of omLC/A into the BoNT holotoxin exhibits its therapeutic potential. The directed-evolution Results can guide the future design of improved proteases and inhibitors.

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Keywords: BoNT/A; Botulinum neurotoxin; Directed evolution; SNAP23; SNAP25

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ASSESSMENT OF THE EFFECTIVENESS OF BOTULINUM TOXIN TYPE A IN A COMPREHENSIVE APPROACH TO TREATMENT OF PATIENTS WITH HEMIFACIAL SPASM

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Introduction: Botulinum toxin type A (BTA) injections are the treatment of choice for idiopathic hemifacial spasm. However, some patients experience minimal or inadequate results from BTA monotherapy, causing doctors to continue exploring an integrated approach to treatment of these patients. Such a comprehensive approach may include kinesiotherapy—including work with an instructor, as well as telerehabilitation—which can help to improve the well-being of patients during the period between BTA injections. However, the effectiveness of these combined treatments has not yet been thoroughly investigated.

Methods: A small case control study was performed. We compared the effectiveness of repeated BTA injections (group I, control, n = 27) and an integrated approach (repeated BTA injections, accompanied by kinesiotherapy (post-isometric relaxation) under full- and part-time video supervision by an instructor (group II, cases, n = 21). The visits were performed before BTA treatment, and at 2 weeks, and 3 months after treatment. The study was performed in patients aged 41 to 80 years with confirmed hemifacial spasm. The severity of hemifacial spasm was assessed on a scale of 0 to 20, mild to severe. In addition, the subjective assessment of "waiting for the next injection", expressed on a 10-point scale, was analyzed.

Results: During the study period and 2 weeks post-study, both groups were comparable on both scales. After 3 months, both groups had positive results, but only the patients in group II showed significant improvement in hemifacial spasm severity (before treatment 15.97 ± 0.72 vs 11.41 ± 2.33) 3 months post-therapy ($P<0.05$). The assessment of "waiting for the next injection" was also significantly lower in this group than in the control group (5.11 + 1.72 vs 7.78 + 1.13 in group II and group I (control group), respectively ($P<0.05$).

Conclusion: A comprehensive approach to treating patients with hemifacial spasm can be integrated into everyday practice to extend the interval between injections, improve patient quality of life, and reduce the financial burden of the disease. Novel instruments can be used to assess the effectiveness of this therapeutic approach, in particular, the hemifacial spasm severity scale and the assessment of "waiting for the next injection". In addition, the effectiveness of indirect support of patients using telerehabilitation has been shown, which is especially important in the context of the COVID 19 pandemic and the increased demand on health care resources.

A RANDOMIZED, OPEN-LABEL, EVALUATOR-BLINDED TRIAL ON THE USE OF INCOBOTULINUMTOXINA FOR THE TREATMENT OF MASSETERIC HYPERTROPHY: POST-THERAPY CHANGES IN THREE-DIMENSIONAL VOLUMETRIC ANALYSES

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Introduction and Objectives: Masseteric hypertrophy (MH) is a condition that arises from excessive development of the masseter muscle(s) and is often the result of hyperfunction.[1] While MH is benign, it is associated with aesthetic complaints; especially in females who prefer a narrower facial width, compared to males.[2] A well-documented treatment option for MH includes the use of botulinum toxin type A.[3] However, it remains unknown whether different injection techniques offer varying levels of treatment efficacy.

Methods: Twenty-four female patients with MH were randomized to bilateral treatment with incobotulinumtoxinA, using either a single- (SIT) or multiple-injection technique (MIT). The SIT group received 40 units directly into the overlapping region of the masticatory heads, while the MIT group received 40 units dispersed at five points throughout the masseter. Assessments were conducted at Baseline and Weeks 4, 16, and 20. Changes over time in three-dimensional volumetric analyses were recorded and analyzed.

Results: An analysis of variance (Table 1) revealed a statistically significant effect of visit ($P=0.002$). Volume decreases at Week 4 ($P=0.018$) and 16

(*P*=0.002) were statistically greater than Week 20. There was no statistically significant effect of lateral side (i.e., right versus left; *P*=0.345). Multivariate tests revealed that Group*Side*Visit and Group*Visit interactions were not statistically significant (*P*=>0.05; Table 2). However, mean volume decreases with the multi-injection technique (MIT) were generally greater than the respective single-injection technique (SIT), at the same timepoint (Figure 1).

Conclusions: The diminutive effects of incobotulinumtoxinA on MH are clinically apparent and statistically significant at Week 4. Maximal MH reduction is maintained after 16 weeks followed by gradual muscle size recovery. Both a SIT and MIT result in a similar time-dependent pattern of effect.

Keywords: Botulinum toxin; Injectables; Maxillofacial aesthetics; Three-dimensional photography; Volumetric analysis; Xeomin Cosmetic®

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Table 1
Group statistics. An analysis of variance revealed a statistically significant effect of visit (*P*=0.002). Volume decreases at Week 4 (*P*=0.018) and 16 (*P*=0.002) were statistically greater than Week 20. There was no statistically significant effect of side (i.e., right versus left; *P*=0.345).

Side	Visit	Mean Decrease (mm) from Baseline (SD)
Right	2 (Week 4)	-0.20 (0.09)
Left		-0.21 (0.10)
Right	3 (Week 16)	-0.21 (0.10)
Left		-0.23 (0.11)
Right	4 (Week 20)	-0.13 (0.07)
Left		-0.15 (0.10)

Table 2
Subgroup analysis. Multivariate tests revealed that Group*Side*Visit and Group*Visit interactions were not statistically significant (*P*=>0.05). However, mean volume decreases with multi-injection technique (MIT) were generally greater than the respective single-injection technique (SIT), at the same timepoint.

Group	Side	Visit	Mean Decrease (mm) from Baseline (SD)
SIT	Right	2 (Week 4)	-0.18 (0.08)
	Left		-0.19 (0.09)
MIT	Right		-0.20 (0.09)
	Left		-0.23 (0.08)
SIT	Right	3 (Week 16)	-0.18 (0.06)
	Left		-0.19 (0.07)
MIT	Right		-0.20 (0.10)
	Left		-0.27 (0.03)
SIT	Right	4 (Week 20)	-0.11 (0.07)
	Left		-0.15 (0.06)
MIT	Right		-0.10 (0.08)
	Left		-0.24 (0.10)

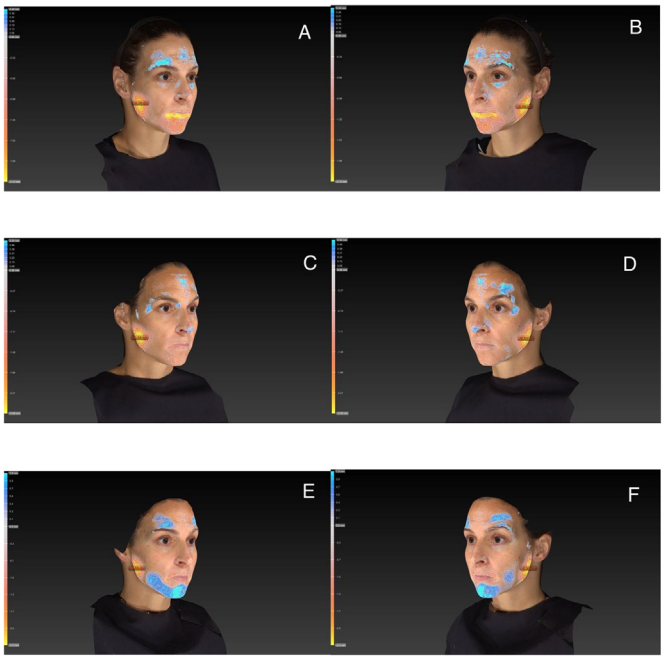

Fig. 1. Changes from baseline in volumetric analyses of a thirty-seven-year–old female at Weeks 4 (A, B), 16 (C, D) and 20 (E, F), following the use of incobotulinumtoxinA for bilateral treatment of masseteric hypertrophy. The subject was randomized to the multi-injection technique and received a total of 40 units per side (i.e., dispersed throughout right and left masseter muscles).

ABOBOTULINUMTOXINA: EVIDENCE FOR LONG DURATION OF RESPONSE FROM 5 PATIENT POPULATIONS

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Introduction: For many patients treated with botulinum neurotoxin type A (BoNT-A), the symptom relief afforded by an injection wears off earlier than the minimum injection interval, leaving patients to experience symptom recurrence with negative impact to quality of life until their next injection.

Methods: In this analysis of data from Phase 3 clinical trials (randomized controlled trials and respective open-label extensions), we assess treatment intervals over repeat cycles for abobotulinumtoxinA in the management of cervical dystonia (CD), adult lower-limb (ALL) and upper-limb (AUL) spasticity, and paediatric lower-limb (PLL) and upper-limb (PUL) spasticity. Flexible study designs allowed patients to be reinjected after Week 12 (or, for PUL, Week 16) according to clinical need.

Results: At doses tested, a long duration of response was observed. Pivotal studies of abobotulinumtoxinA reveal a large proportion of patients did not require retreatment for >12/16 weeks (% patients injected Week-16 or later): CD study: 72.6-81.5%, ALL study: 20.1-32.0%, AUL study: 24.0-36.9%, PLL studies: 72.8-93.8%; % children injected Week-22 or later in PUL study: 19.6-67.0%. Safety was as expected for BoNT-A.

Conclusions: AbobotulinumtoxinA, when dosed optimally, offers many patients a long duration of response. This may give patients sustained

symptom relief between injections and decrease the chance of patients experiencing a waning of effect before the next injection can be provided. Pre-clinical research has established BoNT-A duration of response to be dose-dependent. Supported by other published data (Field, et al, 2018), we hypothesize that the amount of active neurotoxin administered with abobotulinumtoxinA might be a factor in explaining this long duration of response.

Keywords: AbobotulinumtoxinA; Duration; Dysport; Intervals

Reference

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IMPACT OF PATIENT INPUT ON THE STUDY EXECUTION OF AN OBSERVATIONAL STUDY ASSESSING THE EFFECTIVENESS OF ABOBOTULINUMTOXINA TREATMENT IN LEG SPASTICITY MANAGEMENT IN ADULTS

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Introduction: It is increasingly accepted that people living with long-term conditions should be involved in the design, conduct, and dissemination of research that affects them.

Methods: A one-day structured workshop was conducted with a small focus group of patients with leg spasticity. The day was split into presentations and discussions about the study design, the process of goal setting in spasticity, and an open feedback session.

Results: Key learnings were that the clinical research process can be confusing for patients who do not clearly understand medical terminology. For example, when reviewing the informed consent process, patients emphasised the need for clear, jargonless explanations of the study objective to enable participation. The AboLiSh study aims to assess the longitudinal attainment of person-centred and function-related goals following repeat treatment cycles with abobotulinumtoxinA for spasticity management. Patient feedback was that, for optimal engagement with the process, they require training on appropriate goal setting, and that this could be delivered by a well-designed leaflet they can read in advance of their visit. Finally, patients noted that they can feel undervalued when not kept informed of study results.

Conclusions: Following the patient workshops, a goal-setting leaflet has been produced to aid patient and caregiver understanding of the process. Patients will receive regular updates by newsletter (digital and paper) and will be invited to review the results once the study ends.

Keywords: Person-centred; Patient; Observational study; Goal setting

VIDEO AND SPATIO-TEMPORAL PARAMETER ASSESSMENT OF GAIT AFTER ABOBOTULINUMTOXINA TREATMENT: A PILOT STUDY

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Introduction: The use of evaluation with video systems that record movement and visualize postures as well as superficial muscle contraction during walking, although not perfect, can be useful to enhance observation. It also can serve as a proxy to determine with greater confidence which muscles contribute to the observed maladaptive posture and the underlying muscle imbalance from which more specific and appropriate treatment can be inferred. [1-2] The aim of this study was to summarize the principal findings of the use of video-enhanced visual analysis (VEVA) as a muscle selection assistance method for the treatment with botulinum toxin type A (BoNT-A) of abnormal foot postures in a real-world setting in adults with upper motor neuron (UMN) syndrome.

Methods: *Design:* Prospective treatment study. *Setting:* Outpatient, rehabilitation tertiary hospital. *Participants:* Patients with stroke or traumatic brain injury (TBI) of >6-month duration who had a spastic equinovarus ankle foot deformity amenable to treatment with BoNT-A and were able to ambulate a minimum of 10 meters independently. *Intervention:* Participants were evaluated before abobotulinumtoxinA injection (500 U to 1500 U) to the identified lower limb muscles and four to five weeks post-intervention. *Main Outcome Measures:* Spatio-temporal data (self-selected and maximal walking velocities (SSWV/MWV); step length and stance time); Modified Ashworth Scale (MAS); Tardieu Scale (TS) and ankle Passive Range of Motion (PROM) change from baseline to follow up (f/u).

Results: A total of 11 participants were enrolled in the study. One participant withdrew due to pain in the opposite ankle. There was a 25% increase from baseline to follow up in barefoot SSWV condition and no changes in the shoe condition. There was a 14% increase from baseline to follow up in MWV with shoes and no changes in the barefoot condition. Step length and stance time symmetry did not show changes. Ankle MAS scores with knee flexed improved in 70% of subjects, and 90% improved their MAS score with knee extended. Ankle TS scores improved by 80%. Ankle PROM increased by 22% post-intervention.

Fig. Ankle posture deformities. a. Equinus; b. Equinovarus with toe flexion; c. Equinus with toe flexion.

Conclusions: VEVA as a supplement to clinical evaluation appears to facilitate muscle identification and selection for treatment of ankle deformities with BoNT-A with marked improvement in ankle MAS and TS as pharmacological activity indicators and increase in SSWV as a functional marker.
Funding: This study was supported by a researcher-initiated grant from Ipsen.
Keywords: Stroke; Traumatic brain injury; Gait spatio-temporal data; Video-enhanced visual analysis; AbobotulinumtoxinA

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ADHERENCE TO ONABOTULINUMTOXINA TREATMENT IN PATIENTS WITH SPASTICITY FROM THE ASPIRE STUDY

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Introduction and Objectives: To better understand clinical strategies to manage spasticity, we aimed to identify baseline clinical characteristics and treatment-related variables that impact adherence to onabotulinumtoxinA treatment in patients from the Adult Spasticity International Registry (ASPIRE) study.
Methods: International, prospective, observational registry (NCT01930786). Adults with spasticity were treated with onabotulinumtoxinA at the clinician's discretion. Clinically meaningful thresholds for treatment adherence ($\geq$3 treatment sessions in 2-year study) and non-adherence ($\leq$2 sessions) were used. Data were analyzed using univariate and multivariate logistic regression and presented as odds ratios (OR) with 95% confidence intervals (CI). Statistical significance was accepted at $P<0.05$; clinically meaningful non-significant variables of interest at $P<0.10$. Data for treatment-related variables were assessed at sessions 1 and 2 only.
Results: Of the total population (N=730), 523 patients (71.6%) were treatment adherent with 5.3 (1.6; mean [SD]) treatment sessions; 207 (28.4%) were non-adherent with 1.5 (0.5) treatment sessions. In the final model (n=626/730), 522 patients (83.4%) were treatment adherent, 104 (16.6%) were non-adherent. Baseline characteristics associated with adherence: treated in Europe (OR: 1.84; CI: 1.06-3.21; P=0.030) and use of orthotics (OR: 1.88; CI: 1.15-3.08; P=0.012). Baseline characteristics associated with non-adherence: history of diplopia (OR: 0.28; CI: 0.09-0.89; P=0.031) and use of assistive devices (OR: 0.51; CI: 0.29-0.90; P=0.021). Treatment-related variables associated with non-adherence: treatment interval $\geq$15 weeks (session 1 to 2; OR: 0.43; CI: 0.26-0.72; P=0.001) and clinician dissatisfaction with onabotulinumtoxinA to manage pain (OR: 0.18; CI: 0.05-0.69; P=0.012).
Conclusions: These ASPIRE analyses provide real-world insight into variables that impact adherence to onabotulinumtoxinA treatment, which can help optimize spasticity management strategies to improve patient care.
Funding: Allergan, prior to acquisition by AbbVie
Keywords: Botulinum toxin; OnabotulinumtoxinA; Patient compliance; Spasticity; Treatment adherence

MANAGEMENT OF SYMPTOM RE-EMERGENCE IN PATIENTS LIVING WITH SPASTICITY AND CERVICAL DYSTONIA: FINDINGS FROM 2 ONLINE PATIENT SURVEYS

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Introduction: Botulinum toxin type A (BoNT-A) injections are first-line treatment for spasticity and cervical dystonia (CD). Current guidelines suggest that BoNT-A injections should not be given more frequently than every 12 weeks but waning of benefit may occur at a shorter interval allowing for re-emergence of symptoms.
Design: Separate, but parallel, online surveys were developed for adult spasticity and CD including questions assessing current management of symptom re-emergence, and patient expectations for optimal treatment. Both surveys were deployed in France, Germany, Italy, the United Kingdom, and the United States.
Results: A total of 210 respondents with spasticity and 209 with CD completed the online surveys. Following a report of symptom re-emergence, the most common management approaches for patients living with spasticity or CD respectively were to add adjunctive treatments (36% spasticity; 35% CD), increase the BoNT-A dose (28% spasticity; 54% CD), wait for the next injection (27% spasticity; 19% CD) or a reduction in treatment interval (26% spasticity; 23% CD). Of the respondents, 8% with spasticity and 14% with CD said they do not tell their doctor about symptom re-emergence with the most common reason being they *"do not believe they can do anything about it."* While respondents (both conditions) were generally happy with their current injection schedules, 72% of respondents with spasticity and 71% of those with CD suffering symptom re-emergence said they would like a longer-lasting BoNT-A treatment.
Conclusions: Symptom re-emergence between BoNT-A injections in both spasticity and CD is common. Greater patient—and physician—awareness of this therapeutic profile should lead to better informed therapeutic discussions, planning, and outcomes.
Keywords: Botulinum toxin; Cervical dystonia; Patient survey; Spasticity; Symptom re-emergence

PATIENT EXPERIENCES OF SYMPTOM RE-EMERGENCE: FINDINGS FROM 2 ONLINE PATIENT SURVEYS IN SPASTICITY AND CERVICAL DYSTONIA

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Introduction: Botulinum neurotoxin type A (BoNT-A) is a major, effective pharmacological treatment for the management of spasticity and cervical dystonia (CD) that requires repeated administration at variable intervals. Patient perceptions of treatment efficacy and waning of BoNT-A effect over

a treatment cycle have not been well studied.
Design: Separate, but parallel, online surveys were developed for adult spasticity and CD to assess patient perceptions of the waning of BoNT-A effect before the next planned injection and its impact on daily life. Both surveys were deployed in France, Germany, Italy, UK, and USA.
Results: A total of 210 respondents with spasticity and 209 with CD completed the online surveys. Symptom re-emergence between BoNT-A injections was common in both indications (affecting 83% respondents with spasticity and 88% with CD). A majority of patients, both with spasticity and CD, experienced recurrence within 3 months of their injection; the average time from injection to symptom re-emergence was 89.4 days in spasticity and 73.6 days in CD. Treatment was not reported to completely abolish symptoms of either condition, even at peak effect. However, symptom intensity was consistently rated as lowest at the peak of treatment effects, increasing as the effects of treatment start waning and was strongest one day before the next session [Figure]. Most patients (both conditions) reported having BoNT-A injections at 3-4 monthly intervals, which means that many patients spend at least a few weeks with re-emergent symptoms between treatments.
Conclusions: Results will raise awareness as to how the waning of BoNT-A effect can impact daily life to inform future treatment decisions to address patients' needs.
Keywords: Botulinum toxin; Cervical dystonia; Patient survey; Spasticity; Symptom re-emergence

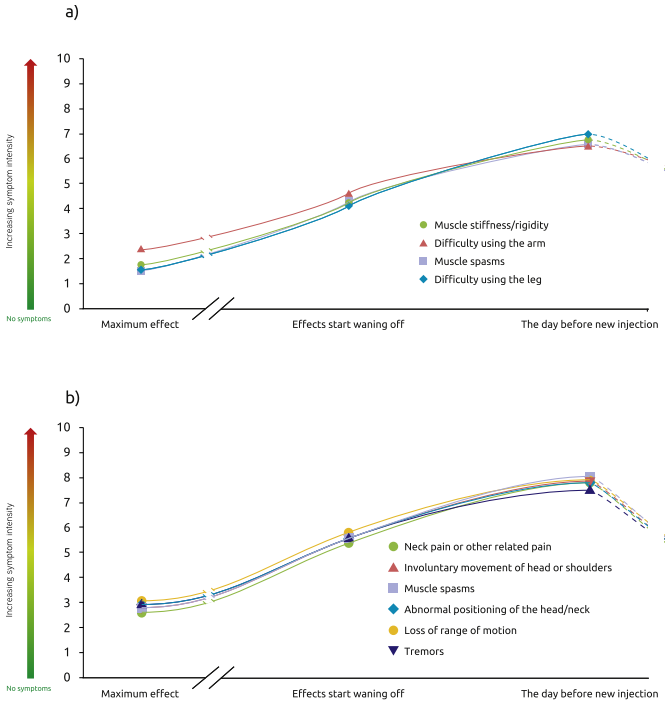

Fig. Symptom intensity across a BoNT-A injection cycle (a) for patients living with spasticity (b) for patients living with CD.

REDUCTION IN INCONTINENCE PRODUCT USE AND ASSOCIATED COST SAVINGS AFTER ONABOTULINUMTOXINA TREATMENT IN PATIENTS WITH OVERACTIVE BLADDER

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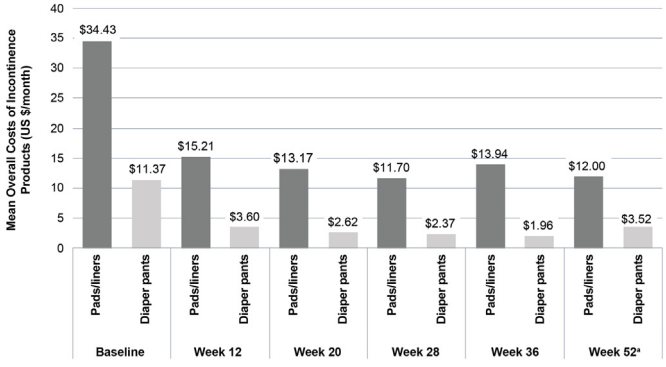
Background: In patients with overactive bladder (OAB), onabotulinumtoxinA 100 U reduces urinary incontinence (UI) and improves quality of life. This analysis aimed to estimate potential cost savings in a real-world clinical setting seen after onabotulinumtoxinA treatment due to a reduced reliance on incontinence products.
Methods: This 12-month prospective, observational, non-randomized multinational phase 4 study was performed in 4 European countries. Outcomes: percent reduction from baseline (BL) in UI episodes/day, proportion of patients achieving ≥50%/100% reduction in UI episodes/day, treatment benefit score (TBS), and number of incontinence products used per month. Incontinence product costs (pads/liners and diaper pants) were estimated using available pricing data filtered by average female waist size (diaper pants) and largest package size.
Results: Overall, 504 patients received onabotulinumtoxinA. Daily UI episodes at BL were (mean ± standard deviation [SD]) 4.9 ± 4.2. UI episodes were significantly reduced by 46.9% ($P < .001$) at week 1 and 61.3% at week 12 ($P<.001$). The proportion of patients achieving a ≥50% and 100% reduction in UI episodes/day was 60.7% and 25.5% at week 1 and 73.9% and 41.8% at week 12. A positive treatment response on the TBS was seen in 87.6% of patients. The mean monthly number of pads/liners and diaper pants used dropped from BL (67.7 and 13.9) to week 12 (29.9 and 4.4) and was sustained until week 52 (23.6 and 4.3). Mean cost for pads/liners was determined to be $0.51/unit and for diaper pants $0.82/unit. Monthly costs of pads/liners and diaper pants decreased substantially from BL to week 12 and was sustained to week 52 (Figure 1).
Conclusions: This analysis is the first of its kind detailing potential cost savings in incontinence product use in OAB patients treated with onabotulinumtoxinA. There was greater than 50% reduction in monthly costs of pads/liners and nearly 70% reduction in diaper pants costs sustained out to 52 weeks.
Funding: Allergan, an AbbVie company
Keywords: Cost analysis; OnabotulinumtoxinA; Urinary incontinence

Figure 1: Estimated monthly cost of incontinence products.



[a]Week 52: only patients without reinjection of onabotulinumtoxinA.

DEVELOPMENT OF A NEW SIALORRHEA TREATMENT SERVICE FOR PATIENTS WITH NEUROLOGICAL DISORDERS WITHIN A COMMUNITY AND DISTRICT HOSPITAL SETTING

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Introduction: A new service was created to provide specialist investigation and treatment of resistant hypersalivation in neurological disorders through the provision of ultrasound-guided botulinum toxin type A (BTX-

A) injections into the salivary glands.
Methods: Over a year the service provided assessment and treatment for eleven (11) patients with neurological disorders. This also included a 5-month clinic closure due to the COVID-19 pandemic. The parotid and submandibular glands of 10 patients were injected with BTX-A using ultrasound guidance. Before injection, the baseline rate of salivation was assessed using a visual analogue scale. In some cases, repeat injections were provided at regular planned intervals.
Results: Of the 11 patients treated, all (100%) reported a subjective reduction in salivation post-treatment. Subjective scores from the 16 episodes of treatment indicated a reduction of 60.8% in the mean rate of salivation for all patients. No serious adverse events occurred, and no procedure-related complications were reported.
Conclusions: This is the first year of data available for our new service and the results show that the treatment is effective, beneficial, and safe. This suggests that the technique is safe and that BTX-A injections are effective for the treatment of sialorrhea in patients with neurological disorders. This service can also be provided in both district and community hospitals with positive effects within a small unit set-up pending availability of appropriate equipment.
Keywords: Community; District; Development; Neurology; Sialorrhoea

IMPACT OF BOTULINUM TOXIN CONSULTATION INTERRUPTION DUE TO THE COVID-19 PANDEMIC ON ADULT PATIENT WITH DYSKINETIC CEREBRAL PALSY PATIENTS' PERCEPTIONS OF THERAPEUTIC GOALS

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Introduction and Objectives: The COVID-19 pandemic has imposed an additional pressure on health systems worldwide, creating an increased challenge to chronic patient management due to consultation shutdown.[1] Spastic patients were especially vulnerable to inadequate care, resulting in a worsened quality of life.[2] This study aims to evaluate the impact of a 10-week consultation interruption due to the COVID-19 pandemic on the patients' perceptions of spasticity evolution and therapeutic goals with botulinum toxin (BT) therapy.
Methods: A cross-sectional study of patients with spasticity followed in the BT clinic at a physical medicine and rehabilitation department who had their consultation delayed due to the COVID-19 pandemic shutdown (March to May 2020). A standardized questionnaire was administered to all patients. Goal Attainment Scaling (GAS) was done for each previous BT treatment goal and patients' perceptions regarding the impact of treatment delay on their spasticity was evaluated using a Likert scale.
Results: A total of 29 patients had their consultation delayed. One patient died during the follow-up period and was excluded from the analysis. The mean patient age was 65.3±11.7 years and the majority were male (57.1%; n=16). Mean disease duration was 8.9±5.5 years,and the majority of patients had a stroke diagnosis (82.1%; n=23) and a spastic hemiparesis (85.7%; n=24). Eighteen patients were usually treated with abobotulinumtoxinA (611.9±275.7 U) while 10 were treated with onabotulinumtoxinA (215.0±83.1 U). Treatment objectives defined according to the World Health Organization's International Classification of Functioning, Disability and Health[3] included mobility facilitation (71.4%; n=20), improved passive function (53.6%; n=15), involuntary movement control (35.7%; n=10), pain control (21.4%; n=6), maintenance of range of motion (ROM) (10.7%; n=3), and improved active function (10.7%; n=3). The majority of patients (92.9%) presented an expected or greater outcome regarding their previous BT GAS assessment (Table 1). The mean GAS outcome T-score was 52.1±5.1, and the mean change in GAS score was 11.2±6.3. BT administration was delayed by 4.3±1.6 months, with a mean inter-treatment period of 8.8±2.2 months. Twenty-four patients (85.7%) reported worsening of symptoms at the time of consultation delay (90% - mobility; 83.3% - pain; 80.0% - involuntary movement; 100.0% - ROM maintenance; 100.0% - passive function; 100.0% - active function). Some patients (35.7%; (n=10) made up for the absence of BT administration with physiotherapy, 7.1% (n=2) with oral antispastic medication, and 7.1% (n=2) with increased orthosis usage, although the majority (57.1%; n=16) adopted no additional adjuvant strategy. In patients reporting a "much worse" impact on mobility and passive function, the median treatment delay was higher (4.7 vs 4.1 and 4.5 vs 3.0 months, respectively) than in patients reporting a "worse" impact.
Conclusions: The COVID-19 pandemic has had a major negative impact on the BT treatment of spasticity, even in patients treated with not very high doses. Consultation shutdown resulted in a treatment delay of 4 months, with the worsening of patient symptoms. As expected, effects seemed to be worse with longer treatment delays. The delay period was greater than shutdown duration due to rescheduling availability. Thus, consultation shutdown severely affected these patients and needs to be avoided.
Keywords: Botulinum Toxin; Coronavirus; COVID-19; Spasticity

Table 1
GAS Results and Impact of Treatment Delay by Goal Area (N=28).

Goal areas according to ICF	Last Treatment GAS		Impact of Treatment Delay (Likert scale)	
Pain	Lower than expected	1	Much Worse	1
	Goal attainment	3	Worse	4
	Greater than expected	2	Equal	1
Involuntary Movement	Goal attainment	8	Much Worse	2
	Greater than expected	2	Worse	6
			Equal	2
ROM Maintenance	Goal attainment	3	Worse	3
Passive Function	Goal attainment	12	Much Worse	6
	Greater than expected	2	Worse	9
	Much Greater than expected	1		
Active Function	Goal attainment	2	Much Worse	2
	Greater than expected	1	Worse	1
Mobility	Lower than expected	1	Much Worse	5
	Goal attainment	14	Worse	13
	Greater than expected	4	Equal	2
	Much Greater than expected	1		

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BOTULINUM TOXIN TREATMENT OF SPASTICITY-RELATED PAIN: A CASE REPORT

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Introduction: Spasticity has many associated symptoms that must be recognized and properly treated. Spasticity-related pain is one of them, and it is considered a major therapeutic challenge. It can be treated with the use of a vast number of pharmacological agents, including botulinum toxin. There is some evidence suggesting that botulinum toxin is effective

in treating this condition.[1-3] However, its mechanism of action is not yet fully understood.[4]
Methods: A 36-year-old Caucasian male, with spastic tetraparesis sequential to a traumatic brain injury, complained of increasing left hamstring muscle pain, refractory to any therapeutic modality. Botulinum toxin was administered to the left lower limb.
A systematic assessment of passive range of motion of both hips, knees, and tibiotarsal joints was performed before injection. Reassessment of the same joints was performed 4 weeks postinjection. The contralateral side was also assessed. Pain status was monitored with the Verbal Numeric Scale (VNS).
Results: Throughout the treatment, there was improvement in range of motion on hip flexion and left foot dorsiflexion beginning after the first injection and persisting through the following evaluations. Gait performance also improved. Pain assessment showed progressive improvement over the course of treatment, with total absence of pain after 6 months.
Conclusion: In addition to spasticity, botulinum toxin has additional applications in management of chronic pain. There is much debate regarding its mechanism of action in this indication: it appears to act by an indirect effect in reducing excess dysfunctional muscle activity and also through a potential direct pharmacological effect on peripheral nociceptors. This case report clearly emphasizes the benefit of considering botulinum toxin in pain control, especially when other therapies have proved ineffective.
Keywords: Botulinum toxin; Pain; Spasticity

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EFFICACY AND SAFETY OF INCOBOTULINUMTOXINA IN THE TREATMENT OF LOWER-LIMB SPASTICITY IN ADULTS: THE PATTERN CUSTOMIZED STUDY DESIGN

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Introduction: The Phase 3 PATTERN study (NCT03992404) will assess the efficacy and safety of incobotulinumtoxinA for lower- or lower- and upper-limb spasticity due to stroke or traumatic brain injury. We introduce the study design and its new innovative features.
Methods: PATTERN is an ongoing randomized, double-blind, placebo-controlled, multicenter study, with an open-label extension (OLEX) period. An estimated 600 adults with lower-limb spasticity (Modified Ashworth Scale [MAS] ankle score 2 or 3), with/without upper-limb spasticity of the same body side, will be enrolled. Subjects will receive incobotulinumtoxinA 400 U or placebo into lower-limb muscles in the main period (1 injection cycle [IC]) and ≤800 U into lower-limb muscles with combined upper-limb treatment, if indicated, in the OLEX (4-5 ICs). A two-stage adaptive design with interim sample size reassessment after 360 subjects will be applied. Primary and co-primary endpoints are change from baseline in derived MAS ankle score and physician's Global Impression of Change Scale score at Weeks 4-6. Safety will be assessed. A goal catalog derived from physicians' and patients' qualitative interviews will be incorporated into the goal attainment scale treatment evaluation.
Results: The PATTERN study design allows for the observation of up to 6 consecutive ICs, with the OLEX including a multi-pattern, patient-centric approach, if clinically indicated.
Conclusions: This novel study design, utilizing pre-specified sample size adaptation based on an interim analysis, demonstrates an innovative approach to the challenge of lower-limb spasticity clinical studies. To our knowledge, this is the first time such an adaptive design has been used in a BoNT study in this indication. A goal catalog specifically developed for this population is expected to overcome the difficulties of comparable high-quality SMART treatment goal setting and follow up in a large Phase 3 trial.
Funding: Merz Pharmaceuticals GmbH, Frankfurt am Main, Germany
Keywords: IncobotulinumtoxinA; Lower-limb spasticity; Stroke

REAL-LIFE USE OF BOTULINUM TOXIN TYPE A IN POSTSTROKE SPASTICITY: IS THERE A TIME WINDOW TO START TREATMENT?

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Introduction: Spasticity can occur following stroke, causing disability and a negative impact on quality of life. Botulinum toxin type A (BoNT-A) is the first-line treatment for post-stroke spasticity. Goal Attainment Scaling (GAS) is a method of rating the extent to which patients' individual goals are achieved. This study aims to compare the therapeutic effects of BoNT-A in spastic patients starting treatment within 1 year after stroke and 3 years after stroke.
Methods: A sample of 216 patients (98 females) diagnosed with poststroke spasticity – encompassing a total of 2083 injection sessions – was divided into 3 groups: patients who started BoNT-A in the first year post-stroke (Group 1; n = 1009 injections); patients who started BoNT-A between 1 and 3 years post-stroke (Group 2; n= 749 injections); and patients who started BoNT-A after 3 years post-stroke (Group 3; n= 308 injections). We excluded 17 injections that lacked valid information. We characterized the sample according to gender, age, and BoNT-A formulation administered. Treatment efficacy was measured by goal achievement/overachievement, using GAS as the primary outcome measure for all goals. Success was defined as a GAS score ≥0 per goal.
Results: BoNT-A was very effective in all groups of patients (GAS≥ 0 in over 80% of the injections), regardless of the time the treatment was started. Group 3 had better treatment efficacy for secondary goals, with statistically significant results. When comparing the results between the various BoNT-A preparations used, we found no statistically significant differences in GAS scores.
Conclusions: Our findings suggest that BoNT-A is very effective in post-stroke spasticity. BoNT-A can be started even after 3 years post-stroke for well-selected patients, with realistic, individualized goals. Patients in Group 3 had long-standing, established, and well recognized deficits, with easily identifiable needs. It may be easier to set viable objectives for these patients, enabling better results in goal attainment.
Keywords: AbobotulinumtoxinA; Botulinum toxin; GAS score; IncobotulinumtoxinA; OnabotulinumtoxinA; Poststroke spasticity

UNIQUE INTRACELLULAR LOCALIZATION OF BOTULINUM NEUROTOXIN LIGHT CHAIN A1 AND A3

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Introduction and Objectives: *Clostridium botulinum* and related clostridia produce seven immunologically distinct serotypes (A-G) of the botulinum neurotoxin (BoNT).[1] BoNTs are composed of a light chain (LC), the catalytic domain, and a heavy chain (HC), which contains the translocation and receptor-binding domains.[2] Within BoNT/A, there are 8 subtypes (BoNT/A1-A8).[3-5] BoNT/A1 has a long-lived intoxication, while BoNT/A3 has a short-lived intoxication.[6] GFP-LC/A1 and GFP-LC/A3 showed unique intracellular localizations.[7] Sequence alignment identified a low homology domain (LHD) between LC/A1 and LC/A3. Earlier studies showed that the N terminus (NT) contributed to the intracellular localization of LC/A1.[8] This study addresses the role of the LHD and N terminus in LC/A intracellular localization.
Methods: Neuro-2A (N2A) cells were transfected with 500 ng of indicated plasmid (Lipofectamine® LTX, Invitrogen). The next day, N2A cells were stained with WGA-647 (membrane marker) and 10 random fields from each transfection were scored for GFP colocalization with WGA, indicating membrane localization. Percent membrane localization was scored by the following equation: [(number of cells with GFP & WGA colocalized)/total number of GFP-positive cells] X 100^{-7} Results were graphed and subjected to statistical analysis to determine significance of LC membrane localization.
Results: At steady state, ~80% of cells transfected with GFP-LC/A1 were membrane localized. Approximately 20% of the cells transfected with GFP-LC/A3, Loch Maree, had membrane localized GFP. Approximately 20% of cells expressing GFP-LC/A3 (A1 NT) possessed membrane localized GFP, while ~35 % of cells expressing GFP-LC/A3 (A1 LHD) possessed membrane localized GFP. Approximately 60% of cells transfected with GFP-LC/A3 (A1 NT and LHD) possessed membrane localized GFP.
Conclusions: The results show that the NT and LHD domains of LC/A1 contribute to LC membrane localization, via an additive mechanism, suggesting that each domain contributes an independent, sequential step in LC/A1 membrane localization.
Funding: AI-139306
Keywords: Botulinum neurotoxin (BoNT); Heavy chain (HC); Light chain (LC); Low homology domain (LHD); N terminus (NT)

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COST-UTILITY ANALYSIS OF FLEXIBLE INTERVALS WITH INCOBOTULINUMTOXINA VERSUS FIXED DOSING WITH ONABOTULINUMTOXINA IN THE MANAGEMENT OF CERVICAL DYSTONIA AND BLEPHAROSPASM IN FOUR MAJOR CANADIAN PROVINCES

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Introduction: The aim was to carry out a cost-utility analysis for incobotulinumtoxinA (INCO) administered with flexible treatment intervals compared to onabotulinumtoxinA (ONA) administered with fixed intervals for the management of cervical dystonia (CD) and blepharospasm (BEB) from the payer perspective in four major Canadian provinces. INCO and ONA have marketing authorization in Canada, among others, for the treatment of CD and BEB. INCO is the only botulinum toxin in Canada with marketing authorization for administration at flexible intervals between 6-20 weeks based on the patients' individual clinical needs. Prospective comparison trials have demonstrated that INCO and ONA result in comparable safety and efficacy when a 1:1 clinical conversion ratio is used.
Methods: A Markov state transition model was developed to estimate costs (CDN$, 2019) and benefits (Quality Adjusted Life Years, QALYs) of the management of CD and BEB with INCO administered at flexible intervals (6 to 20 weeks) vs ONA administered at fixed intervals (12 weeks); a 5-year time horizon was used. It was assumed that patients experience a re-emergence of symptoms after toxin treatment effect wanes. Utilities were extracted from a published study in patients with focal dystonia. Costs were based on Canadian public databases.
Results: For all provinces studied (Quebec, Ontario, Alberta, and British Columbia) and for both indications, INCO was a dominant strategy vs ONA, with higher effectiveness and lower cost. Patients treated with INCO at flexible intervals had less symptomatic weeks, while receiving comparable numbers of injection sessions.
Conclusions: The treatment of CD and BEB with INCO at flexible intervals determined by the patients' individual clinical needs is a cost-effective therapeutic alternative compared to the administration of ONA in four major Canadian markets.
Keywords: Blepharospasm; Cervical dystonia; IncobotulinumtoxinA; OnabotulinumtoxinA

DAXIBOTULINUMTOXINA FOR INJECTION – TREATED SUBJECTS SHOW PROGRESSIVE IMPROVEMENT IN STATIC GLABELLAR LINES WITH REPEATED TREATMENT

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Introduction: Static glabellar lines result from repeated contraction of the glabellar complex muscles. While botulinum toxin treatments are most commonly employed to treat dynamic glabellar lines, a demonstrated secondary benefit has been observed on static glabellar lines. DaxibotulinumtoxinA for Injection (DAXI) is a novel botulinum toxin type A formulation in development for the treatment of glabellar lines. The Phase 3 SAKURA clinical program demonstrated the efficacy of DAXI on dynamic glabellar lines with therapeutic benefit sustained over baseline for 28 weeks.
Methods: Two pivotal studies and an open-label safety study enrolled adults with moderate or severe glabellar lines who received 40 U DAXI in a standardized 5-point injection pattern into the corrugator and procerus

muscles. Glabellar line severity (at maximum frown and at rest) was assessed using validated 4-point rating scales. In the Phase 3 program, a subset of subjects received 3 DAXI treatments. Retreatment was permitted when the subject's glabellar line severity returned to baseline based on both investigator and subject ratings.
Results: Overall, 568 subjects received 3 DAXI treatments; 91% had static glabellar lines at baseline based on subject self-rating. At week 4 after DAXI treatment 1, 57.9% of subjects had no static glabellar lines. At week 4 after DAXI treatments 2 and 3, the proportion increased to 68.7% and 71.5%, respectively. Similar progressive improvement was observed based on investigator assessment.
Conclusions: DAXI-treated subjects showed substantial and progressive improvement in the severity of static glabellar lines with repeated treatment. The extended duration of therapeutic benefit observed with DAXI in dynamic glabellar lines provides a longer window of muscle hypoactivity, permitting an extended period for dermal remodeling to occur. This can potentially account for the progressive improvement in static glabellar lines observed over repeated DAXI treatments.
Keywords: Aesthetics; Botulinum toxin; Facial rejuvenation; Glabellar lines; Injectable

BILATERAL HEMIMASTICATORY SPASM: REPORT OF A 4-YEAR FOLLOW-UP CASE TREATED WITH BOTULINUM TOXIN

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Introduction and Objectives: Hemimasticatory spasm (HMS) is a rare movement disorder.[1] Our goal is to describe a case of bilateral involvement successfully treated with botulinum toxin**.**
Methods: We describe a patient with bilateral HMS in a movement disorders center, followed up periodically between 2016 and 2020.
Results: A 40-year-old female presented with a 3-year history of abnormal sensation of the jaw on the left side, at times causing her jaw to lock. She experienced dozens of sometimes painful episodes in a single day, and she had several biting injuries to her tongue and cheek.
She also noticed that episodes were more frequent during eating and talking. Trials of carbamazepine and lamotrigine were unsuccessful. We observed bilateral hypertrophy of the masseter and temporalis muscles, significantly worse on the left side, and involuntary contractions of those muscles lasting for periods of a few seconds to a minute. During these spasms, she could not open her mouth. There was no associated facial hemiatrophy. Brain magnetic resonance imaging scans were normal, and electromyography showed irregular bursts of motor unit potentials correlated with muscle spasms, but incompatible with dystonia.
The diagnosis of idiopathic bilateral HMS was made. Treatment with onabotulinumtoxinA reversed the spasms and pain after a few days. Sixty units (U) of onabotulinumtoxinA were injected in the left masseter and 40 U in the right masseter and each temporalis muscle. Since then she has been treated every 3-4 months with a good response maintained. At 4-year follow up, muscle hypertrophy had improved, and lower doses of botulinum toxin were needed.
Conclusions: HMS is a very rare disorder characterized by paroxysmal involuntary contraction of the jaw-closing muscles, typically unilaterally.[1,2] Since the original description by Gowers in 1897, few cases have been described in the literature, and no case of bilateral involvement to the authors' knowledge.[3,4] Botulinum toxin type A injection is the most effective treatment available.[4,5]
Keywords: Botulinum toxin; Hemimasticatory spasm; Movement disorder

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EFFICACY AND SAFETY OF ABOBOTULINUMTOXINA IN PEDIATRIC LOWER LIMB SPASTICITY (PLLS): 2ND INTERIM RESULTS FROM A PHASE IV, PROSPECTIVE, OBSERVATIONAL, MULTICENTER STUDY

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Introduction: The primary objective was to assess subject-centered, function-related Goal Attainment Scale (GAS) T-score after repeated aboBotulinumtoxinA (aboBoNT-A) injections. A 1st interim analysis included efficacy results from treatment cycle 1. We report updated data after up to 5 cycles, including long-term safety results assessed for ≤18 months.
Methods: Eligible patients aged 2-17 years were recruited from the investigators' clinical practices. Prescription decisions were made prior to/independent of study enrollment. Functional goals were identified at baseline by patient/parent/caregiver in consultation with investigators. Adverse events were reported.
Results: This interim analysis included 201 patients, of whom 78.1% (n=157) had received prior botulinum neurotoxin (BoNT) treatment. At enrollment, 69.2% were aged 2-9. Average time to the 2nd and 3rd treatments was 24.79 (SD 12.38) and 42.58 (12.85) weeks, respectively. The cumulative GAS T-score for the total population was 51.60 (9.69). By the last treatment assessed (in this 2nd interim analysis), mean T-score for the total population (N=201) was 48.14 (8.08); BoNT-naïve (n=44) had a T-score of 52.07 (3.58) vs 47.52 (8.47) in BoNT–non-naïve (n=157) patients aged 2-9; the T-score was 46.66 (8.31) vs 52.07 (6.41), respectively, in patients aged 10-17. In the safety population (N=243), 44 treatment-emergent adverse events (TEAEs) were reported in 26 patients; most were mild to moderate, with 1 severe. Extremity pain, limb discomfort, muscle swelling, and myalgia reported in 3 patients were deemed treatment related. No reported TEAEs led to study drug withdrawal or death.
Conclusions: Goal attainment outcomes reflect overachievement (T-score >50.0) for the overall PLLS population, potentially due to goals being overcautious. AboBoNT-A was well tolerated, with a low incidence of TEAEs. These results support aboBoNT-A as an effective treatment option with a positive risk-benefit profile for patients with PLLS.
Funding: Ipsen, Inc. (Cambridge, MA, USA)
Keywords: AbobotulinumtoxinA; Pediatric lower limb spasticity; T-score

RESCUE STRATEGIES IN DYSTONIA BEFORE DEEP BRAIN STIMULATION: THE VALUE OF SWITCHING BOTULINUM TOXIN FORMULATIONS

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Introduction and Objectives: Dystonia is characterized by abnormal, often repetitive movements and/or postures that are caused by sustained or intermittent muscle contractions.[1] Patients with refractory dystonia are extremely challenging to treat and pose a significant burden to the medical system. Two serotypes of botulinum neurotoxin (BoNT) are used for dystonia treatment: A and B.[2,3] In Canada, only serotype A is available, and three formulations are used in clinical practice: onabotulinumtoxinA (Ona-A), incobotulinumtoxinA (Inco-A) and—more recently—abobotulinumtoxinA (Abo-A). Despite the widespread use of different BoNT formulations in the dystonia population, there is a lack of comparative data on the efficacy of different formulations in patients with refractory dystonia, such as the ones who are referred for deep brain stimulation (DBS). We hypothesized that some patients might experience significant improvement by switching to a different BoNT formulation, specifically Abo-A, before surgery is considered.
Methods: This is a single-center, retrospective study of dystonia patients who were referred to the DBS program between January 2014, and December 2018. Patients with other movement disorders were excluded. Demographic data, dystonia classification, BoNT regimen, and clinical outcomes were collected. Clinical course was chronologically documented from the referral date until the last clinic follow up.
Results: Sixty-seven patients were included (30 males [45%]; mean age: 48.3±20.1 years; disease duration: 16.9±15.3 years). Generalized dystonia was the most common pattern of distribution (32%), followed by segmental (30%) and focal (22%) dystonia. The majority of the patients received BoNT prior to DBS referral (Ona-A in 65%; Ona-A and Inco-A in 5%) for a treatment duration of 6.1±5.8 years and a mean number of BoNT sessions of 3.3±1.3 in the year prior to referral. Thirty-three (49%) patients underwent DBS: 29 (85%) targeting the globus pallidus pars interna (GPI), four (12%) the unilateral ventral intermediate nucleus (VIM), one (3%) the bilateral VIM, and one (3%) both the GPI and VIM. Four (6%) patients were awaiting the procedure while the remaining 30 patients (45%) did not undergo DBS. The reasons DBS was not performed were: patient refusal (17 [53%]), presence of functional dystonia (6 [20%]), and successful use of Abo-A (3 [10%]) in patients who had failed other BoNT-A formulations.
Conclusions: Our study highlights the importance of careful patient selection for DBS, specifically excluding functional dystonia. Also, some patients might experience significant clinical benefit by switching BoNT formulations.
Funding: None.
Keywords: Dystonia; Deep brain stimulation; Botulinum neurotoxins; OnabotulinumtoxinA; IncobotulinumtoxinA; AbobotulinumtoxinA

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THE ROLE OF BOTULINUM TOXIN TYPE A IN TRIGEMINAL NEURALGIA

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Introduction and Objectives: The International Headache Society defines trigeminal neuralgia (TN) as recurrent paroxysms of unilateral facial pain in the distribution(s) of one or more divisions of the trigeminal nerve, with no radiation beyond, and precipitated by innocuous stimuli within the affected region. The conventional treatment includes anticonvulsive drugs (particularly carbamazepine, gabapentin, lamotrigine, or phenytoin) and baclofen.
We are reporting the case of a 49-year-old female patient presenting with TN refractory to the standard treatment. As a consequence, the patient underwent treatment with injection of botulinum toxin type A (BoNT-A).
Methods: After initial assessment, BoNT-A injection (incobotulinumtoxinA) was proposed and accepted. The patient was injected in the V2 and V3 territories. The technique was intradermal (2.5 U/point), with each injection point separated by 10 mm-15 mm. Two subcutaneous points were injected with 5 U in close proximity to the exit sites of the terminal sensory branches of the trigeminal nerve of the targeted regions (infraorbital and mental nerves). A total of 60 U were injected.
Results: The treatment showed a latency of 7-10 days. The duration was 10 weeks, after which the patient gradually returned to the previous symptom severity and frequency. The only adverse event the patient reported was temporary mild xerostomy. Overall, the treatment resulted in a 50% decrease in pain intensity. The BPI-Facial rating decreased to 72/180 and the numeric pain scale score decreased to 4/10. After 12 weeks, the frequency of pain crises per month was reduced by 75%, to 3 per month on average. No immediate complications following the treatment (hemorrhage or increased pain) were reported.
Conclusion: IncobotulinumtoxinA therapy was successful in controlling pain in a patient with trigeminal pain refractory to standard treatment. BoNT-A appears to be a safe treatment.
Keywords: Adverse events; Botulinum Toxin; Pain; Trigeminal neuralgia

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USING OVER-THE-LABEL DOSES IN REAL-LIFE MANAGEMENT OF POST-STROKE SPASTICITY WITH BOTULINUM TOXIN TYPE A: TREATING MORE MUSCLES TO ENSURE SUCCESS

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Introduction: Poststroke spasticity is common and very disabling. This study aims to compare the number of muscles targeted and therapeutic results of different formulations of botulinum toxin type A used in real-life management of poststroke spasticity.
Methods: A sample of 216 patients (108 females) with poststroke spasticity was divided into 2 groups: on-label dose (Group 1) and over-the-label dose (Group 2). Each group was categorized by gender, age, BoNT-A formulation used (abobotulinumtoxinA [ABO]), incobotulinumtoxinA [INCO]), and onabotulinumtoxinA [ONA]), treatment outcomes (evaluated with Goal Attainment Scale [GAS]), and number of muscles injected per session. A total of 2083 treatment sessions were evaluated. On-label doses considered were: ABO 1500 U, ONA 400 U, and INCO 500 U. Treatment success was defined as a GAS score ≥ 0 per goal.
Results: Group 2 patients were treated in more upper limb muscles than Group 1 (ABO Group 1 vs Group 2: 5.17 ± 2.46 vs 6.89 ± 1.95, $P<0.001$; ONA Group 1 vs Group 2: 3.98 ± 2.62 vs 5.93 ± 2.12, $P<0.001$; INCO Group 1 vs Group 2: 4.59 ± 2.78 vs 6.83 ± 2.37, $P<0.001$), as well as in lower limb muscles (ABO Group 1 vs Group 2: 3.08 ± 2.32 vs 5.21 ± 2.15, $P<0.001$; ONA Group 1 vs Group 2: 2.14 ± 2.27 vs 4.78 ± 1.79, $P<0.001$; INCO Group 1 vs Group 2: 2.92 ± 2.55 vs 4.69 ± 1.49, $P<0.001$).
A comparison of treatment outcomes, showed no difference between groups, independent of the formulations used (ABO Group 1 vs Group 2: X^2 (1) = 0.071, P =0.79; ONA Group 1 vs Group 2: X^2 (1) = 0.259, P =0.61; INCO Group 1 vs Group 2: X^2 (1) = 0.10, P =0.92).
Conclusion: Our data suggest that higher total doses allowed clinicians to increase the number of targeted muscles per limb in selected cases. On the other hand, Group 2 showed the same treatment success rate as Group 1. Hence, if Group 2 had received lower total doses it would mean either fewer muscles treated or lower doses per muscle, with predictably lower rates of success, ie, the use of over-the-label doses was worthwhile.
Keywords: Botulinum Toxin; GAS score; Poststroke spasticity; Targeted muscles

SPASTICITY MANAGEMENT OF INDIVIDUALS WITH SPINAL CORD INJURY IN ERA OF SOCIETAL RESTRICTIONS DUE TO COVID 19 PANDEMIC

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Introduction: With the emergence of the novel coronavirus (SARS-CoV-2), the COVID-19 pandemic has swept the world and left us with serious public health concerns and the need for significant modifications in healthcare operations all around the globe. These unprecedented times have been hard for everyone with or without disability; however, we feel that there has been a serious lack of information targeted specifically to spinal cord—injured (SCI) individuals. Inaccessibility to hospitals or health care systems for their routine follow up visits and treatments and psychological distress due to quarantine measures are among the common problems that persons with SCI may face during the COVID-19 pandemic. Most of the outpatient services, including botulinum toxin type A (BoNT-A) injections for the management of spasticity, were postponed.[1] We aimed to study the spasticity management and activities of daily living of individuals who have spasticity due to SCI in this socially restricted era.
Methods: A telephone interview was conducted with individuals with SCI who had moderate and severe spasticity. Twenty-four volunteers participated in this cross-sectional study. All of the patients received ultrasound-guided BoNT-A injections at two rehabilitation centers in Istanbul, between 15th September, 2019 and 15th March, 2020. A questionnaire prepared by the authors was used and all participants were interviewed by the same author (KG) between 15th June and 15th July, 2020.
Each participant was asked about increases in spasticity during the societal restrictions of the COVID-19 pandemic and the need for a new BoNT-A injection. The spasticity severity of the previous week was rated on a numeric rating scale (NRS). Activities of daily living complicated by spasticity were addressed with open-ended questions. Accessibility to the health care system and medications was also queried.
Results: The demographic characteristics of the 24 participants are summarized in Table 1. Eighteen (75%) patients reported a moderate increase in spasticity, while 3 (12.5%) patients reported a severe increase and 3 (12.5%) reported no difference during this time period. The m ean NRS score was 6 (±2). Twenty-one (87.5%) of the patients reported a need for BoNT-A treatment because of symptom re-emergence.
In relation to spasticity-induced deterioration in activities of daily living, 10 (42%) individuals reported difficulty in walking. Ten (42%) patients reported difficulty in sitting in the wheelchair, and 11 (46%) experienced lack of sleep due to spasticity symptoms.
Thirteen (54.2%) of the patients had difficulty with keeping doctors'

appointments. All of the patients reported that they obtained their medications easily.

Conclusions: Most (87.7%) of the individuals with SCI reported a moderate or severe increase in spasticity due to the societal restrictions enforced to cope with the COVID-19 pandemic. Spasticity also affected their activities of daily living. During the pandemic, the global and local health authorities have neglected people with SCI and other disabilities to some degree.[2] People with disabilities are a vulnerable group and deserve more attention in extraordinary conditions such as pandemics, hurricanes, or earthquakes.

Table 1
Demographic and Clinical Characteristics of the Participants.

Male/Female n(%)	20(833)/4 (16.7)
Age (years) mean±SD	43.1±13.6
Time since injury (years) mean±SD	13.8±10.3
Married/Single n(%)	16 (66.7)/8(33.3)
Employment	
Not officially employed	19(79.2)
Employed	3(12.5)
Student	2(8.3)
Etiology n(%) Traumatic/Nontraumatic	17(70.8)/7(29.2)
Paraplegia/Tetraplegia n(%)	9(37.5)/15(62.5)
AIS Grade N(%)	
A	7(29.2)
B	1(4)
C	4(16.7)
D	12(50)
Ambulation n(%)	
Wheelchair dependent	12(50)
Household ambulator	4(16.7)
Community ambulator	8(33,3)
Spasticity Medication n(%)	
Baclofen oral	23(95.8)
Tizanidine	2(8.3)
Botulinum toxin type A injection	24(100)
Baclofen pump	0 (0)
Spasticity NRS mean±SD	60±20

AIS=American Spinal Injury Association Impairment Scale.

Keywords: Disability; Lockdown; Societal restrictions; Spasticity; Spinal cord injury

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A NEW APPROACH TO STUDY THE INFLUENCE OF THE COURSE OF CERVICAL DYSTONIA SEVERITY BEFORE BOTULINUM TOXIN ON LONG-TERM OUTCOME AFTER BOTULINUM TOXIN THERAPY

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Introduction and Objectives: The aim of the study was to analyse the influence of the course of severity of idiopathic cervical dystonia (CD) before botulinum toxin (BoNT) on long-term outcome of BoNT therapy.
Methods: Seventy-two CD patients who were treated on a regular basis in the botulinum toxin outpatient department of the University of Düsseldorf and had received at least 3 injections were consecutively recruited after written informed consent was obtained. While waiting for their next injection patients had to rate the amount of improvement of CD in percent of severity of CD at start of BoNT therapy (IMP). Then they had to draw the course of severity of CD (CoD graph) from onset of symptoms until the start of BoNT therapy in a 10x10cm square. The residual CD severity was estimated by the treating physician using the Tsui score. Demographic and treatment-related data were extracted from the charts of the patients.
Results: Depending on the curvature, three different types of CoD graphs could be distinguished. Time to BoNT therapy, severity of CD at start of BoNT therapy, dose used at start of therapy, increase of dose during therapy, and outcome (IMP) were significantly different ($P<0.05$) when patients were split up according to these three different graph types. Patients with a more rapid development of CD responded better although lower doses were used.
Conclusions: The course of severity of CD before BoNT therapy influences the long-term outcome. This has implications for patient management and information on the efficacy of BoNT therapy.
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Funding source: Diesbach-Stiftung.
Keywords: Botulinum toxin therapy; Cervical dystonia; Course of disease; Long-term outcome; Natural history; Secondary treatment failure

IMPROVEMENT OF SPASTICITY-RELATED PAIN WITH INCOBOTULINUMTOXINA TREATMENT IN CHILDREN/ADOLESCENTS WITH CEREBRAL PALSY: POOLED ANALYSIS OF 3 PHASE 3 STUDIES

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Introduction: Spasticity-related pain (SRP) in children and adolescents with cerebral palsy (CP) is common, often neglected, and impacts daily quality of life. We assessed the effect of incobotulinumtoxinA on SRP using pooled data from 3 large Phase 3 pediatric studies.
Methods: Ambulant and non-ambulant patients (2-17 years of age; uni- or bilateral CP; Ashworth Scale score ≥2 in clinical patterns for treatment) were enrolled. Patients received total body incobotulinumtoxinA doses of ≤16 U/kg (≤400 U) for lower-limb (LL) treatment in 2 injection cycles (ICs) in TIM (NCT01893411). In TIMO (NCT01905683), TIM completers and new recruits received 4 ICs with 16-20 U/kg (≤400-500 U) for LL or combined LL and upper-limb (UL) treatment. In XARA (NCT02002884), patients received 4 ICs with 16-20 U/kg (≤400–500 U) for UL or combined LL/UL treatment. Changes in self-reported (child/adolescent) or observed (parent/caregiver) SRP were assessed using the Questionnaire on Pain caused by Spasticity (QPS) in patients with LL (TIM, TIMO, and XARA) and UL treatment (TIMO and XARA).
Results: Assessments for 849 patients with LL and 454 patients with UL treatment were included. Of these, 340 (40.0%, LL: 61.2% male, mean [SD] age 9.3 [3.8], body weight [BW] 32.6 [14.8] kg) and 160 (35.2%, UL: (61.9% male, mean [SD] age 10.3 [3.7] years, BW 36.8 [16.5] kg) were able to assess

SRP by interviewer- or self-administered QPS. Most (81.9% LL; 69.7% UL) reported pain at baseline for ≥1 activity. SRP increased with activity demands. Complete SRP relief at Week 4 post-treatment in each IC was seen (Table) and was highest in IC4. Observed SRP frequency was consistent with self-reported SRP and was supported by respective QPS item scores (all $P<0.001$ for responder rates).

Conclusions: In this large, pooled analysis, repeated incobotulinumtoxinA injections led to sustained pain reduction in children and adolescents with spasticity, with complete pain relief in the injected limb during activities in ≤54.8% of patients.

Keywords: Cerebral palsy; IncobotulinumtoxinA; Lower-limb spasticity; Pediatric; Pain; Upper-limb spasticity

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Table
Proportion of Patients With SRP at Baseline and With Complete Pain Relief According to the Children/Adolescents' QPS.

Child/adolescent QPS self- and interviewer-reported spasticity-related pain intensity (FAS)	Overall population baseline N	Pain population[a] baseline		Patients with complete pain relief after incobotulinumtoxinA treatment, %[b]			
		n	%	Cycle 1, Week 4	Cycle 2, Week 4	Cycle 3, Week 4	Cycle 4, Week 4
Lower Limb							
QPS item 3, general pain							
Hurt when tight	330	178	53.9	35.3***	48.1***	45.7***	49.4***
QPS item 5, pain at rest							
Sitting, watching TV, or trying to sleep	324	86	26.5	43.4***	46.2***	39.5***	51.4***
QPS item 7, pain with usual activities							
Moving, walking, or playing	328	178	54.3	45.3***	45.2***	44.2***	53.3***
QPS item 9, pain with exercises							
Physical therapy or stretching exercises	330	234	70.9	32.3***	33.0***	39.4***	41.6***
QPS item 12, pain with hard task							
Self-defined hard task	330	202	61.2	28.4***	33.3***	30.7***	33.8***
Upper Limb							
QPS item 3, general pain							
Hurt when tight	152	69	45.4	39.7***	40.0***	43.1***	41.8***
QPS item 5, pain at rest							
Sitting, watching TV, or trying to sleep	148	34	23.0	35.3***	25.0*	32.3***	36.7***
QPS item 7, pain with usual activities							
Getting dressed, eating, or playing	150	52	34.7	34.0***	33.3***	45.2***	54.8***
QPS item 9, pain with exercises							
Physical therapy or stretching exercises	153	99	64.7	35.7***	41.4***	42.4***	44.3***
QPS item 12, pain with hard task							
Self-defined hard task	155	83	53.5	26.6***	43.7***	43.1***	48.4***

*$P<0.025$; **$P<0.01$; ***$P<0.001$, exact one-sample binomial test, one-sided, significance level alpha=0.025 for responder rates significantly above 10%.
FAS, full analysis set; N, overall population at baseline; n, number of non-missing observations; QPS, Questionnaire on Pain caused by Spasticity; SRP, spasticity-related pain.
[a] Baseline pain population defined as children/adolescents that reported SRP with an item score >0 (no hurt) at baseline.
[b] Percentage of children/adolescents from observed cases in the baseline pain population.

POOLED EFFICACY ANALYSIS OF INCOBOTULINUMTOXINA IN THE MULTIPATTERN TREATMENT OF UPPER- AND LOWER-LIMB SPASTICITY IN CHILDREN AND ADOLESCENTS WITH CEREBRAL PALSY

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Introduction: This pooled analysis assessed the efficacy of incobotulinumtoxinA for lower-limb (LL) and upper-limb (UL) spasticity in children and adolescents with cerebral palsy (CP) using data from the first controlled injection cycle of 2 large Phase 3 studies, TIM (NCT01893411) and XARA (NCT02002884).

Methods: Ambulant and non-ambulant pediatric patients with spasticity due to CP (2-17 years of age; uni- or bilateral CP; Ashworth Scale [AS] score ≥2 in clinical patterns for treatment) were enrolled. Patients were randomized (2:1:1) to 3 incobotulinumtoxinA dose groups: 8, 6, 2 U/kg body weight (BW), maximum 200, 150, 50 U per LL clinical pattern in TIM and per UL in XARA. Additional multipattern treatment was allowed in both studies with total body doses up to 16-20 U/kg BW (≤400-500 U) depending on study and Gross Motor Function Classification System (GMFCS) levels I-V. Changes from baseline in AS score and Global Impression of Change Scale (GICS) scores at Week 4 were assessed in patients with LL treatment (TIM and XARA) and in those with UL treatment (XARA).

Results: In total, 603 patients with LL treatment from both studies (58.9% male, mean [SD] age 6.8 [4.2] years, BW 23.6 [13.5] kg, 27.2% GMFCS IV-V) and 350 patients with UL treatment from XARA (62.9% male, mean [SD] age 7.3 [4.4] years, BW 25.0 [15.0] kg, 30.9% GMFCS IV-V) were included in this analysis. Improvements in AS score for the main LL and UL clinical patterns were seen with all incobotulinumtoxinA doses at Week 4 (all $P<0.0001$ vs baseline except adducted thigh at 8 U/kg; Table). Significantly greater improvement in AS score for the main UL clinical pattern was noted in the 8 U/kg versus the 2 U/kg dose group ($P=0.004$). Investigator's (Table), child/adolescent's and parent/caregiver's GICS scores confirmed improvement in LL and UL spasticity at Week 4.

Conclusions: IncobotulinumtoxinA provides effective multipattern treatment of LL and UL spasticity in pediatric patients with CP (GMFCS I-V).

Funding: Merz Pharmaceuticals GmbH, Frankfurt am Main, Germany

Keywords: Cerebral palsy; IncobotulinumtoxinA; Lower-limb spasticity; Multipattern; Pediatric; Upper-limb spasticity

Table. Change from baseline to Week 4 in AS score for the main clinical patterns treated in the lower and upper limbs, and in the investigator's GICS score for lower and upper limbs

	IncobotulinumtoxinA					
Total dose per limb or pattern[a]	**8 U/kg, ≤200 U**		**6 U/kg, ≤150 U**		**2 U/kg, ≤50 U**	
AS score	**n**	**LS-mean (SE) [*P* value]**	**n**	**LS-mean (SE) [*P* value]**	**n**	**LS-mean (SE) [*P* value]**
Lower limb: pes equinus	191	−0.78 (0.058) [*P*<0.0001]	190	−0.85 (0.055) [*P*<0.0001]	147	−0.74 (0.059) [*P*<0.0001]
Upper limb: flexed elbow/wrist	144	−1.19 (0.060) [*P*<0.0001]	114	−1.01 (0.071) [*P*<0.0001]	85	−0.92 (0.077) [*P*<0.0001]
Investigator's GICS score	**n**	**LS-mean (SE) [*P* value]**	**n**	**LS-mean (SE) [*P* value]**	**n**	**LS-mean (SE) [*P* value]**
Lower limb	166	1.50 (0.077) [*P*<0.0001]	260	1.45 (0.051) [*P*<0.0001]	177	1.35 (0.060) [*P*<0.0001]
Upper limb	192	1.66 (0.059) [*P*<0.0001]	86	1.39 (0.095) [*P*<0.0001]	72	1.55 (0.091) [*P*<0.0001]

[a]Doses noted are per upper-limb (XARA) or per lower-limb clinical pattern (TIM).

P values are based on change from baseline analyzed by ANCOVA.

ANCOVA, analysis of covariance; AS, Ashworth scale; GICS, Global Impression of Change Scale; LS, least squares; n, number of non-missing observations; SE, standard error.

REMOTE CENTRAL EFFECTS OF BOTULINUM TOXIN TYPE A AS ADJUVANT TO INTENSE OCCUPATIONAL THERAPY IN THE EARLY STAGE OF STROKE: A TYPE II FMRI RANDOMISED CONTROLLED TRIAL

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Introduction: Improvements in motor function following interventions incorporating botulinum toxin type A (BTX-A) remain controversial, with existing studies yielding contrasting results.[1-3] The mechanisms underlying BTX-A remote central effects are still under investigation. It is hypothesized that the toxin administration strategy may play a role in producing such differing outcomes. We tested a strategy based on modulating muscle synergies.

Aim: The aim of the study was to investigate the clinical and remote central effects of an occupational therapy intervention combined with adjunctive BTX-A compared to the same occupational therapy without the adjuvant application of the toxin.

Methods: A two-group, parallel, pre-post, randomized controlled trial was performed. The clinical effects of occupational therapy when performed following BTX-A injections to disinhibit finger flexors (n=5) was compared to those of an equal dose of occupational therapy alone (n=6). Motor dexterity and function were assessed using the Fugl-Meyer Scale, Motor Index, Arm Activity Measure, 9-Hole Peg Test, and Box and Block Test, and differences were analysed using ANCOVA. Brain activity was examined using functional magnetic resonance imaging (fMRI), and between-group differences were analysed using contrast statistical parametric mapping.

Results: Both groups started in statistically similar conditions. Both treatments provided significant clinical improvements compared to baseline. The total differences in change score on the Fugl-Meyer Scale and Motor Index were larger, though not significantly, in the toxin-treated group than in the control group (Figure). When the toxin is administered, activity in the brain is more localised and appears more in the right hemisphere in subjects in the toxin-treated group and more in the left in those in the control group.

Conclusions: Functional improvements were observed in the toxin-treated group, but the effect size compared to the control group was too small to provide definitive results.

Post-treatment contrast analysis of the fMRI scans suggests that the toxin reduces cortical and cerebellar overexcitation. Findings are limited by the small cohort size.

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Keywords: Neurorehabilitation; Botulinum toxin; fMRI; Stroke

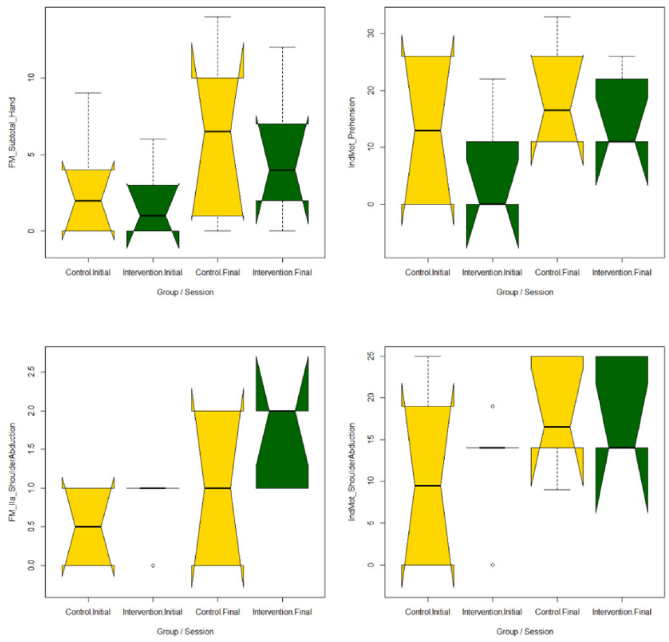

Fig. Functional changes for hand and shoulder abduction which have been determined to be particularly relevant for favorable prognosis. Top, left. Fugl-Meyer subscale for the hand. Top, right. Motor index prehension subscale. Bottom, left. Fugl-Meyer item for shoulder abduction. Bottom, right. Motor index shoulder abduction subscale.

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ASSESSMENT OF THE EFFECTS OF ABOBOTULINUMTOXINA INJECTIONS IN PATIENTS WITH CHRONIC MIGRAINE: A PILOT STUDY

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Introduction: The treatment of patients with chronic migraine (CM) using local injections of botulinum toxin type A into the head and neck area according to the international standard protocol started in Belarus in 2018. AbobotulinumtoxinA (AboBoNT-A) is the only botulinum neurotoxin agent with regulatory approval in Belarus, but it is not indicated for treatment of

CM in its instructions for use.
Methods: As use of AboBoNT-A injections for treatment of CM is off label, a discussion about possible positive results and side effects was conducted between the investigator and patients, and informed consent was obtained from patients before the administration of injections.
Eighteen patients (15 females, 3 males; median age 37 (range: 31-46) years) received AboBoNT-A injections at an initial dose of 300 units. Repeat injections were performed in 8 patients. Patients independently kept headache diaries and recorded the intensity of the headaches using a visual analogue scale (VAS). They also filled out Headache Impact Test (HIT-6) questionnaires, assessing headache-related disability, and Headache Attributed Lost Time (HALT) questionnaires, assessing productive time lost due to headache. Statistical analysis of the results was carried out using the STATISTICA 8.0 software program.
Results: Patients reported 18 (15-21) headache days on average before injection according to their headache diaries and a headache intensity of 7 (5-9) according to the VAS. A significant decrease in the number of headache days within a month to 10 (7-12; Wilcoxon test, T=0.00; Z=2.520; P=0.012) and a decrease in headache intensity to 4 (2-6; Wilcoxon test, T=0.00; Z=2.201; P=0.028) were obtained as a result of treatment. The average score on the HIT-6 scale preinjection was 65 (63-65), which corresponds to severe impact. The HIT-6 score decreased to 52 (50-57; Wilcoxon test, T=0.00; Z=2.665; P=0.008) at 1 month post-injection, which corresponds to moderate impact. The HALT score before the injection was 26.9 (severe impact); it decreased to 12.9 (moderate impact; P<0.05) at 1 month postinjection.
Conclusion: These study findings indicate the need for further research.
Keywords: AbobotulinumtoxinA; Chronic migraine; HALT; HIT-6; VAS

PATIENT PERCEPTIONS OF SPASTICITY AND TREATMENT SATISFACTION OVER THE COURSE OF A BOTULINUM NEUROTOXIN TYPE A (BONT-A) TREATMENT CYCLE: AN ETHNOGRAPHIC STUDY OF STROKE SURVIVORS

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Introduction: While BoNT-A is an acknowledged treatment for spasticity, many patients experience recurrence of symptoms before their next injection. However, there is a paucity of data on patients' and caregivers' perspectives on the "rollercoaster" of symptoms and its impact on their quality of life. In this ethnographic study we investigated changes in symptom burden and their effects on patient functioning and quality of life throughout the duration of a BoNT-A treatment cycle.
Methods: The REBOT study (NCT03995524) was a prospective observational study conducted in 30 eligible stroke survivors who were ambulatory and receiving regular BoNT-A treatment (≥2 prior injection cycles). Informal caregivers of post-stroke patients with spasticity were also followed. The study comprised 3 stages: (1) In-depth qualitative interviews; (2) a 16-week ethnography observation period using a dedicated smartphone application for collation of questionnaire data (3); and an additional qualitative in-depth interview to assess satisfaction with treatment course.
Results: A total of 30 patients (21 females, 9 males; mean age 48.7 years) with varying severity and patterns of impairment were followed over the course of a BoNT-A cycle. Patients rated stiffness as their worst symptom/impairment, and this clearly improved with treatment and then started to return to near baseline levels at around 9-11 weeks post-injection. By contrast, responders reported little impact of treatment on aspects such as enjoyment of activities. Qualitative analysis of patients' answers indicated that they were generally satisfied with BoNT-A treatment but wished for better symptom coverage over their treatment cycle.
Conclusions: A recent patient survey (Jacinto J, et al, 2020) suggested a "rollercoaster" of symptomatology and functional impact within a single injection cycle. These ethnographic data provide additional novel insights into the impact of these fluctuations on patients' lives.
Keywords: Botulinum toxin; Ethnography; Quality of life; Symptom re-emergence; Spasticity

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REAL-LIFE DATA ON THE TIME TO RETREATMENT WITH BOTULINUM TOXIN TYPE A IN UPPER LIMB SPASTICITY MANAGEMENT

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Introduction: The Upper Limb International Spasticity (ULIS)-III study aims to describe the real-life management of upper-limb spasticity using an integrated approach, including repeat botulinum toxin type A (BoNT-A) injection cycles. One way to understand the duration of BoNT-A benefit is to evaluate the time to reinjection, which is often tailored to patient needs.
Methods: ULIS-III (NCT02454803) was an international, observational study that examined longitudinal outcomes in adult (≥18 years old) patients with upper-limb spasticity.
A multivariate additive linear regression model was built to evaluate treatment intervals adjusting on multiple baseline and treatment covariates and depending on significance. Potential factors included toxin type, age, sex, previously treated for upper-limb spasticity with BoNT, time from event onset to injection Cycle 1, spasticity distribution, dominance of affected limb, indication of lower limb spasticity, duration of BoNT-A therapy prior to study, use of systemic antispastic medications, guidance technique, indexed dose, any physiotherapy during the study, and number of therapy visits.
Results: Of the 1004 enrolled patients, 832 remained on the same BoNT-A product throughout the study and had their injection intervals assessed. Across the study, the mean ±SD injection interval (all BoNT-A products) was 213.1 ± 164.7 days; treatment intervals with abobotulinumtoxinA (222.8 ± 167.2 days, n=555) were numerically longer than with onabotulinumtoxinA (204.4 ±173.6 days, n=198) and incobotulinumtoxinA (166.9 ±105.1 days, n=79). After conducting multivariate linear regression analyses, statistically significant differences in the overall injection intervals between abobotulinumtoxinA and incobotulinumtoxinA (difference of 36.4 days, P=0.002) and abobotulinumtoxinA and onabotulinumtoxinA (difference of 18.1 days, P=0.03) remained.
Conclusions: These real-world injection data from a large, observational study support the clinical experience that there are clinical differences between BoNT-A products that should be taken into account when designing patient treatment plans.
Keywords: AbobotulinumtoxinA; OnabotulinumtoxinA; IncobotulinumtoxinA; Interval; Spasticity.

A PHASE 3 TRIAL EVALUATING THE EFFICACY, DURATION OF EFFECT, AND SAFETY OF DAXIBOTULINUMTOXINA FOR INJECTION IN THE TREATMENT OF CERVICAL DYSTONIA

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Introduction: DaxibotulinumtoxinA for Injection (DAXI) is a novel botulinum toxin type A product formulated with a proprietary excipient peptide. A Phase 2 study demonstrated promising efficacy and duration of effect of DAXI for treatment of cervical dystonia. A large, multicenter, phase 3 double-blind, placebo-controlled trial was subsequently conducted to evaluate efficacy and safety of two doses of DAXI compared to placebo for cervical dystonia.
Methods: Adults with moderate-to-severe cervical dystonia were randomized 1:3:3 to placebo, DAXI at 125 Units (125 U), or DAXI at 250 Units (250 U), and followed for up to 36 weeks after a single treatment. Subjects were evaluated using the Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS). The primary outcome measure was average change from baseline in TWSTRS total score at Weeks 4 and 6. Safety was evaluated at all study visits.
Results: Three hundred one subjects were randomized to placebo (n=46), DAXI 125 U (n=125), or DAXI 250 U (n=130). Demographics were similar across cohorts. Mean baseline TWSTRS total score was 43.3. Mean improvement from baseline in TWSTRS total score at the primary timepoint was 4.3±1.82 for placebo, 12.7±1.30 for DAXI 125 U ($P<0.0001$ vs placebo), and 10.9±1.25 for DAXI 250 U ($P=0.0006$ vs placebo). There was no statistical difference in TWSTRS total score reduction between DAXI dose groups. Median duration of effect was 24.0 and 20.3 weeks for DAXI 125 U and DAXI 250 U, as determined by time to loss of 80% peak treatment benefit. The most commonly reported treatment-related adverse events were injection site pain, headache, injection site erythema, muscular weakness, and musculoskeletal pain. Dysphagia was reported in 1.6% and 3.9% of subjects treated with DAXI 125 U and DAXI 250 U, respectively.
Conclusions: Treatment with DAXI 125 U and 250 U was demonstrated to be safe and efficacious with meaningful reduction in signs/symptoms associated with cervical dystonia and median duration of effect of 20.3-24.0 weeks.
Keywords: Botulinum toxin; Cervical dystonia; Clinical trial; Efficacy

NEUTRALIZING ANTIBODY CONVERSION WITH ONABOTULINUMTOXINA FROM GLOBAL STUDIES ACROSS MULTIPLE INDICATIONS IN NEARLY 30,000 PATIENT RECORDS: A META-ANALYSIS

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Introduction and Objectives: Neutralizing antibodies (NAbs) may reduce the effectiveness of onabotulinumtoxinA treatment. An extensive clinical trial database across 10 indications in different patient populations has been accumulated for onabotulinumtoxinA over the past 3 decades. A meta-analysis of these data was performed to assess the incidence of NAb formation as a function of subject gender, indication (dose route and location), dose level, dosing interval, and number of treatment cycles.
Methods: This analysis was based on placebo-controlled or prospective, open-label trials across 10 therapeutic and aesthetic indications with immunogenicity assessment in 6118 patients treated up to 15 cycles. Total onabotulinumtoxinA doses per treatment ranged between 10 U (glabellar lines) and 600 U (post-stroke spasticity). Detection of NAb at baseline and post-treatment was by mouse protection assay, either as a single step or following positive binding Ab assay results.
Results: Frequency of subjects who had positive NAb results (with either a NAb negative result or unknown status at baseline) at any post-treatment time point ranged from 0% (crow's feet lines, migraine, and pediatric neurogenic overactive bladder) to 1.4% (neurogenic overactive bladder). Overall, across all 10 indications, 27/5846 subjects (0.5%) fell into this category. By the time of final assessment at study exit, only 17/5846 subjects (0.3%) out of the 27 subjects still had positive NAb results. Due to the low incidence and lack of consistent pattern, no clear correlation was observed between positive NAb results and onabotulinumtoxinA dose level, dosing interval, subject gender, indication (dose route and location), or number of treatment cycles.
Conclusions: This comprehensive and robust meta-analysis confirms the low frequency of NAb formation following onabotulinumtoxinA treatments across multiple indications.
Funding: AbbVie
Keywords: Aesthetics; Migraine disorders; Muscle spasticity; Neutralizing antibodies; Overactive; Stroke; Urinary bladder

BONT-As FOR ADULT SPASTICITY AND CERVICAL DYSTONIA: COST-EFFECTIVENESS ANALYSIS AND THE COST OF RESPONSE IN THE UNITED KINGDOM

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Introduction: For adults with spasticity or cervical dystonia (CD), treatment with botulinum neurotoxin type A (BoNT-A) has been shown to improve achievement of treatment goals. Considering the costs of available BoNT-A therapies could increase the efficiency of healthcare spending. The objective of this analysis was to evaluate the average expenditures per response obtained with abobotulinumtoxinA (aboBoNT-A) and onabotulinumtoxinA (onaBoNT-A) for spasticity and CD.
Methods: Cost-effectiveness analyses were conducted to compare aboBoNT-A and onaBoNT-A for treatment of upper limb spasticity (ULS), lower limb spasticity (LLS), and CD, in the United Kingdom. Effectiveness was defined as response to therapy. Response rates and dosing were based on observational studies for ULS and CD. For LLS, real-world observational data were not available, so response rates were based on systematic literature reviews with meta-analysis of clinical trials and dosing based on UK product labels. BoNT-A treatment intervals were based on real-world data when available (ULS), or otherwise were assumed to follow similar patterns to those observed in clinical trials (LLS and CD). Resource use for responders and non-responders was based on a UK physician survey. Unit costs were informed by UK fee schedules.
Results: Compared with onaBoNT-A, aboBoNT-A resulted in lower annual costs per patient for the management of ULS, LLS, and CD (savings of £302, £584, and £422, respectively). Results were driven by differences in injection intervals and a higher treatment response rate for people receiving aboBoNT-A compared with onaBoNT-A. Total cost per responder was lower for patients receiving aboBoNT-A compared with onaBoNT-A (ULS: £43,266 vs £55,416; LLS: £98,962 vs £209,376; CD: £13,883 vs £21,817). Results were robust to sensitivity analyses.
Conclusion: With high response rates and low costs, aboBoNT-A may be an efficient choice for treating adult spasticity and cervical dystonia in the UK.
Funding: Ipsen.
Keywords: Botulinum neurotoxin type A; Cost-effectiveness analysis; Quality of life; Spasticity costs

USE OF BOTULINUM NEUROTOXIN IN POST-MASTECTOMY PAIN SYNDROME

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Introduction and Objectives: Botulinum neurotoxin (BoNT) is a therapeutic option to treat post-mastectomy pain syndrome (PMPS), a common complication after mastectomy.
Aim: To evaluate the improvement of pain, mobility, state of mind, quality of life, and goal achievement after BoNT injection of pectoralis major in patients with PMPS.
Methods: A quasi-experimental study was performed. Thirty-seven patients participated from July 2014 to December 2017. Variables analyzed were: age, sex, presence of lymphedema, pain intensity (assessed using a Visual Analogue Scale), type of pain (on the DN4 Questionnaire), mobility limitation, physical activity, anxiety and depression (assessed using the Goldberg scale), quality of life (assessed using the 12-Item Short Form Health [SF-12] Survey), Patient Global Impression of Improvement (PGI-I) scores, and outcomes on the Goal Attainment Scale (GAS). All questionnaires were completed prior to BoNT therapy and at 1-month follow up.
Results: Pain improvement was significant in all patients (P=0.00). Neuropathic pain was noted in 30.5% of patients, nociceptive and mixed pain types in the remainder. Patients with neuropathic pain reported a greater decrease in pain intensity (difference in VAS score: -4,00). The median duration of pain was 24 months. The decrease in pain was greater for the group of patients with ≥24 months' pain (p50 VAS pretreatment: 8; p50 VAS posttreatment: 3.5), but the improvement in pain was significant for both the group of patients with ≥24 months' pain ((P=0.00), and those with ≤24 month's pain (P=0.001). Patients with lymphedema had a greater decrease in pain (p50 VAS pretreatment: 7; p50 VAS posttreatment: 3.5; P=0.003), but there was significant improvement in pain for both those with and without lymphedema (P=0.002). Similarly, there was significant improvement in pain for the group of patients that engaged in sports activity and those that did not (P=0.0049 and P=0.002, respectively).
The range of motion of the shoulder improved by 10º in flexion (P=0.00) and 17.5º in abduction (P=0.00). Comparison of Goldberg scale results before and after BoNT injection showed that 37.5% of patients no longer manifested anxiety (P=0.003), and 26.09% were no longer experiencing depression (P=0.00).
The SF-12 showed statistically significant results in physical and mental components before and after BoNT therapy: 37.94±7.11 vs 41.41±7.38 (P= 0.017) and 41.68±10.844 vs 45.41±8.76 (P= 0.038). The SF-12 also showed statistically significant results in the domains of physical activity, limitations in usual role due to physical problems, bodily pain, vitality, social functioning, limitations in usual role due to emotional problems, and mental health (Table).

Table. Results of the SF-12 Survey Before and After BoNT Therapy.

Domain	Before BoNT injection	After BoNT injection	*P* value
Physical Activity	35.80±7.11	40.45±10.31	0.007
Limitations in usual role due to physical problems	36.36±8.48	40.76±8.24	0.008
Bodily pain	38.92±7.73	42.62±7.68	0.047
General Health	43.54±7.22	45.09±7.98	0.240
Vitality	40.38±9.94	43.14±9.56	0.039
Social functioning	38.87±11.00	43.75±10.67	0.040
Limitations in usual role due to emotional problems	38.17±11.26	41.92±10.29	0.048
Mental health	41.41±8.35	45.77±7.66	0.008
Physical	37.94±6.59	41.41±7.38	0.017
Mental	41.68±10.84	45.41±8.76	0.038

The PGI-I responses showed improvement in 93.93% of patients post-therapy: 51.51% of patients reported feeling a little better, 36.36% much better, 6.06% very much better, and 6.06% no change after treatment. On the GAS scale regarding pain improvement, 51.35% of patients reached the expected level (GAS T-score: 50), 29.72% scored more than expected (GAS T-score:>60), and 18.91% less than expected (GAS T-score:40).
Conclusions: The treatment of post-mastectomy pain syndrome with botulinum neurotoxin is effective in cases involving contracture of the pectoralis major muscle and pain.
Keywords: Botulinum neurotoxin; Post-mastectomy pain syndrome; Quality of life

POOLED EFFICACY AND SAFETY ANALYSIS OF INCOBOTULINUMTOXINA IN THE TREATMENT OF UPPER- AND LOWER-LIMB SPASTICITY IN CHILDREN WITH SEVERE CEREBRAL PALSY (GMFCS LEVELS IV AND V)

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Introduction: This pooled analysis assessed the efficacy and safety of incobotulinumtoxinA for lower-limb (LL) and upper-limb (UL) spasticity in children and adolescents with cerebral palsy (CP) and Gross Motor Function Classification System (GMFCS) level IV or V using data from the first injection cycle in 2 randomized Phase 3 studies, TIM (NCT01893411) and XARA (NCT02002884).
Methods: Non-ambulant patients (pts; aged 2-17 years; uni- or bilateral CP; Ashworth Scale [AS] score ≥2 in clinical patterns for treatment) with GMFCS level IV or V were analyzed. Pts were randomized to 3 incobotulinumtoxinA dose groups: 8, 6, 2 U/kg body weight, maximum 200, 150, 50 U, per LL clinical pattern in TIM, and per UL in XARA. Additional multipattern treatment was allowed in both studies with total body doses up to 16 U/kg (≤400 U). Changes from baseline in AS score and Global Impression of Change Scale (GICS) scores at Week 4 were assessed in pts who had LL treatment (TIM and XARA) and pts who had UL treatment (XARA). Adverse events (AEs) were assessed.
Results: Of pts with GMFCS level IV or V, 164 had LL treatment and 108 had UL treatment. Statistically significant improvements in AS score for the pes equinus and flexed elbow/wrist and investigator's GICS for the LL and UL were seen with all incobotulinumtoxinA doses at Week 4 (P<0.0001 vs baseline, Table). AS and GICS improvements were numerically greatest in the high-dose group. Efficacy was largely similar in patients with GMFCS level I-III. AE frequency was generally <30.0% across dose groups and GMFCS levels I-V. The most common AEs for pts with GMFCS level IV or V were nasopharyngitis (7.0%) and pharyngitis (3.2%). Few treatment-related AEs (n=1), serious AEs (n=5), or AEs of special interest (n=2) occurred in pts with GMFCS level IV or V.
Conclusions: In children with severe CP (GMFCS level IV or V), incobotulinumtoxinA is effective, safe, and well-tolerated for multipattern/multilevel treatment of LL and UL spasticity.
Funding: Merz Pharmaceuticals GmbH, Frankfurt am Main, Germany
Keywords: GMFCS levels IV-V; Cerebral palsy; IncobotulinumtoxinA; Spasticity; Pediatric; Safety

Table. Change from baseline in AS score and investigator's GICS for the main clinical patterns treated in the lower and upper limbs of patients with GMFCS level IV or V

	IncobotulinumtoxinA					
Total dose per limb or pattern[a]	**8 U/kg, ≤200 U**		**6 U/kg, ≤150 U**		**2 U/kg, ≤50 U**	
Lower limb	n	LS-mean (SE) [*P* value]	n	LS-mean (SE) [*P* value]	n	LS-mean (SE) [*P* value]
AS for plantar flexors (pes equinus)	39	−0.81 (0.157) [*P*<0.0001]	43	−0.74 (0.123) [*P*<0.0001]	38	−0.63 (0.127) [*P*<0.0001]
LL GICS	39	1.59 (0.169) [*P*<0.0001]	75	1.30 (0.121) [*P*<0.0001]	50	1.34 (0.129) [*P*<0.0001]
Upper limb	n	LS-mean (SE) [*P* value]	n	LS-mean (SE) [*P* value]	n	LS-mean (SE) [*P* value]
AS for elbow/ wrist flexors	42	−1.22 (0.118) [*P*<0.0001]	27	−0.98 (0.134) [*P*<0.0001]	36	−0.91 (0.125) [*P*<0.0001]
UL GICS	57	1.66 (0.132) [*P*<0.0001]	20	1.49 (0.198) [*P*<0.0001]	31	1.33 (0.159) [*P*<0.0001]

[a]Doses noted are per upper-limb (XARA), or per lower-limb clinical pattern (TIM).

Improvements are indicated by decreases in AS and increases in GICS.

P values designate statistically significant changes relative to baseline at Week 4. There were no statistically significant differences between doses ($P>0.05$).

AS, Ashworth scale; GICS, Global Impression of Change Scale; GMFCS, Gross Motor Function Classification System; LL, lower limb; LS, least squares; n, number of non-missing observations; SE, standard error; UL, upper limb.

DURATION OF TREATMENT EFFECT USING INCOBOTULINUMTOXINA FOR UPPER-LIMB SPASTICITY: A POST HOC ANALYSIS

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The efficacy and safety of incobotulinumtoxinA ≤400 U was demonstrated in subjects with post-stroke upper-limb spasticity in a randomized, double-blind Phase 3 study with an open-label extension (OLEX; EudraCT number 2005-003951-11, NCT00432666). We report a post hoc analysis of the duration of the treatment effect. Subjects completing the placebo-controlled main period (single injection cycle with 12−20-week observation) entered the OLEX and received a maximum of five further treatments (maximum duration 69 weeks) with incobotulinumtoxinA ≤400 U at flexible intervals with a minimum duration of 12 weeks, based on clinical need. Intervals between two consecutive incobotulinumtoxinA injections, excluding treatment intervals prior to the end-of-study visit, were evaluated. Of 437 incobotulinumtoxinA treatment intervals, 415 received by 136 subjects were included in the post hoc analysis. More than half (52.3%; 217/415) of all incobotulinumtoxinA reinjections were administered at Week ≥14, 31.1% (129/415) at Week ≥16, 19.0% (79/415) at Week ≥18, and 11.6% (48/415) at Week ≥20. The duration of effect may vary and can exceed 20 weeks or more, which was observed in at least one injection cycle in 29.4% (40/136) subjects over the course of their treatment. Data show that incobotulinumtoxinA retreatment for upper-limb spasticity may not be required at fixed 12-week intervals and provides evidence for flexible treatment intervals beyond this time frame.

Keywords: Duration of effect; IncobotulinumtoxinA; Post-stroke; Treatment interval; Upper-limb spasticity

SUSTAINED EFFICACY OF INCOBOTULINUMTOXINA IN UPPER-LIMB POST-STROKE SPASTICITY: A POST HOC ANALYSIS

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Introduction: This post hoc analysis assessed the impact of repeated incobotulinumtoxinA injections on muscle tone, disability, and caregiver burden in adults with upper-limb post-stroke spasticity.
Methods: Data from the double-blind, placebo-controlled main period and three open-label extension cycles of two Phase 3, randomized, multicentre trials were pooled. Subjects received incobotulinumtoxinA 400 Units at 12-week intervals (±3 days) (study 3001, NCT01392300) or ≤400 Units at ≥12-week intervals based on clinical need (study 0410, NCT00432666). Ashworth Scale (AS) arm sumscore (sum of elbow, wrist, finger and thumb flexor, and forearm pronator AS scores), Disability Assessment Scale (DAS), and Carer Burden Scale (CBS) scores were assessed.
Results: Among 465 subjects, from study baseline to 4 weeks post-injection, mean (standard deviation) AS arm sumscore improved continuously: main period, −3.23 (2.55) (placebo, −1.49 [2.09]); extension cycles 1, 2, and 3, −4.38 (2.85), −4.87 (3.05), and −5.03 (3.02), respectively. DAS principal target domain responder rate increased from 47.4% in the main period (placebo 27.2%) to 66.6% in extension cycle 3. Significant improvements in CBS scores 4 weeks post-injection accompanied improved functional disability in all cycles.
Conclusions: IncobotulinumtoxinA conferred sustained improvements in muscle tone, disability, and caregiver burden in subjects with upper-limb post-stroke spasticity.
Keywords: Botulinum neurotoxin; Caregiver burden; Duration of effect; IncobotulinumtoxinA; Rehabilitation; Spasticity; Upper limb

DECREASED THERAPEUTIC EFFECT OVER TIME AMONG BOTULINUM TOXIN AGENTS

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Introduction: Sufficient long-term data comparing botulinum neurotoxin type A (BoNT-A) agents for antibody formation, resistance, and loss of effect is lacking. IncobotulinumtoxinA is a pharmaceutical BoNT-A agent that contains only the 150 kDa active component. Here, we utilize the US Food and Drug Administration (FDA) Adverse Event Reporting System (FAERS) database to explore decreased therapeutic effect over time amongst BoNT-A agents.
Methods: The FAERS database was utilized. The analysis was conducted on data between March 2014 and June 2019. BoNT-A cases were included when it was considered the "Primary Suspect" drug. The primary outcome was relative rate of decreased therapeutic effect over time by drug, defined as presence of "therapeutic response decreased" and/or "drug effect decreased" being reported as an adverse event. This relative rate methodology has been well described previously in the pharmacovigilance literature.
Results: A total of 23,789 unique BoNT-A cases was included for analysis across a wide array of cosmetic and therapeutic indications. The relative incidence of decreased therapeutic effect for patients on ≥ 1 year of

treatment versus < 1 year was significantly higher for onabotulinumtoxinA [14.4% (609/4219) vs 7.6% (753/9909); $P>0.001$] and abobotulinumtoxinA [9.9% (59/597) vs 3.3% (38/1144); $P<0.001$]. This phenomenon was not observed with incobotulinumtoxinA [0.0% (0/34) vs 3.7% (18/485); $P=0.62$]. Rates remained steady for treatments beyond 1 year and did not continue to increase (ie, 2+ years, etc.), potentially negating the effect of time on market for the present analysis.
Conclusion: A safety signal was detected in this analysis of one of the largest BoNT-A safety data sets analyzed to date. Long-term differences in decreased BoNT-A therapeutic effect by agent warrants further study, including whether even lower dose indications are not immune from this phenomenon over time. Important limitations include that causal relationships cannot be established from pharmacovigilance analyses and that incobotulinumtoxinA had the lowest sample size relative to other agents.

BOTULINUM TOXIN OVERDOSES AND ASSOCIATION WITH MEDICATION ERRORS

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Introduction: The aim of this analysis was to update a previous publication that assessed the respective botulinum neurotoxin type A (BoNT-A) agents for associations with overdose, as well as explore any association with medication errors. The previous study showed a 73-fold higher relative odds ratio for overdose with abobotulinumtoxinA versus other BoNT-A agents; this analysis includes two additional years of data that have been published since the original analysis.
Methods: The Food and Drug Administration Adverse Event Reporting System (FAERS) was utilized. The analysis was conducted of data submitted between March 2014 and June 2019. Cases were included in the analysis when BoNT-A agents were considered the "Primary Suspect" drugs. Overdose was defined as incidence of "Overdose" being reported as an adverse event. The primary outcome was incidence of "Overdose" compared within the respective agents. Rates of overdose having a concomitant medication error were also reported between agents. Medication error was defined as having a concomitant report of "Product preparation error" and/or "Wrong technique in product usage process".
Results: A total of 6,432,493 unique adverse events were reported during the study period for all drugs in the FAERS database. Of these, 23,789 were BoNT-A cases. The rate of adverse events involving overdose for abobotulinumtoxinA (14.2%; 342/2,415) was significantly higher than for both onabotulinumtoxinA (0.4%; 78/20,113; $P<0.0001$) and incobotulinumtoxinA (<0.1%; 1/1,261; $P<0.0001$). Additionally, a large percentage of abobotulinumtoxinA overdoses (37.1%; 127/342) had concomitant reports of medication errors. This phenomenon was not seen with onabotulinumtoxinA (1.3%; 1/78) or incobotulinumtoxinA (0.0%; 0/1) overdoses.
Conclusion: The analysis validates earlier findings that showed abobotulinumtoxinA adverse events were significantly associated with overdose versus other BoNT-A agents. Furthermore, abobotulinumtoxinA overdoses were associated with medication errors. The FAERS database cannot establish causal relationships; however, the analysis did identify safety signals that future studies should venture to confirm.
Keywords: Botulinum toxin; Overdose; IncobotulinumtoxinA; AbobotulinumtoxinA; OnabotulinumtoxinA

DAXIBOTULINUMTOXINA FOR INJECTION FOR LATERAL CANTHAL LINES: 4-WEEK INTERIM ANALYSIS OF A PHASE 2A STUDY

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Introduction: DaxibotulinumtoxinA for Injection (DAXI), a novel formulation of botulinum toxin type A in development, has shown an extended duration of efficacy in glabellar lines. This study evaluated the efficacy and safety of DAXI for treatment of lateral canthal lines (LCL).
Methods: This prospective, multicenter, open-label, dose-escalation study (NCT03911102) evaluated a total of 12 U, 24 U, 36 U, or 48 U DAXI for treatment of dynamic LCL. Subjects received 3 injections bilaterally in the lateral orbicularis oculi and were observed for up to 36 weeks following treatment. The primary outcome was proportion of subjects achieving none or mild on the Investigator Global Assessment Lateral Canthal Wrinkle Severity (IGA-LCWS) scale at maximum smile 4 weeks after DAXI treatment. Herein we present interim 4-week efficacy and safety data.
Results: Sixty-three subjects were enrolled. Demographics were broadly similar between cohorts. In total, 87.3% of subjects were female. Subjects' LCL severity at max smile in the 12 U, 24 U, and 36 U cohorts was predominantly severe at baseline (60.0%, 70.6%, and 66.7%, respectively), whereas 25.0% in the 48 U cohort were severe at baseline. The proportion of subjects achieving none or mild on the IGA-LCWS scale at 4 weeks after DAXI treatment was 60.0% (12 U, n=15), 58.8%, (24 U, n=17), 57.1% (36 U, n=15), and 87.5% (48 U, n=16). The proportion of subjects reporting a $\geq$2-point change on the Global Aesthetic Improvement Scale at week 4 after DAXI treatment was 66.7%, 58.8%, 50.0%, and 81.3%, in the 12 U, 24 U, 36 U, and 48 U cohorts, respectively. In the 4 weeks following treatment, 20.6% of subjects reported an adverse event (AE) and 14.3% reported a treatment-related AE. There were no unexpected AEs and no dose-response relationship was observed for any of the AEs. One serious AE was reported (deemed unrelated to treatment).
Conclusions: At 4 weeks following treatment, DAXI substantially improved the appearance of LCL. DAXI was well tolerated at all studied doses.
Keywords: Aesthetics; Botulinum toxin; Clinical trial; Efficacy; Injectable

EFFICACY AND SAFETY OF AN ALTERNATIVE ONABOTULINUMTOXINA INJECTION PARADIGM FOR OVERACTIVE BLADDER: FINAL DOUBLE-BLIND AND OPEN-LABEL RESULTS IN FEMALE PATIENTS

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Introduction: In patients (pts) with overactive bladder (OAB), onabotulinumtoxinA (onabotA) 100 U, given as 20 evenly spaced intradetrusor injections avoiding the trigone, reduced urinary incontinence (UI) and improved quality of life (QoL). Clean intermittent catheterization (CIC) use by females was 5.2%. This study (NCT03052764) assessed if an alternative paradigm could reduce CIC. We report final double-blind (DB; treatment [T_x]1) and open-label (OL; T_x2) results in females.
Methods: Pts with OAB and UI inadequately managed with an anticholinergic were randomized 2:1 to 2 trigonal + 8 peri-trigonal injections of onabotA 100 U or placebo (pbo). Eligible pts received OL onabotA 100U.
Results: One hundred fifteen women received T_x1 (onabotA n=75; pbo n=40). Ninety received T_x2 (onabotA/onabotA n=57; pbo/onabotA n=33). At week (wk) 12 following T_x1, the reduction from baseline (BL) in UI episodes/day was: onabotA −3.07 vs pbo −0.14; $P<.0001$. At wk 12 following T_x2, the mean change from DB BL was: onabotA/onabotA −4.18 vs pbo/onabotA −3.36. Pts with $\geq$75% reduction in UI episodes after T_x1 was: onabotA 38.6% vs pbo 8.1%. Corresponding OL proportions were: onabotA/onabotA 57.7% vs pbo/onabotA 41.9%. The change in incontinence

(I)QoL total score after T_x1 was: onabotA 18.3 vs pbo −6.3; *P*=.0009. In the entire OL population, (1 additional male, onabotA/onabotA), the change from DB BL in IQoL total score was: onabotA/onabotA 31.8 vs pbo/onabotA 27.7. No females used CIC in first 12 wks after T_x1. In the first 12 wks after T_x2 CIC was used by 3 females (3.3%). Urinary tract infection (UTI) rates for onabotA vs pbo after T_x1 were: overall, 31.5 vs 17.9%; symptomatic, 17.8 vs 7.7%. After T_x2, incidences were: overall 20.5%; symptomatic 8.9%.
Conclusions: The efficacy of the alternative onabotA injection paradigm was similar to the standard paradigm; CIC use was lower (0 vs 5.2%), UTI rates were higher (31.5 vs 20.9%). Efficacy and safety after T_x2 was similar to T_x1; the QoL improvement was maintained.
Funding: Allergan, an AbbVie company
Keywords: OnabotulinumtoxinA; Overactive bladder; Trigone; Urinary incontinence

INCOBOTULINUMTOXINA DEMONSTRATES SAFETY AND PROLONGED DURATION OF EFFECT IN A DOSE-RANGING STUDY FOR GLABELLAR LINES

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Background: Recent data provides evidence that increasing the dose of botulinum toxin type A increases the duration of efficacy (Polacco, et al, 2020). A 2-stage Phase 2, randomized, double-blind study investigated the duration of effect and safety of incobotulinumtoxinA (INCO, Merz Pharmaceuticals GmbH) at doses higher than the approved 20 units (U) for glabellar frown lines (GFL). Primary endpoints of Stage 1 are reported here.
Methods: One hundred fifty-one subjects with moderate-to-severe GFL were randomized 1:2:2 to receive a single treatment with 20 U, 50 U, or 75 U INCO. The primary efficacy endpoint was median duration of at least 1-point improvement from baseline as assessed by investigator at maximum frown on the Facial Wrinkle Scale.
Results: The median duration of effect was 185 days for the 50 U dose group (95% CI: [182, 205]) and 210 days for the 75 U dose group (95% CI: [182, 217]). Duration of effect was significantly longer for 75 U vs 50 U (*P*=0.0400) and 20 U (*P*=0.0166) despite the study not being powered for confirmatory statistical significance testing between the dose groups. Duration of effect was also longer for 50 U vs 20 U; however, statistical significance was not reached (*P*=0.4349). The incidence of treatment-related adverse events was low across all doses (20 U:2[6.7%], 50 U:6 [10.0%], and 75 U:8[13.1%]).
Conclusions: These results demonstrate a dose effect of at least 6 months' duration with higher doses in the majority of GFL subjects. All doses were well-tolerated, and safety was consistent with the known safety profile of 20 U INCO for GFL.
Funding: Merz Pharmaceuticals GmbH, Frankfurt am Main, Germany
Keywords: Duration; Glabellar frown lines; IncobotulinumtoxinA

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EFFECTIVENESS OF CHEMODENERVATION WITH INCOBOTULINUMTOXINA IN THE TREATMENT OF CONCOMITANT STRABISMUS IN A GROUP OF CHILDREN WITH HYPEROPIC REFRACTION

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Introduction: Strabismus occurs in 1-3% of children. The efficacy and safety of conservative and surgical treatments are inadequate, while chemodenervation with botulinum toxin is minimally invasive with a short recovery period.
Objective: To investigate the effectiveness of incobotulinumtoxinA (incoBTA) treatment of concomitant strabismus in children with hyperopia.
Methods: One hundred fifteen children (230 eyes; 52 boys, 63 girls; average age: 4 years and 2 months) were included in the study over a 24-month follow-up period. Children with concomitant strabismus and hypermetropic refraction were included; those with paralytic strabismus and myopia were excluded. IncoBTA at an average dose of 3.4 UI was injected into the extraocular muscles using specially designed surgical tweezers to hold the oculomotor muscles under visual guidance in the operating room using mask induction of anesthesia with sevoflurane. The procedure lasted ≤1-2 minutes.
Results: In 94.9% of patients, leveling of the strabismus angle was noted throughout the entire observation period. The majority of patients (68.7%) experienced a positive result after one injection, which indicates the adequacy of the chosen dose. A decrease in the angle of deviation was noted in the other patients, but a second injection was performed, after which the angle of strabismus leveled. On average, the second injection was performed after 9.18 months. Most often (19.13%), repeat incoBTA injections were required in patients with an angle greater than 25 degrees.
Conclusions: The effectiveness of chemodenervation depended both on the initial strabismus angle and on the doses used. IncoBTA can be offered as an effective, safe, minimally invasive method of treating strabismus in children with hyperopia.
Keywords: Binocular vision; Chemodenervation; Hyperopia; IncobotulinumtoxinA; Strabismus

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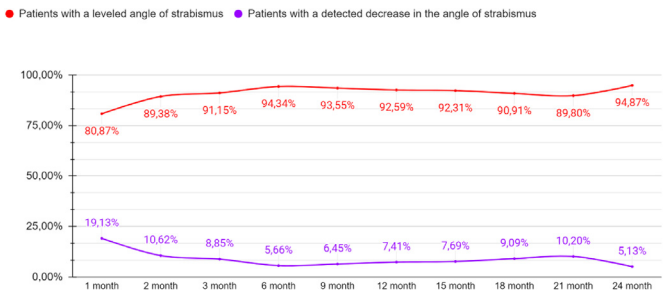

LONG-TERM EFFICACY AND SAFETY OF ABOBOTULINUMTOXINA SOLUTION FOR THE TREATMENT OF MODERATE-TO-SEVERE GLABEL LAR LINES: A PHASE III, DOUBLE-BLIND, PLACEBO-CONTROLLED AND OPEN-LABEL REPEAT INJECTION STUDY

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Introduction: Powder formulations of botulinum toxin type A (BoNT-A) require reconstitution before injection. We assessed long-term efficacy and safety of abobotulinumtoxinA (aboBoNT-A) solution, a new ready-to-use liquid formulation for glabellar line (GL) treatment.
Methods: Multicenter, phase III study, with randomized double-blind, placebo-controlled (DBPC) and open-label (OL) phases (NCT02493946; 2014-003841-86). Patients: 18-65 years old; BoNT-naïve; dissatisfied/very dissatisfied with moderate/severe vertical GLs at baseline maximum frown (MF). Patients were randomized 2:1 (aboBoNT-A solution 50 U: placebo) in DB phase and received ≤4 OL cycles; OL phase included de novo patients. Long-term analysis (LTA) included patients who received ≥1 aboBoNT-A solution injection during the DB or OL phases.
Results: LTA included 595 patients (DBPC phase: aboBoNT-A solution n=126, placebo n=64) from 24 sites. Mean (SD) age in DBPC aboBoNT-A solution and placebo, and LTA groups was 47.8 (9.4), 47.2 (9.0) and 46.6 (10.0) years, and 91.3%, 90.6% and 89.1% were female, respectively. At Day 29 of DBPC phase, per Investigators Live Assessment of GL severity at MF, 81.6% vs 0.8% were responders in the aboBoNT-A solution vs placebo group, respectively (primary endpoint; $P<0.0001$). This was consistent across LTA cycles 1-4 (82.2-87.8%; Figure 1). Responders for Subject-Self Assessment of GL severity and patient satisfaction with GL appearance with aboBoNT-A solution vs placebo at Day 29 were 68.1% vs 2.3% and 83.1% vs 5.7%, respectively (both $P<0.0001$), consistent with LTA cycles 1-4: 72.5-80.6% and 85.2-87.8%, respectively. No new/unexpected adverse events vs the known profile of aboBoNT-A (powder) or neutralizing antibodies were seen across cycles.
Conclusions: These data support the long-term safety and efficacy of aboBoNT-A solution for moderate-to-severe GL treatment. Injectors and patients may benefit from convenient, consistent, and precise dosing of a ready-to-use liquid formulation.
Funding: Ipsen
Keywords: AbobotulinumtoxinA; Botulinum toxin; Glabellar lines; Liquid formulation; Solution

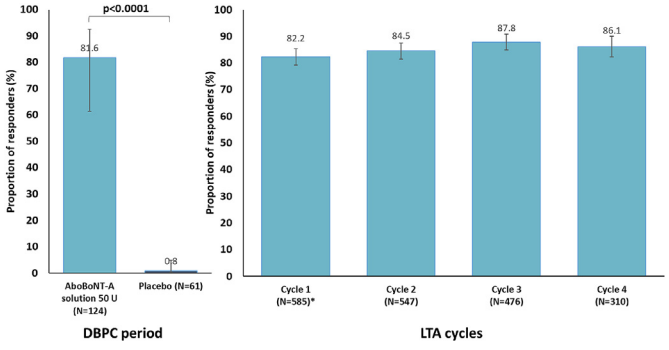

Fig. 1. Proportion of responders by ILA of GL severity at maximum frown (rating of mild or none) on Day 29 of each treatment cycle.DBPC period, mITT population; LTA population. AboBoNT-A, abobotulinumtoxinA; CI, confidence interval; DBPC, double-blind placebo-controlled; ILA, investigator's live assessment; LTA, long-term analysis; mITT, modified intention-to-treat. Responders were defined as patients with a rating of 'none' or 'mild' on the glabellar line severity scale at post-injection time points, when (per the inclusion criteria) rated 'moderate' or 'severe' at study baseline. *LTA Cycle 1 included data for patients who received aboBoNT-A solution 50 U during the DBPC period. Error bars represent the 95% CI.

SLEEP IN FOCAL CRANIOCERVICAL DYSTONIA PATIENTS

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Introduction and Objectives: Nonmotor symptoms, including sleep disturbances, are highly prevalent in craniocervical dystonia (CCD) and affect quality of life. Botulinum toxin (BT) is considered the most effective treatment for focal dystonias and is highly efficacious in controlling motor symptoms.[1] Studies suggest that sleep aberrations in cervical dystonia do not improve following BT treatment.[2,3] The authors systematically investigated motor symptom severity and sleep quality in patients with CCD and controls. The severity of motor symptoms and sleep quality was also analyzed in patients with CCD in relation to botulinum toxin type A (BTA) treatment and its regularity.
Methods: The study included 76 patients with CCD and 31 control group patients with hemifacial spasm (HFS). Severity of motor manifestations was rated using the Unified Dystonia Rating Scale (UDRS), and sleep quality was assessed with the Pittsburgh Sleep Quality Index (PSQI). Patients with CCD were divided into three subgroups: A) patients who had never received BTA injections; B) patients regularly receiving BTA injections (3-4 times a year); and C) patients receiving BTA injections less than 3 times per year.
Results: The mean estimated total PSQI score in the CCD group was 9.6 ± 4.9, ranging from 0 to 19 points. In the control group, the mean value was 4.6 ± 1.1 points, ranging from 3 to 6 points. The sleep quality index indicators were higher in the CCD group than in the control group, indicating a poorer quality of sleep in these patients compared with the HFS patients ($P=0.000$). No correlation between the severity of motor manifestations and the quality of sleep was found in either the CCD or control groups ($r=0.178$, $P=0.123$; $r=-0.088$, $P=0.646$). There was no significant difference in PSQI scores among the group of patients who had never received BTA treatment and either of the BTA-treated subgroups. However, there was significant difference in UDRS scores between the group of patients who had never received BTA treatment and those receiving regular BTA treatment ($P=0.039$).
Conclusions: These results suggest that sleep aberrations cannot be viewed as secondary complications of motor manifestations of CCD. The findings support the hypothesis that sleep disturbances are one of the nonmotor manifestations of CCD.
Keywords: Craniocervical dystonia; Blepharospasm; Cervical dystonia; Botulinum toxin; Sleep disturbances

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LOWER LIMB INJECTIONS OF ONABOTULINUMTOXINA: IMPROVEMENT IN GAIT AND TREATMENT GOAL ACHIEVEMENT IN PEDIATRIC PATIENTS WITH CEREBRAL PALSY

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Introduction: In a phase 3, double-blind, placebo-controlled study, onabotulinumtoxinA was demonstrated to be well tolerated and effective for the treatment of lower-limb spasticity in children with cerebral palsy (CP). Here we report the specific functional outcomes from that study.
Methods: Children (aged 2-<17 years) were included who had CP and an ankle plantar flexor Modified Ashworth Scale-Bohannon (MAS) score ≥2. OnabotulinumtoxinA 8 U/kg, 4 U/kg, or placebo was injected into the ankle plantar flexors; patients also received standardized physiotherapy (PT). Functional outcomes include physician-assessed Goal Attainment Scale (GAS) and the Edinburgh Visual Gait (EVG) score, which included a subset of patients (n=65).
Results: Overall, 384 patients were randomized (onabotulinumtoxinA: 8 U/kg, n=128; 4 U/kg, n=126; placebo, n=130). At baseline, 265 patients (70%) set active goals related to walking and moving; 202 patients (53%) set passive goals related to symptoms of pain, spasm, or orthosis/brace wear. Improvements in GAS were significant for onabotulinumtoxinA + PT vs placebo + PT for both active and passive goals at Weeks 8 and 12 for 8 U/kg ($P \leq 0.010$) and at week 8 for 4 U/kg ($P \leq 0.047$). In the subset of patients evaluated for EVG, 4 and 8 U/kg demonstrated dose-dependent improvement (statistical significance at 8 U/kg vs placebo at Week 8; $P = 0.018$) in the total score and select individual items (associated with foot stance and swing) over placebo.
Conclusions: OnabotulinumtoxinA and PT led to greater improvement in gait and significant achievement in active and passive treatment goals compared with placebo and PT alone.
Keywords: Cerebral palsy; Children; Lower limb; OnabotulinumtoxinA; Spasticity

AGE-RELATED DYNAMICS OF LOWER LIMB SPASTICITY TREATMENT WITH ABOBOTULINUMTOXINA IN PATIENTS WITH CEREBRAL PALSY AT GMFCS IV-V LEVELS

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Objectives: To evaluate the age-related changes in lower limb spasticity patterns in patients with cerebral palsy (CP) with Gross Motor Function Classification System (GMFCS) IV-V levels (according to the frequency of botulinum toxin type A (BTA) injections).
Methods: Six hundred seventy-seven repeat multilevel injections of abobotulinumtoxinA for 333 patients with CP were retrospectively analyzed. All patients were observed regularly and received multidisciplinary rehabilitation and BTA injections in two specialized neurologic departments. Two hundred fifteen (64.6%) patients were at GMFCS IV level and 118 (35.4%) at GMFCS level V; 210 (63.1%) were boys and 123 (36.9%) were girls. The median age at the first injection was 4.8 (range: 1.3-17.9) years without significant difference between GMFCS levels. The proportion of target muscles was calculated separately in different GMFCS age groups: 2-4 years, 4-6 years, 6-12 years, and 12-18 years. The efficacy of injections was evaluated on the basis of the Modified Tardieu and Ashworth scales.
Results: In the overall group of patients, the gracilis muscle was injected most frequently— in 221 (66.4%) patients. The adductors were injected in 164 (49.2%) patients; the medial hamstrings in 144 (43.2%) patients; the rectus muscles in 121 (36.3%) patients; and the gastrocnemius muscle in 87 (26.1%). The adductor muscles of the thigh were injected predominantly in children aged 2-4 years, the gracilis and gastrocnemius muscles in those aged 4-6 years, the rectus muscle approximately equally in those aged 2-4 and 4-6 years, and the medial hamstring muscles in those aged 6 to 12 years (Figure 1).
Conclusions: According to our data, the proximal thigh muscles are injected much more frequently than the distal muscles in all age groups of patients at GMFCS levels IV-V. The dynamics of target muscle choice may reflect the natural evolution of spasticity patterns and contractures in severely disabled CP patients, as well as the goals of antispasticity treatment using BTA.
Keywords: AbobotulinumtoxinA; Botulinum toxin; Cerebral palsy; Children; GMFCS levels IV-V; Spasticity

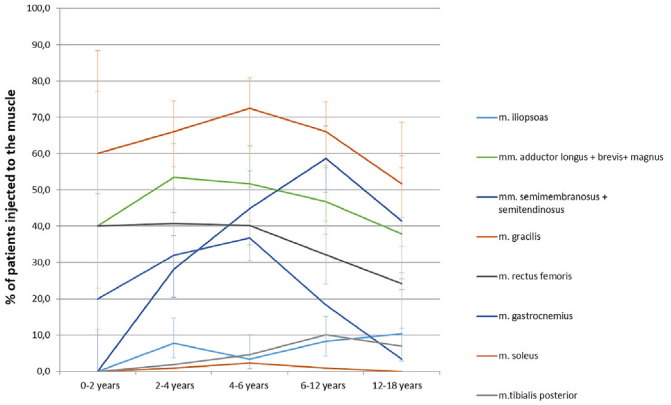

Fig. 1. Age-related distribution of the target muscles for BTA injections in patients at GMFCS level IV-V.

REAL-LIFE USE OF ONABOTULINUMTOXINA FOR SYMPTOM RELIEF IN PATIENTS WITH CHRONIC MIGRAINE: REPOSE STUDY GERMAN POPULATION

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Introduction: In Germany, chronic migraine (CM) has been associated with substantial disability and increased healthcare resource utilization (HRU). We assessed HRU and patient-reported reductions in headache days after onabotulinumtoxinA treatment for CM in German patients from the REPOSE study.
Methods: REPOSE, a 2-year, prospective, noninterventional, observational, open-label, real-world study, included adults with CM who received onabotulinumtoxinA ~every 12 weeks. Patients estimated their mean headache-day frequency for the last month at each treatment visit. HRU, including family doctor or specialist visits, inpatient acute treatment, acute treatment for headache, acupuncture, technical investigations, and use of nonpharmacologic remedies, were collected at baseline and at 6, 12, 18, and 24 months.
Results: Of 641 enrolled patients, 633 received ≥1 onabotulinumtoxinA dose (mean age, 45 years; 85% female; 60% (n=377) German). Baseline mean monthly headache days for German patients were 18.9 and declined to 8.6 at 12 months and 7.6 at 24 months; significant reductions were observed at all follow-up visits (range: 11.3-6.0; $P<0.0001$). At baseline, 41.7% and 61.7% of patients saw a family doctor and a specialist, respectively; these percentages declined to 12.2% and 8.9% at 12 months and 9.2% and 4.6% at 24 months. Inpatient acute treatment and acute treatment for headache also declined from 6.4% and 71.2% of patients at baseline, respectively, to 0.8% and 65.9% of patients at 12 months and 0.0% and 55.4% of patients at 24 months. Use of acupuncture declined from 15.5% at baseline to a range of 2.2%-0.0%. Technical investigations and use of remedies decreased from 19.7% and 30.6% at baseline to ranges of 1.6%-0.0% and 15.4%-4.6%, respectively.

Conclusions: In German REPOSE patients, onabotulinumtoxinA treatment for CM is associated with reduced monthly headache days and decreased HRU.
Keywords: Chronic; Headache days; Health resource utilization; Migraine; OnabotulinumtoxinA; Real life
Funding: Allergan (prior to its acquisition by AbbVie)

CRYSTAL STRUCTURES OF BOTULINUM NEUROTOXIN X AND *WEISSELLA ORYZAE* BOTULINUM TOXIN–LIKE CATALYTIC DOMAINS

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Background: Botulinum neurotoxins (BoNTs) are one of the most poisonous proteins known to man. All seven serotypes (BoNT/A-G) act as zinc-dependent endopeptidases. They cleave SNARE proteins, which inhibits neurotransmitter release and eventually leads to paralysis. Recently, a new botulinum toxin serotype, BoNT/X, and several botulinum toxin–like proteins were identified. BoNT/X was shown to cleave the canonical targets, vesicle-associated membrane protein (VAMP) 1/2/3 at a unique site. Moreover, it showed the unique ability to cleave VAMP4/5 and Ykt6, which are not cleaved by any other BoNT serotype.[1] The first botulinum toxin–like protein, BoNT/Wo, was discovered during bioinformatical analysis of the genome of *Weissella oryzae*, a non-spore–forming anaerobic bacteria isolated from fermented rice grains.[2] The natural targets of BoNT/Wo are yet to be determined; however, the toxin was shown to cleave VAMP2 at a unique cleavage site.[3]
Methods: Structural studies of BoNT/X and BoNT/Wo were performed by X-ray crystallography.
Results and conclusions: Here we report the 1.35 Å structure of the light chain of BoNT/X (LC/X)[4] and the 1.6 Å structure of the light chain of BoNT/Wo (LC/Wo).[5] The core folds of both LC/X and LC/Wo are similar to those of other BoNTs, which demonstrates that LC/X and LC/Wo are truly members of the BoNT-LC family. Interestingly, access to the catalytic site of LC/X is more restricted compared to other BoNTs, while access to that of LC/Wo is more open. Both structures display several unique features. The structural information collected provides the molecular basis for understanding the evolution of BoNTs and engineering new scientific tools and therapeutic toxins targeting distinct SNARE proteins in cells.[4,5]
Keywords: BoNT; BoNT/X; BoNT/Wo; Catalytic domain; X-ray crystallography

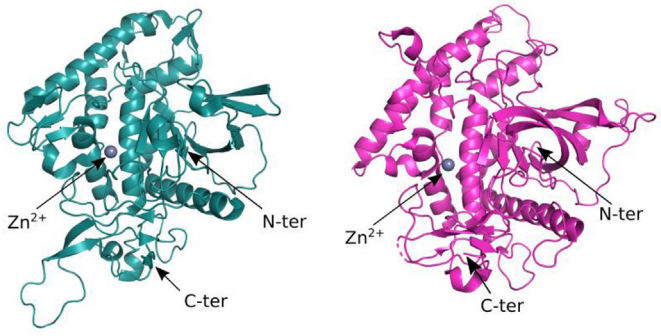

Fig. 1. Crystal structures of LC/X (cyan) and LC/Wo (magenta); Zn ion is shown as grey sphere.

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DEVELOPMENT AND EVALUATION OF MANUAL TESTING OF SPASTIC MUSCLES FOR BOTULINUM TOXIN THERAPY

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Introduction: Skills and techniques to identify spastic muscles remain a weak point for botulinum toxin therapists. Developing a method for determining spastic muscles is an urgent task. The economic and clinical benefits of such a development are of great interest.
Methods: One hundred twenty patients with post-stroke spasticity were enrolled in the study. Patients were randomized into two groups of 60 each. In the first group, botulinum toxin (BoNT) was administered by a specialist trained in spasticity testing methods, and in the second group by an injector of average skill. Clinical evaluation of patients was performed using standardized measures: for spasticity, the Tardieu Scale (TS) and Modified Ashworth Scale (MAS); for muscle force, the Medical Research Council Scale (MRCS); for mobility, the 10-Meter Walk Test (10MWT) and Rivermead Mobility Index (RMI); for arm function, the Leeds Arm Spasticity Impact Scale (LASIS); for performance in activities of daily living, the Barthel Scale; for motor skills, the Modified Rankin Scale; and for assessing patient satisfaction with treatment, the Visual Analog Scale (VAS). Goals were set using a SMART approach (specific, measurable, achievable, realistic, and time limited), and evaluated using Goal Attainment Scaling.
Twenty-seven muscles were tested for spasticity using manual muscle testing techniques; muscle selection was made in conjunction with the determined treatment goals. IncobotulinumtoxinA (Xeomin®) 300-850 U was injected under ultrasound guidance. Clinical evaluations of patients were performed at 3 timepoints: baseline (Day 1), and 25 and 100 days post-injection.
Results: At Day 25, the first group showed better results than the second on the TS (P<0.01), MAS (P <0.05), LASIS (P <0.05), 10MWT (P <0.05), and RMI (P <0.05). At Day 100, the first group also showed better results than the second on the TS (P <0.01), MAS (P <0.05), LASIS (P <0.01), 10MWT (P <0.001), RMI (P <0.01), Barthel (P <0.05), Modified Rankin (P <0.05), and VAS (P <0.01). Patient goals were achieved in both groups, measured by GAS with rating at +2 in the first group and +1 in the second.
Analysis of the injection protocols in group 2 showed frequent unwarranted injections of the biceps brachii (BB), flexor carpi ulnaris (FCU), flexor carpi radialis (FCR), biceps femoris (BF), rectus femoris (RF), soleus (S), tibialis anterior (TA), flexor digitorum brevis (FDB), and in the lateral head of the gastrocnemius (G/c). Frequent non-recognition of spasticity in the subscapularis (S/s), flexor digitorum superficialis (FDS), flexor digitorum profundus (FDP), pronator teres (PT), tibialis posterior (TP), and medial head of the gastrocnemius (G/c) was also noted. Overdosing of BoNT reached 15-20% in the upper and 30-40% in the lower extremities. The frequency of using mid-range dosages in the first group increased by 15%.
Conclusions: Correct selection of target muscles for BoNT injection is highly important to increase treatment effectiveness and reduce treatment cost. These study results will make it possible to develop a guideline for

muscle testing to determine spastic muscles.
Keywords: Botulinum neurotoxin (BoNT); Post-stroke rehabilitation; Spasticity; Spasticity testing

EXOSKELETON "EXOATLET" AND BOTULINUM TOXIN THERAPY IN REHABILITATION OF STROKE PATIENTS

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Introduction: Walking disorders are a frequent consequence of stroke. New technologies, such as the use of robotic exoskeletons, can help with recovery, but their effectiveness has not yet been sufficiently proven.
Methods: Sixty-two patients with spasticity and walking disorders (stroke duration from 1.5 to 4 years) were included in the study. The Tardieu Scale (TS), Modified Ashworth scale (MAS), Medical Research Council Scale (MRCS), 10 Meter Walk Test (10MWT), Rivermead Mobility Index (RMI), Berg Balance Scale (BBS), Modified Rankin scale, and a Visual Analog Scale (VAS) (to assess patient satisfaction with treatment) were used in assessments .
The patients were randomized into 2 groups (n=31): the first group received exoskeleton walk training with the powered exoskeleton "ExoAtlet" and the second group received physical therapy sessions, each for 1 hour daily over 10 days. Then all patients were injected with incobotulinumtoxinA (Xeomin®) 300-400 U in lower limb spastic muscles under ultrasound guidance. Injections were made into spastic muscles defined individually. Target muscles were determined by manual testing techniques. Clinical evaluations of patients were performed at 3 timepoints: baseline (Day 1), and 12 and 21 days post-injection.
Results: Comparison of both groups at the second timepoint showed significantly better results (P<0.05) in the first group vs the second group on the 10 MWT (0.43 and 0.47 meter per second [m/s]), BBS (42 and 44.5), and TS hamstrings (132° and 137.5°) (Table 1). Walking speed increased due to balance training, correction of postural disorders, spastic muscle stretching, and stretch reflex suppression. BoNT injections were performed at the second timepoint (Day 12). At the third timepoint (Day 33), the best results were observed in the first group (P<0.05) on the 10 MWT (0.49 and 0.56 m/s), BBS (46 and 49), and TS (1440 and 1550). When comparing the difference between the two groups at the first and third timepoints, the absolute gains in test scores (P<0.01) were: 10 MWT (0.07 and 0.12 m/s), BBS (3.5 and 8.5), and TS (14.5° and 22°). The improvement in walking performance at the third timepoint demonstrates the potentiating effect of BoNT injections and exoskeleton walk training.
Conclusions: The wearable powered "ExoAtlet" exoskeleton is a promising technology for improving walking, and in combination with BoNT injection produces a pronounced potentiating effect.
Keywords: Botulinum neurotoxin (BoNT); Exoskeleton; Exoskeleton "ExoAtlet"; Exorehabilitation; IncobotulinumtoxinA; Post-stroke rehabilitation; Spasticity; Walking disorder

Table 1. Results of Comparison of Absolute Increments of Evaluation Scales After Rehabilitation in the Study and Control Groups; Median [Q25; Q75%]; (n=number of patients).

Rating scales	Comparison groups		Mann-Whitney U-test	Significance level
	1st Group n=31	2nd Group n=31		
Modified Ashworth Scale	0.5 [0.0; 1,0]	0.0 [0.0; 0.5]	130.0	P<0.05
Tardieu Scale, degrees	7.0 [6.0; 8.0]	4.0 [3.0; 5.0]	58.0	P<0.001
10-Meter Walking Test (m/s)	0.06 [0.05; 0.07]	0.04 [0.03; 0.05]	56.0	P <0.001
Berg Balance Scale	3.0 [3.0; 3.0]	2.0 [20; 3.0]	137.0	P<0.05
Rivermead Mobility Index	1.0 [1.0; 2.0]	1.0 [0.0; 1.0]	135.0	P <0.05
Modified Rankin Scale	0.0 [0.0; 0.0]	0.0 [0.0; 1.0]	216.0	P=0.91
Medical Research Council Scale	0.0 [0.0; 0.0]	0.0 [0.0; 1.0]	201.0	P=0.98
Visual Analogue Scale	3.0 [3.0; 5.0]	2.0 [1.0; 2.5]	83.5	P < 0.001

DETERMINATION OF THE INTRAMUSCULAR MOTOR ENDPOINTS FOR EFFECTIVE ADMINISTRATION OF BOTULINUM TOXIN IN THE TREATMENT OF SPASTICITY

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Introduction: Neuromuscular transmission occurs between the axon terminals and intramuscular motor endpoints (IMEs). Accurate targeting of IMEs can improve the effectiveness of botulinum neurotoxin (BoNT) injections.
Methods: Fifty-nine healthy people were examined. Twenty-five muscles of the shoulder girdle, and the upper and lower limbs were examined in full using electromyography (EMG) and ultrasound (US) scanning. Forty-two IMEs were identified and localized using EMG. Then patients with post-stroke spasticity (n=32) were randomized into two groups (16 people each), and they were injected with 1500 U abobotulinumtoxinA. In group 1, injection of IMEs was performed using US guidance, and in group 2, using both US and EMG guidance. Spasticity was evaluated using the Tardieu Scale (TS). Assessments were performed at baseline (Day 1), and 12, 16, 21, and 28 days post-injection.
Results: IMEs were localized on the surface of the body. The location of the IME is identical and not dependent on the gender, age, or dominant limb of the subject. Results were confirmed in all 59 patients and IME locations were mapped (Figure). Group 2 showed better results on the TS (P<0.05) 16 days after BoNT injection. At 28 days post-injection, the difference between the groups on the TS decreases (P=0.1).
Conclusions: This study showed an approach to determination of the IME locations of the upper and lower limb and shoulder girdle muscles and allowed us to create a surface map of these locations. With IME injection of BoNT, the effect develops faster than with the usual method, but was equated to Day 28 post-injection. This data might improve the clinical efficacy and the feasibility of IME targeting, when injecting BoNT in treating spasticity. Further research is required.
Keywords: Botulinum neurotoxin (BoNT); Intramuscular motor endpoint (IME); Targeted BoNT injection; Post-stroke rehabilitation; Spasticity

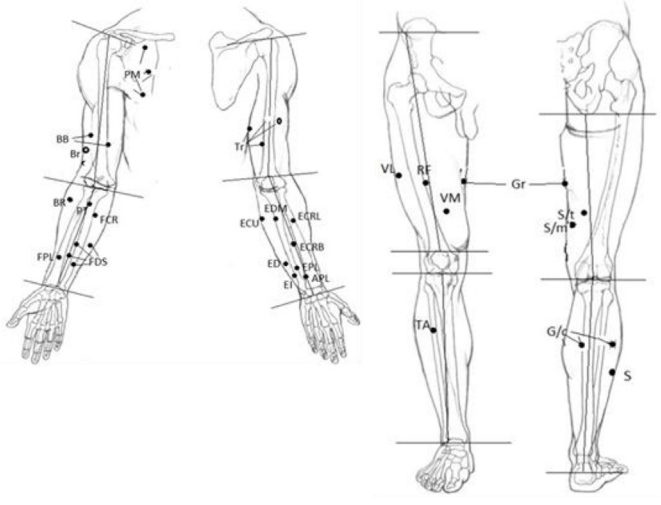

Fig. Map of the IME muscle locations for BoNT injections.

BOTULINUM TOXIN TYPE A (INCOBOTULINUMTOXINA) IN CEREBRAL PALSY: COMBINED TREATMENT OF SPASTICITY AND SIALORRHEA

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Objective : A retrospective analysis of incobotulinumtoxinA injections in the combined treatment of spasticity and sialorrhea in children with cerebral palsy (CP).
Methods: One hundred and eighty-five children with spastic forms of CP, including 114 boys (61.6%), were studied. The average age of the patients was 3.8±2.5 years; the average weight was 14.2±6.9 kg. The patients received incobotulinumtoxinA injections according to approved labeling or specialist recommendations and after voluntary informed consent of the patient's representative was obtained. All children received incobotulinumtoxinA injections for anti-spastic purposes and 17 of them also received injections to correct sialorrhea. At least a 1-point decrease of muscle tone according to the Modified Ashworth Scale and 10-point decrease in the Drooling Impact Scale (DIS) were used as the criteria for assessing the effect of incobotulinumtoxinA 2 weeks post-injection.
Results: The total dose of incobotulinumtoxinA injected in treatment of spasticity in these CP patients was 154.5±67.7 U and 11.6±4.7 U per kg of body weight and in combined treatment of spasticity and sialorrhea 196.8±78.3 U and 14.7±5.9 U per kg of body weight. The gracilis muscle (65.4% of cases, 95% CI: 58.1-72.2) and the gastrocnemius muscle (49.4% of cases, 95% CI: 41.8-56.6) were the most frequently injected muscles in the lower extremities, and the pronator teres muscle (58.9% of cases, 95%CI: 51.5-66.1) in the upper extremities. A decrease in DIS scores by 20 or more points was observed in 10 (58.8%) patients, and by 10-19 points in 7 (41.2%) patients. Adverse events were observed in 13 patients (7.0%). They were mild in 9 patients and moderate in 4 patients.
Conclusion: The data obtained in our study indicate the effectiveness and safety of incobotulinumtoxinA for reducing spasticity and sialorrhea in children with cerebral palsy.
Keywords: Botulinum toxin type A; Cerebral palsy; IncobotulinumtoxinA; Sialorrhea; Spasticity

PAIN-RELATED POTENTIATION BY NGF OF CAPSAICIN-EVOKED INTRA-NEURONAL CALCIUM SIGNALS IN DORSAL ROOT GANGLION (DRG) FROM *PIRT-GCAMP3* MICE IS RELIANT ON SNAP-25

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Introduction and Objectives: Chronic pain is highly prevalent and current therapies are too often ineffective, poorly tolerated and/or addictive. Pain is initiated via ion channels in peripheral neurons that sense noxious chemicals, heat or mechanical force, the best characterized being transient receptor potential vanilloid 1 (TRPV1). Nerve growth factor (NGF), released in injured and inflamed tissues, enhances TRPV1 responsiveness so disruption of this process is a prime therapeutic goal.
The aims of this study were to: (1) quantify intensity and duration of Ca^{2+} signals evoked by capsaicin in DRG neurons expressing the Ca^{2+} sensor GCaMP3, (2) demonstrate an increase in such capsaicin-evoked TRPV1 activity after acute pre-exposure to NGF, and (3) establish whether this enhancement is inhibited by recombinant light chain of botulinum neurotoxin type E fused to N-terminus of whole botulinum neurotoxin type A (LC/E-BoNT/A), an attractive chimera (Wang at el, 2017).
Methods: *Pirt-GCaMP3*$^{+/-}$ mice expressing the genetically-encoded Ca^{2+} indicator, GCaMP3, in >95% of DRG neurons were kindly provided by Prof. Xinzhong Dong. Confocal fluorescence imaging was performed with a 488 nm laser on whole L3/4 DRG explants from the adult (4-6 weeks) male and female mice. Pixel intensities were measured in manually selected regions of interest in videos imported into ImageJ. Results were exported to Microsoft Excel for processing and values that satisfied a minimal response threshold [$(F-F_0)/F_0 > F_0 + 10$ x s.d., where F and F_0 = average pixel intensity after and before capsaicin application, respectively, and s.d. = standard deviation in F_0 measured over 2 min prior to capsaicin application] were transferred to GraphPad Prism 8 for peak analysis and creation of graphs. Expression, purification and functional characterization of LC/E-BoNT/A have been described before (Wang et al, 2017). Significance values were calculated by Student's t-test.
Results: Raising capsaicin concentrations evoked responses from significantly larger numbers of neurons [$P<0.05$ (0.3 v 1 μM), $P<0.01$ (10 v 1 μM); n = 4)]. NGF pre-treatment accelerated responses and significantly increased the number of cells activated by 1 ($P<0.05$) and 10 μM ($P<0.01$) capsaicin, and raised intensity ($P<0.05$) and prolonged duration ($P<0.0001$) of Ca^{2+} signals elicited by 10 μM capsaicin. Prior treatment with LC/E-BoNT/A produced detectable /E-cleaved SNAP-25 and abolished all these NGF-induced increments in the number of 10 μM capsaicin responders, Ca^{2+} signal intensity ($P<0.0001$) and duration ($P<0.0001$), but had no effect on the faster activation of cells.
Conclusions: NGF enhances nociceptor responses to capsaicin by at least two different mechanisms: signal potentiation and acceleration of cell activation.
Increased numbers of capsaicin responders and enlargement of their Ca^{2+} signals by NGF seems to be mediated by SNAP-25—dependent exocytotic trafficking of TRPV1 to the cell surface, as they were inhibited by LC/E-BoNT/A which cleaved this protein. Such transport of TRPV1 seems not to be involved in the faster activation of neurons by capsaicin after pre-treatment with NGF.
Funding: Science Foundation Ireland, Investigator Program Award (15/IA/3026) to JOD.
Keywords: Ca^{2+} imaging; Chronic pain; Nerve growth factor; Neurotherapeutics; Recombinant botulinum neurotoxins; Sensitization of nociceptors

Reference

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MANAGEMENT OF FOCAL NEUROMYOTONIA WITH BOTULINUM TOXIN TYPE A: A CASE REPORT

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Introduction and Objectives: Neuromyotonia is a rare disorder characterised by progressive hypertonia, cramping, muscle weakness, and often hyporeflexia. Abnormal firing of nerve impulses peripherally results in continuous muscle fibre activity, giving a mixed upper and lower motor neuron lesion picture. We present a rarer variation of this condition, focal neuromyotonia (FN), which results in persistent isolated finger flexion.
We report functional improvement noted 6 weeks after botulinum toxin type A (BoNT-A) injection in a patient with FN.
Clinical details: A 71-year-old woman noticed progressive curling of her fingers for 12 months. This started with her left middle and ring fingers,

followed by her right ring finger. She had flexion at interphalangeal joints of the left fingers with passive extension limited to -90 degrees. Her right fingers started following similar patterns. There was no muscle wasting or weakness. She gets occasional pins and needles sensation in the left hand and passive movements of her fingers were painful. She has chronic obstructive pulmonary disease (COPD) and is on a salbutamol inhaler/nebulizer.

As she reported altered sensation in her fingertips, a spine MRI was arranged, which revealed significant cervical canal stenosis at C3-C4 and C4-C5 and myelomalacia (C5-C7). Electromyography (EMG) reported persistent high-frequency discharges in the upper limbs consistent with FN. Antibody (Ab) screening, including voltage-gated potassium channel (VGKC) antibody test, was negative.

Management: The patient is currently under a treatment plan that aims to improve comfort, prevent deformity, and regain hand function. BoNT-A was injected into the left flexor digitorum superficialis (FDS), left flexor digitorum profundus, and right FDS under ultrasound and EMG guidance. BoNT-A inhibits presynaptic release of acetylcholine at the neuromuscular junction, which is expected to ease contractions in FN. Pregabalin was started for neuropathic pain. Finger exercises and stretches were advised. A carrot splint for the left hand and a rest wrist splint for the right were arranged.

Salbutamol has been reported as a contributing factor in FN in previously published case reports[1,2] and hence was changed to ipratropium under respiratory team advice. Neurosurgical referral was done for the cervical spine. The Arm Activity measure (ArmA) was used to monitor treatment outcome. This demonstrated improvement in completing daily activities such as writing and picking up objects, as seen in reduction of difficulty scoring from 31 to 17 at 6 weeks post-BoNT-A.

Conclusion: Holistic management to enhance the patient's quality of life and independence in activities of daily living is only possible through close collaboration among rehabilitation physicians, neurophysiologists, and neurologists. Mixed results are reported with effectiveness of BoNT-A in neuromyotonia in the literature. Our patient showed signs of functional improvement at 6 weeks after BoNT-A injection. However, clinical outcome will also depend on management of neuropathic pain and extent of established soft tissue shortening. We will continue to follow up with her progress.

This presentation shares similarities with Dupuytren's contracture, which may lead to potential misdiagnosis and missing the therapeutic window to treat with BoNT-A before joint range is lost permanently. Fixed finger flexion deformity that clinically appears as contractures should be investigated for neuromyotonia. FN has been reported in association with lumbosacral radiculopathy. Hence, cervical spinal disease is a potential contributing factor to FN.

Keywords: Botulinum; Degeneration; Neuromyotonia; Outcome; Salbutamol; Toxin

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LONG-TERM ADHERENCE AND SELF-PERCEIVED THERAPY EFFECT OF BOTULINUM NEUROTOXIN IN DIFFERENT NEUROLOGICAL DISORDERS

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Introduction: Botulinum neurotoxin type A (BoNT/A) is applied for long-term treatment of different neurological disorders. We aimed to analyze the adherence and self-perceived treatment response to long-term BoNT-A treatment in different neurological indications.

Methods: In this retrospective, monocentric, observational study, we analyzed cross-sectional and longitudinal data of 1351 patients treated at the BoNT outpatient clinic of Heinrich Heine University Düsseldorf between 1989 and 2014. Patients had been treated for neurological conditions, including cervical dystonia (CD), blepharospasm (BSP), other dystonia (ODT), hemifacial spasm (HFS), and spasticity (SPAS). Longitudinally, therapy duration as well as the mean and cumulative BoNT-A dose were analyzed. Cross-sectionally, for subgroups of at least 721, patients' global self-perceived quality of health and life, global self-perceived reduction of symptoms due to BoNT-A treatment, as well as the Clinical Global Impression Scale (CGI) ratings were evaluated. In a subset, mouse hemidiaphragm assay neutralizing antibodies (MHDA-NABs) were analyzed in a subgroup. Furthermore, we include a subgroup analysis of more detailed patient-reported outcomes (PROs) based on the EQ-5D-5L scale and disease-specific quality of life scores for the different treatment indications assessed more recently.

Results: The mean treatment duration was 4.58 years (95% CI: 4.32-4.84), and 678 (50.2%) therapy dropouts of a total 1351 patients occurred within the first eight years. Therapy adherence and self-perceived symptom reduction in long-term BoNT-A treatment over the years were significantly longer in BSP, HFS, and CD patients than in ODT and SPAS patients. A detailed comparative analysis of the different PROs is provided. MHDA-NABs had a significant impact on global self-perceived symptom reduction, but only with a limited generalizability due to a low effect size and statistical power.

Conclusions: In BoNT-A therapy, the treatment indication determines long-term adherence and self-perceived symptom reduction, which are better in BSP, HFS, and CD patients than in ODT and SPAS patients.

Keywords: Adherence; Botulinum neurotoxin therapy; Hemifacial spasm; Focal dystonia; Long-term treatment; Spasticity,

LONG-TERM AMELIORATION OF PERSISTENT PAIN STATES WITH A NOVEL BOTULINUM–SUBSTANCE P CONSTRUCT AND REINSTATEMENT OF ANALGESIA WITH A SECOND INJECTION OF THE CONSTRUCT

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Introduction: We have shown that a single injection of substance P conjugated to the silencing domain of botulinum toxin (SP-BOT) produced long lasting pain relief for up to 40 days (d) in mouse models of inflammatory and neuropathic pain (Maiarú, et al 2018). Here we investigated the longevity of botulinum construct–based neuronal silencing and associated pain relief and also if a second injection of conjugate could reinstate analgesia.

Methods: To characterise the time course and long-term effects of SP-BOT on pain signalling, we used the spared nerve injury (SNI) model of neuropathic pain. SP-BOT (100 ng/3 uL) was injected intrathecally (i.t.) over the lumbar spinal cord and the neuropathic injury made 30-120 d later. We tested both the sensory "reflex" responses to hind paw stimulation with Von Frey hairs as well as the later "affective" response to stimulation using a single Von Frey filament. Finally, we also investigated the response to cold stimulation.

A second intrathecal injection was given when pain behaviours had returned to levels of neuropathic pain seen in vehicle-injected mice.

Results: Our results show that a single injection of SP-BOT given before SNI produced a long-lasting reduction in reflex, cold, and affective coping responses that develop after neuropathic injury. Most measures of pain relief had been lost by 120 d after injection of SP-BOT. However, a second intrathecal injection of SP-BOT at 128 d after the first injection restored relief from chronic neuropathic pain and to levels seen previously.
Conclusion: Our data strongly indicate that botulinum-conjugated molecules provide unusually long periods of relief from neuropathic pain. The reinstatement of analgesia following a second injection of botulinum conjugate indicated that the key neurons in the dorsal horn have not been damaged by months of silencing but had recovered and remained integrated into nociceptive networks.
Funding: MRC UK grant MR/S025847/1
Keywords: Analgesia; Botulinum; Neuropathy; Pain; Substance P

Reference
Maiarù M, Leese C, Certo M, et al. Selective neuronal silencing using synthetic botulinum molecules alleviates chronic pain in mice. *Sci Trans Med.* 2018;10: eaar7384.

NOT ONLY GUSTATORY SWEATING AND FLUSHING: SIGNS AND SYMPTOMS ASSOCIATED WITH FREY'S SYNDROME AND THE ROLE OF BOTULINUM NEUROTOXIN TYPE A THERAPY

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Introduction: The classic symptoms of Frey's syndrome are gustatory sweating and flushing.[1] The aims of the study were to: 1) describe the prevalence, severity, and affected areas observed in typical and atypical presentations of the disorder, and 2) assess the effects of botulinum neurotoxin type A (BoNT-A) therapy in patients with Frey's syndrome after parotidectomy.[2]
Methods: In this prospective, observational study of 18 patients, we assessed symptom severity before therapy; after 15 days and at 1, 3, and 6 months' follow up with the Sweating-Flushing-Itch-Paresthesia-Pain (SFIPP) Frey Scale (overall score range: 0-20) specifically designed for this study (Figure 1). The post-treatment score gain was calculated from the scores before therapy and at follow-up examination. The affected areas were observed and recorded, and the following regions were identified: preauricular, retroauricular, temporoparietal, cheek, and retroangulo mandibular.
Results: Before BoNT-A injection, all patients (100%) complained of gustatory sweating, 80% paresthesia, 77% gustatory flushing, 60% pain, and 60% gustatory itch. The facial and cranial areas involved (in decreasing order of occurrence) were: preauricular (18/18-100%), temporoparietal (14/18-77%), retroauricular (12/18- 66%), retroangulomandibular (4/18-22%), and cheek (2/18-11%) (Figure 2). The SFIPP Frey score decreased significantly at 15 days post-therapy (10.4 vs 6.2; $P<0.05$). The mean overall score obtained 3 months after BoNT-A therapy was significantly lower compared to that calculated 1 month post-BoNT-A (4 vs 5.6; $P<0.05$) (Figure 3).
The mean pre- and post-treatment scores for each symptom are shown in Figure 4. After treatment, the highest mean gain was observed for the gustatory sweating score (1.53 ±1.81), followed by paresthesia (1.13 ± 1.55), flushing (1.00 ± 1.51), pain (0.60 ± 1.06), and itch (0.60 ± 1.06). The mean improvement in gustatory sweating severity was statistically significantly greater compared to the other symptoms ($P<0.05$).
Conclusions: The prevalence of "unusual" manifestations of Frey's syndrome is not negligible. BoNT-A improves the severity of symptoms. The SFIPP Frey Scale may be useful to assess symptoms and monitor post-therapy outcomes.
Keywords: Frey's syndrome; Auriculotemporal syndrome; Gustatory sweating; Botulinum neurotoxin type A; OnabotulinumtoxinA; Parotidectomy

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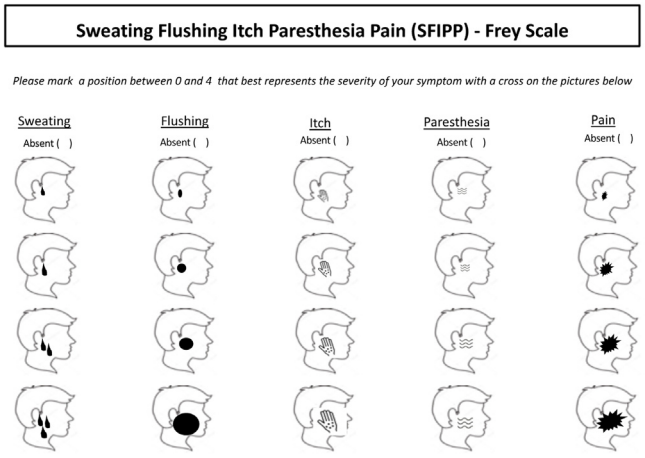

Figure 1. The all-inclusive self-assessment card to score the severity of typical and atypical symptoms of Frey's syndrome

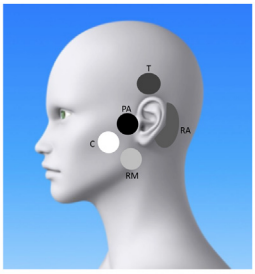

Figure 2. The map of the affected areas. The darkness of the color is directly related to the frequency of the involvement. PA = PreAuricolar (100%); T = Temporal (77%); RA = RetroAuricolar (66%); RM = RetroMandibular (22%); C = Cheek (11%)

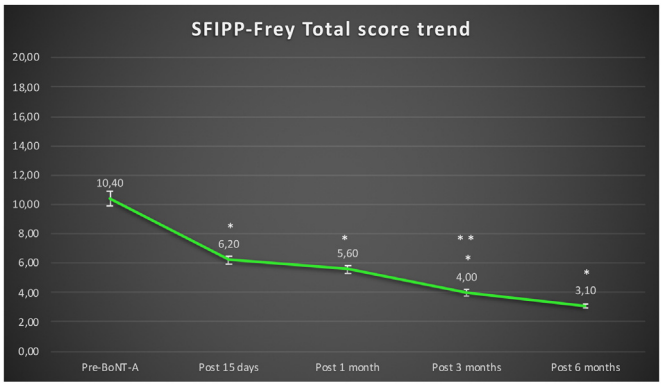

Figure 3. The mean SFIPP-Frey score obtained before and at each time point of control after BoNT-A injection. * = p<0.05 (vs pre-therapy); ** = p<0.05 (vs post-1 month)

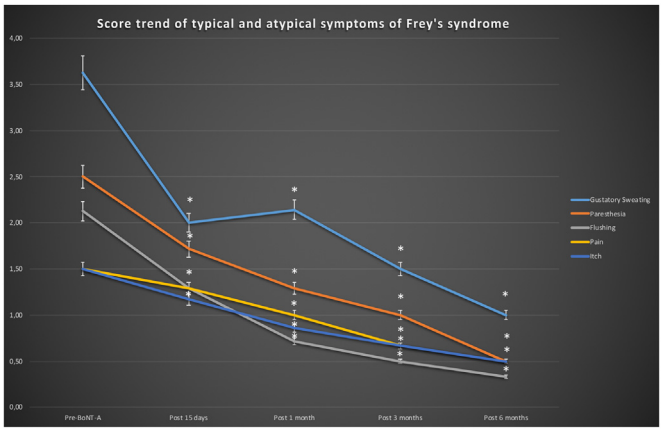

Figure 4. The mean pre- and post-therapy scores obtained for each symptom at the different time points of follow-up. * = p<0.05 (vs pre-therapy)

BONT-A EFFICACY IN HIGH-FREQUENCY MIGRAINE: AN OPEN-LABEL, SINGLE-ARM, EXPLORATORY STUDY APPLYING THE PREEMPT PARADIGM

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Introduction: OnabotulinumtoxinA (BoNT-A) has proven efficacy in the treatment of chronic migraine. In this exploratory, open-label, single-arm trial we evaluated its efficacy and safety in the prevention of high-frequency episodic migraine.
Methods: We enrolled 32 subjects with high-frequency migraine (8-14 migraine days/month in the previous 3 months). After a 28-day baseline period, subjects underwent 4 BoNT-A treatments delivered 12 weeks apart according to the PREEMPT paradigm. The primary outcome measure was the reduction in the number of migraine days in the 12-week period (normalized to 28 days) after the last BoNT-A treatment as compared to baseline. At baseline, the population enrolled had a mean age of 44.8 ± 11.9 years, 11.1± 2.3 migraine days, and 11.5 ± 2.2 headache days; 81% of them were women. Ten patients were on stable concomitant preventive therapy (beta-blockers: 6, calcium channel antagonists: 1, and antiepileptic drugs: 3). Exclusion criteria included the failure of >2 preventive treatments for migraine and any other chronic pain condition.
Results: BoNT-A reduced the number of migraine days by 3.73 days (-33.57%, *P*<0.001). Thirty-four percent of the patients had at least a 50% reduction in migraine days. BoNT-A also significantly reduced the number of headache days (-34.28%, *P*<0.001) and the intake of acute medications (-24.24%, *P*=0.029). Migraine Disability Assessment Test (MIDAS) and Migraine-Specific Quality-of-Life Questionnaire (MSQ) scores improved markedly (-39.75%, *P*=0.001 and -31.20%, *P*<0.001, respectively). Adverse events were transient and mild to moderate in severity. Only one patient discontinued the study drug due to a cutaneous adverse reaction.
Conclusions: BoNT-A is effective in reducing migraine days, acute pain medication intake,and burden of disease in high-frequency migraine. Larger multicentric controlled studies are needed to confirm the signal detected in this study in order to bring more effective therapeutic options to a subgroup of migraine patients who are at high risk of chronification.
Keywords: BoNT-A; Episodic migraine; High frequency migraine; OnabotulinumtoxinA
Funding: This study is an investigator-initiated research study, partially supported by Allergan-Abbvie.

Table. Results: Comparison of the 28-Day Run-in Period With the Average of the Last Trimester After the Fourth Administration.

	Change from baseline (%)	*P* value
Headache days	-3,95 (-34,28)	<0.001
Migraine days	-3.73 (-33.57)	<0.001
Moderate/severe migraine, days	-2.70 (-27.38)	0.008
Acute headache pain medications, intake	-2.47 (-24.24)	0.029
Acute headache pain medications, intake days	-2.29 (-25.44)	0.021
MIDAS score	-10.07 (-39.75)	0.001
MSQ total score	-14.50 (-31.20)	<0.001

Midas=Migraine Disability Assessment Questionnaire; MSQ=Migraine-Specific Quality-of-Life Questionnaire.

STRUCTURE AND PH STABILITY OF BOTULINUM NEUROTOXIN X IN COMPLEX WITH NTNH

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Botulinum neurotoxins (BoNTs) are the most potent toxins known to man and are also used to treat an increasing number of medical disorders. They target the neuromuscular junction and inhibit synaptic vesicle exocytosis in motor neurons, thereby causing paralysis. The molecular architecture of BoNTs comprises the receptor-binding domain, translocation domain, and zinc-dependent protease domain. BoNTs are naturally co-expressed with a non-toxic non-hemagglutinin partner (NTNH) with which they form the minimal progenitor toxin complex to resist the low pH and proteases in the intestine, before they cross the intestinal barrier in the host. The full-length structures of BoNT/A, BoNT/B and BoNT/E have been determined and the structures of minimal progenitor toxin complexes of BoNT/A and BoNT/E are also known.
We have recently identified and characterized a new botulinum neurotoxin serotype, BoNT/X. It shares the lowest sequence identity with other BoNTs and is not recognized by antisera against known BoNTs. BoNT/X cleaves its substrates at a novel site and is the only BoNT that also cleaves

other non-canonical substrates. The only structural information currently available for this novel toxin is the structure of its protease domain (light chain).
We have determined the structure of the 300 kDa BoNT/X-NTNH complex at 3.12 Å resolution using single-particle cryo-electron microscopy. This structure together with the pH stability analysis of the complex provides the molecular basis to understand the toxin's interactions with its protective partner and also the evolutionary relationships between BoNT serotypes.
Keywords: BoNT; Cryo-EM; NTNH; Structure

SUCCESS OF BOTULINUM TOXIN TREATMENT IN POSTSTROKE SPASTICITY IN REAL-LIFE CLINICAL PRACTICE: DO THE FORMULATIONS AND DOSES MATTER?

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Introduction: Intramuscular injection of botulinum toxin type A (BoNT-A) is an effective treatment for poststroke spasticity, and Goal Attainment Scaling (GAS) is a method of assessing patient success in reaching individual goals. This study aimed at investigating the relationship of BoNT-A formulations and doses to the achievement of treatment goals in post-stroke spasticity.
Methods: Data regarding formulations, doses, and GAS scores were collected from patients' records in our spasticity clinic. Success was defined as primary goal achieved/overachieved (GAS scores = 0, 1, 2). Average total doses are stated as mean ± standard deviation, in units (U) of each formulation.
Results: Data from 2083 records of BoNT-A injections in 216 patients (55% male and 55% with right hemiparesis) were analyzed. The mean age at time of stroke was 55 years. AbobotulinumtoxinA (ABO) accounted for 68%, onabotulinumtoxinA (ONA) 15%, and incobotulinumtoxinA (INCO) 17% of injections.
Overall, average doses were 1123±369 U ABO, 408±155 U ONA, and 397±156 U INCO. The global success rate was similar (P=0.815) for each group. Average doses were not significantly different between successful and unsuccessful treatments across formulations.
Doses used for upper limb (UL) injections were: 815±302 U ABO, 294±96 U ONA, and 298±122 U INCO; for lower limb (LL), 610±292 U ABO, 217±115 U ONA, and 251±103 U INCO. The success rates were not statistically significantly different among the 3 formulations for either UL (P=0.642) or LL (P=0.117) injections, nor were the doses for successful and unsuccessful treatments. In the treatments of both UL and LL, the doses were ABO 1240±329 U, ONA 463±135 U, and INCO 443±146 U. The success rates were similar (P=0.636) among the groups, and the doses were not significantly different between successful and unsuccessful treatments.
Conclusions: The achievement of primary goals measured by GAS was similar among the 3 BoNT-A formulations, and we found no statistically significant relationship between the doses used and the success rates of the treatment sessions.
Keywords: Botulinum toxin; Spasticity; Stroke; Upper limb; Lower limb.

EFFICACY AND SAFETY OF INCOBOTULINUMTOXINA IN THE TREATMENT OF LOWER-LIMB SPASTICITY IN JAPANESE PATIENTS

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Objective: To confirm the efficacy and safety of treatment with incobotulinumtoxinA (INCO; Xeomin®, Merz Pharmaceuticals GmbH) in Japanese patients with post-stroke spasticity in the lower limb (LL), using the Modified Ashworth Scale spasticity score for the plantar flexors (MAS-PF).
Methods: This Phase 3, double-blind, placebo-controlled, randomized, multi-center study (CTI-153030) started with an open-label lead-in tolerability period (LITP) of 1 injection cycle of 400 U INCO in 11 patients. At the end of the LITP, a decision was made based on a safety assessment by an independent data monitoring committee to continue in the main period (MP) with the 400 U dose. In the MP, 208 patients were randomized to receive 1 injection of INCO (N=104) or placebo (N=104) into the LL muscles. Two hundred two patients were then enrolled in an open-label extension (OLEX) of 3 injection cycles, 10–14 weeks in duration (cycle 3 was fixed at 12 weeks). Changes in MAS-PF in both groups from baseline to Weeks 1, 4, 6, 8, and 12 and at each cycle in OLEX were assessed and compared.
Results: Tolerability of INCO was assessed as "good" or "very good" in all LITP patients. The efficacy of INCO (400 U) vs placebo was confirmed by means of area under the curve (AUC) of the changes in MAS-PF throughout the MP. INCO patients' AUC of the changes from baseline in the MAS-PF was statistically significantly greater compared to placebo (LS mean: −8.40 and −5.81; respectively [P=0.0041]; Table 1). The largest improvement was seen at Week 6. The mean (standard deviation) changes in MAS-PF from study baseline to end of OLEX cycle 1, 2, and end of study were −0.51 (0.63), −0.60 (0.65) and −0.83 (0.77), respectively, showing improvement across repeated injection cycles. No safety concerns were observed with INCO treatment.
Conclusions: This study confirmed the efficacy of INCO in a Japanese population with LL spasticity and showed that INCO at 400 U had a favorable safety and tolerability profile in this population.
Funding: Merz Pharmaceuticals GmbH, Frankfurt am Main, Germany
Keywords: IncobotulinumtoxinA; Japanese patients; Lower-limb spasticity; Post-stroke spasticity

Table 1
Summary statistics and ANCOVA of AUC for the change from baseline in MAS plantar flexors score to the end of the MP (BOCF, FAS).

	INCO (N=104)	Placebo (N=104)
Mean ± SD	−7.74 ± 7.01	−4.76 ± 5.84
LS mean ± SE	−8.40 ± 0.661	−5.81 ± 0.713
LS mean difference (INCO − placebo) ± SE	−2.59 ± 0.892	
95%CI	−4.35; −0.83	
P value	0.0041	

AUC, area under the curve; BOCF, baseline observation carried forward; CI, confidence interval; FAS, full analysis set; INCO, incobotulinumtoxinA; LS, least squares; MAS, Modified Ashworth Scale; MP, main period; N, number of subjects in each group; SD, standard deviation; SE, standard error.

DIGITIZATION OF TOXIN DEVELOPMENT

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Introduction: The biotechnology sector is moving towards digitization, with "Industry 4.0" becoming a common theme for 2020. Development of

drug substance manufacturing processes involves generation of large and complex data sets. Manufacturing processes typically contain several process steps, with multiple stages and analytical equipment that may in turn generate many outputs. The transition to digital data management therefore requires close collaboration between service provider and customer in order to suit the customer's needs.
Methods: We describe the process from project initiation to initial data analysis as follows:
(1) design and generation of the Discoverant® platform,
(2) integration of instrumentation and automation of data retrieval, analysis and sharing,
(3) data visualization to allow identification of batch abnormalities and to highlight areas that are off target or out of normal ranges and limits,
(4) identification of correlations within a process for a given product but also between products,
(5) characterization of the manufacturing process to assist process understanding and support future product manufacture.

Results: Through implementation of a digital data management platform, the automation and standardization of data retrieval have been realized. Data analysis and sharing capabilities have been enhanced and will aid future development of toxins and streamline development of new molecules as they enter the development pipeline. The platform can highlight trends and correlations not just for one toxin but for all toxins within a given portfolio.
Conclusions: The implementation of a digital data management platform has enhanced many areas of toxin bioprocess development. Information gained during the earlier stages of process development will aid decision making as projects move forward through clinical phases towards commercial manufacturing and lifecycle management.
Funding: Ipsen BioPharm Ltd
Keywords: Bioprocess; Drug substance; Industry 4.0; Manufacture

LONG-TERM EFFECTS OF BOTULINUM NEUROTOXIN TYPE A ON EXPERIMENTAL MUSCLE HYPERTONIA

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Introduction and Objectives: Long-term effects of botulinum neurotoxin type A (BoNT-A) are the basis of its beneficial effects on neurological disorders characterized by muscle hyperactivity, such as spasticity and dystonias. Clinical reports suggest that BoNT-A–mediated normalization of muscle hypertonia does not necessarily correlate with or outlast the duration of muscular flaccid paralysis. The present aim was to reassess these clinical observations in a preclinical model of muscle hypertonia by characterizing the duration of toxin anti-spastic activity in relation to the duration of its muscular effects.
Methods: In male Wistar rats, the muscle hypertonia was evoked by tetanus neurotoxin (TeNT) injection into the gastrocnemius (1.75-2 ng total dose). After the development of spastic hypertonia, BoNT-A was similarly injected into the ipsilateral gastrocnemius muscle 7 days post-TeNT (1, 2 and 5 U/kg//20 μL). The effects of TeNT and BoNT-A were further examined by measurements of resistance to passive ankle dorsiflexion, digit abduction score (DAS), and Basso-Beattie-Bresnahan (BBB) Locomotor Rating Scale. Muscle atrophy was quantified by measurement of the medio-lateral calf diameter. After complete recovery of BoNT-A–mediated peripheral flaccid paralysis, the animals were re-injected with TeNT (day 49 after BoNT-A injection) and assessed behaviorally for another 19 days.
Results: In the immediate period following the first TeNT injection, all BoNT-A doses employed exhibited a similar reduction of the muscle hypertonia. The TeNT-evoked muscle hypertonia ended by day 28 post-TeNT, while the remaining BoNT-A–mediated flaccid paralysis recovered in a dose-dependent manner by day 42 post–BoNT-A. After the second TeNT injection, the anti-spastic activity of BoNT-A was still present up to day 68; however, it was less prominent in rats injected with the lowest BoNT-A (1 U/kg) dose. The level of persisting muscle atrophy, which did not show signs of recovery by the end of the experiment, was similar at all doses employed.
Conclusions: In line with clinical reports, we found that the antispastic effect of BoNT-A in rats persists after the lower limb recovery from flaccid paralysis in a dose-dependent manner. Long-term actions of BoNT-A on muscle hypertonia might be mediated by a mechanism distinct from its local muscular paralytic action.
Funding: Croatian Science Foundation (project ID: UIP-2019-04-8277)
Keywords: Botulinum neurotoxin type A; Flaccid paralysis; Muscle atrophy; Muscle hypertonia; Tetanus neurotoxin

REAL-LIFE CRITERIA FOR USE OF MAXIMAL AND SUPRAMAXIMAL DOSES OF ABOBOTULINUMTOXINA IN POSTSTROKE SPASTICITY: IS IT WORTH IT?

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Introduction: Spasticity is a major disabling complication of stroke that may also lead to increased pain, joint contractures, skin problems, sleep impairment, and hence, reduced quality of life. Botulinum toxin type A (BoNT-A) intramuscular injection is the gold standard treatment for focal/multifocal spasticity. This study compares the use of maximal and supramaximal versus submaximal doses of abobotulinumtoxinA (AboBT) in the upper limb.
Methods: One hundred fifty-three adult patients with poststroke spasticity, who were treated with AboBT injections in the upper limb, with a total of 1261 injections were included in this study. They were divided by administered dose: $\geq$ 1000 U in group A (n = 320) and <1000 U in group B (n = 941). The sample was characterized by age, gender, number of treatment sessions, number of injected muscles, and AboBT doses used. The primary outcome was goal achievement measured with Goal Attainment Scaling (GAS). Treatment was successful when scores $\geq$ 0 per goal were achieved.
Results: Group A had a greater number of injected muscles per treatment session (7.60 ± 1.41, P<0.001) than Group B (4.99 ± 1.76, P<0.001). There was a small positive correlation between the increase in dose and the number of injected muscles (rho 0.264, P<0.001). There was no significant difference between the groups in treatment success measured by GAS $\geq$ 0 (primary goal: Group A vs Group B: X^2 (4) = 4.518, P=0.34; all goals: Group A vs Group B: X^2 (4) = 3.029, P=0.55). We did not record significant adverse events in any of the groups.
Conclusion: BoNT-A is a safe and effective treatment for focal poststroke spasticity. The use of maximal/supramaximal doses of AboBT may be an option in cases where more than 5 muscles need to be targeted, by allowing the achievement of the same success rates as within-SMPC (Summary of Product Characteristics) doses do, in the cases where fewer muscles need treatment.
Keywords: AbobotulinumtoxinA; Botulinum toxin; Spasticity; Stroke; Upper Limb

DURATION OF EFFECT OF INCOBOTULINUMTOXINA FOR THE TREATMENT OF BLEPHAROSPASM IN BOTULINUM TOXIN-NAÏVE SUBJECTS: RESULTS FROM A PHASE III STUDY

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Introduction: The efficacy of incobotulinumtoxinA, a botulinum neurotoxin type A free from complexing proteins, was investigated in a randomized, placebo-controlled, prospective, Phase III study in toxin-naïve subjects with benign essential blepharospasm (BEB) (NCT01896895). We report a sub-analysis of the flexible re-injection interval that was employed in this study to mirror clinical practice.
Methods: Treatment-naïve subjects (18-80 years) with bilateral BEB and Jankovic Rating Scale severity subscore $\geq$2 were enrolled in Greece, Malaysia, and Sri Lanka. In the double-blind main period, subjects were randomized (1:1:1) to single intramuscular injections of incobotulinumtoxinA 25 U (12.5 U/eye), 50 U (25 U/eye), or placebo. During a subsequent observation period of 6-20 weeks, the patient's subjective assessment of onset and waning of effect was recorded.
Results: Overall, 61 subjects were randomized (mean 55.0 years; 59.0% female) and included in this analysis. Data are reported as median (first quartile, third quartile). The treatment interval was longer with incobotulinumtoxinA 50 U (20 [6.1, 20.6] weeks) and 25 U (11 [6.1, 19.6] weeks) compared with placebo (6 [0, 19.2] weeks). Time to onset was shorter with incobotulinumtoxinA 50 U (5.0 [3.0, 10.0] days) and 25 U (7.0 [4.0, 22.0] days) compared with placebo (14.0 [4.0, 42.5] days). Time to waning was similar between treatment groups (incobotulinumtoxinA 50 U: 12.0 [8.0, 17.9] weeks; 25 U: 11.0 [3.0, 18.0] weeks; placebo: 11.5 [5.0, 18.2] weeks).
Conclusions: IncobotulinumtoxinA had a sustained duration of effect in toxin-naïve subjects with BEB, with a median treatment interval of 20 weeks and time to waning of 12 weeks with 50 U.
Keywords: Blepharospasm; Duration; IncobotulinumtoxinA

DELIVERY OF SINGLE-DOMAIN ANTIBODIES INTO NEURONS USING A CHIMERIC, TOXIN-BASED PLATFORM CURES BOTULISM IN MOUSE MODELS

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Efficient penetration of cell membranes and specific targeting of a cell type represent major challenges facing the development of therapeutics toward intracellular targets. One example of these hurdles is how to develop post-exposure treatment for botulinum neurotoxins (BoNTs), a group of bacterial toxins (BoNT/A-G) that are major potential bioterrorism agents. BoNTs enter motor neurons, block neurotransmitter release, and cause botulism, a paralytic disease. BoNTs, such as BoNT/A, exhibit extremely long half-lives within neurons, resulting in persistent paralysis for months, yet there are no therapeutics that can inhibit BoNTs once they enter neurons. Here we developed a chimeric, toxin-based delivery platform by fusing the receptor-binding domain of a BoNT, which targets neurons, with the membrane translocation domain and inactivated protease domain of the recently discovered BoNT-like toxin BoNT/X, which can deliver cargoes across endosomal membranes into the cytosol. A therapeutic protein was then created by fusing a single-domain antibody (nanobody) against BoNT/A with the delivery platform. In vitro characterization demonstrated that nanobodies were delivered into cultured neurons and neutralized BoNT/A in neurons. Administration of this protein in mice shortened the duration of local muscle paralysis, restoring muscle function within hours, and rescued mice from the systemic toxicity of lethal doses of BoNT/A. Finally, fusion of two nanobodies, one against BoNT/A and the other against BoNT/B, created a multi-valent therapeutic protein able to neutralize both BoNT/A and BoNT/B in mice. These studies provide an effective post-exposure treatment for botulism and establish a platform for intracellular delivery of therapeutics targeting cytosolic proteins and processes.

RELIABILITY AND VALIDITY OF THE MODIFIED HECKMATT SCALE IN EVALUATING MUSCLE CHANGES WITH ULTRASOUND IN SPASTICITY

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Introduction and Objectives: Despite being designed and validated in patients with Duchenne Muscular Dystrophy, the Heckmatt scale is often used more widely. Appreciating pathologic muscle changes may be important in the use of neurotoxins for spasticity management. This study assesses the reliability and validity of the Modified Heckmatt scale in assessing muscle echotexture under ultrasound, in patients with spasticity.
Methods: This is a prospective, observational study across two academic centres. Participants included 45 adults, diagnosed with upper and/or lower extremity spasticity, and 5 healthy adult references. Main outcome measures include Modified Heckmatt scale ratings and quantitative grayscale scores. Ultrasound images were obtained for muscles, de-identified, and reviewers blinded. Two trained resident and two ultrasound experienced staff physicians applied the Modified Heckmatt to grade 100 ultrasound images. Using quantitative grayscale software, clinician grades were further compared to software-generated scores.
Results: Interclass correlation coefficients of intra-rater 0.81, and inter-rater 0.76, (P<0.001) indicate good-to-excellent reliability within and between clinicians, respectively. A strong, significant relationship was found between the clinician Modified Heckmatt scores and quantitative grayscale scores ($r = 0.829$ (P<0.001)).
Conclusions: For patients with spasticity, the Modified Heckmatt scale shows good reliability and validity to assess pathologic muscle changes. This allows clinicians and researchers to differentiate and grade muscle echotexture under ultrasound for spasticity management. This impacts research and clinical decision-making around focal chemodenervation for potential treatment options, guiding procedure targets, and assessing patient outcomes.
Funding: This study was supported by an educational grant from New Westminster Rehabilitation Medicine.
Keywords: Botulinum toxins; Fibrosis; Muscle; Muscle spasticity; Reproducibility of results; Striated; Ultrasonography

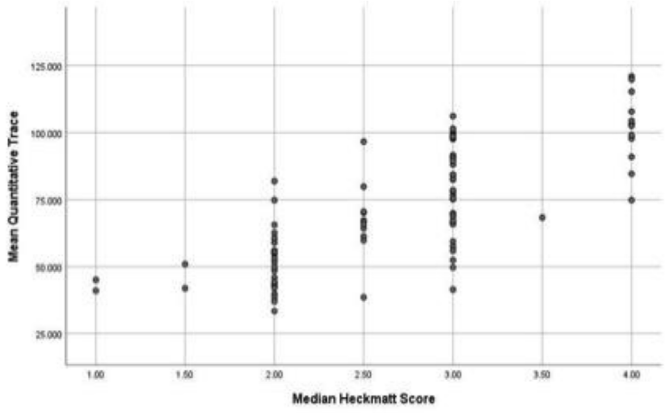

Figure. This graph demonstrates excellent correlation between quantitative grayscale data and median Modified Heckmatt scores.

RELAXATION METHODS IN REHABILITATION OF CHRONIC MIGRAINE

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Introduction: Chronic migraine (CM) is the most common and most severe form of chronic cephalgia, and is most often associated with psychiatric comorbidities. The use of relaxation methods after botulinum therapy in rehabilitation of CM addresses both muscular and emotional pain triggers. The aim of our study was to develop a strategy to improve the effectiveness of rehabilitation using relaxation methods.
Methods: The study included 24 patients, 19 (79.2%) of whom were women and 5 (20.8%) were men. CM was diagnosed using the criteria of The International Classification of Headache Disorders, 3rd edition. All patients were examined using specially designed headache diaries, the Headache Impact Test (HIT-6) scale, Leeds Dependence Questionnaire (LDQ), Migraine Disability Assessment (MIDAS) Questionnaire, Headache-Attributed Lost Time (HALT) Indices, the Beck Depression Inventory, and situational and personal anxiety scales. After botulinum toxin therapy, all patients underwent 10 relaxation training classes, which included progressive muscle relaxation, autogenic training, visualization, and diaphragmatic breathing.
Results: After the patients completed the relaxation sessions, the following statistically significant changes were observed: there was a decrease in indicators on the HALT indices from 43.1 ± 1.57 to 27.7 ± 2.2 (P<0.05); HIT-6 scale from 64.1 ± 2.1 to 59.8 ± 3.5 (P<0.05); MIDAS test from 43.1 ± 1.5 to 30.3 ± 2.0 (P<0.05); and LDQ from 11.5 ± 5.2 to 7.89 ± 2.97 (P<0.05). The level of depression on the Beck scale decreased from 20.0 ± 4.5 to 11.76 ± 4.5 (P<0.05).
Conclusion: The use of relaxation methods in rehabilitation therapy of CM resulted in a reduction in the frequency of headaches, improvement in the quality of life, and a favorable impact on psychiatric comorbidities.
Keywords: Botulinum toxin therapy; Chronic migraine; Relaxation methods

FIRST RESULTS OF AN OUTPATIENT REHABILITATION CLINIC FOR FACIAL PALSY SEQUELAE TREATED WITH BOTULINUM TOXIN: PROFILE AND PROTOCOLS

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Introduction and Objectives: The sequelae of facial palsy are a common consequence of Bell's palsy, neurosurgery, and head trauma. Although frequent, it is an undervalued and often undertreated condition, in the context of rehabilitation. The need for quality care for these patients motivated us to initiate an outpatient rehabilitation service, specifically aimed at the sequelae of facial palsy. This article presents the profile of our service, highlighting the characteristics of the patients and protocols used, to help other services offer and improve the rehabilitation of facial palsy sequelae worldwide.
Methods: We delineate how we manage patients: from admission, therapeutic planning, assessment scales, treatment objectives, and the methods employed. We also describe the characteristics of the patients evaluated between September 1, 2019, and September 30, 2020, along with the tools used to evaluate the treatment results.
Results: On admission, patients were evaluated by a neurologist and a physiotherapist, using active movement evaluation and the Sunnybrook Facial Grading System. The goals of treatment were to reduce synkinesis and improve facial symmetry using individualized doses of botulinum toxin. An additional objective was to enhance facial motor control, using specialized physiotherapy after botulinum toxin injection, without electric stimulation. After evaluation, a request for the procedure was submitted to the health insurance companies that supply the medication and authorize the medical procedures. IncobotulinumtoxinA (Xeomin®) injections at doses of 30-100 UI were administered by a neurologist, according to the individualized plan for each patient, and applied to both the synkinesis-affected and the unaffected sides of the face (to treat hypercontraction and improve symmetry). Fifteen days after the injections, the patients were re-evaluated, and results were assessed using the Sunnybrook Facial Grading System and compared to previous results. From September 1, 2019, to September 30, 2020, a total of 77 patients with facial palsy sequelae participated: 75% were female, 25% were male. The median age was 62.5 years.
Conclusions: Facial palsy sequelae constitute a frequent medical condition that requires adequate evaluation and treatment. Botulinum toxin injections followed by physiotherapy appears to be an effective treatment option for these patients. Structured services are needed to offer adequate treatment to these patients.
Keywords: Facial palsy; Paralysis; Sequelae; Synkinesis; IncobotulinumtoxinA

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INCOBOTULINUMTOXINA COMBINED WITH PHYSIOTHERAPY IN THE TREATMENT OF FACIAL PALSY SEQUELAE: RESULTS

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Introduction and Objectives: Facial palsy is a devastating condition. Facial expressions communicate feelings, reactions, and emotions. The perception of beauty is closely related to the concept of symmetry, and facial palsy disrupts this facial feature. We present the results of a sample of patients who attended our service, and received treatment with incobotulinumtoxinA injections combined with facial physiotherapy.
Methods: The Sunnybrook Facial Grade System (SFGS) was used to assess patients before and after injection of incobotulinumtoxinA (Xeomin®) at doses of 30-100 UI. We evaluated facial weakness, facial function, and synkinesis, and, in planning the injections, targeted the muscles that initiate or sustain synkinesis. Based on an evaluation of facial symmetry, we injected toxin in the normal side of the face, if necessary, to minimize hyperexpression or hypercontraction of the face. Physiotherapy was applied according to the needs of the patients to improve facial motor control. These exercises were done in front of the mirror, under the direction of the physiotherapist, who taught patients to activate the muscles more consciously, isolating muscles in each movement as much as possible.
Results: Twenty-three patients (17 females, 6 males; average age: 39.2 years) participated.
All patients showed improvement on their individual SFGS scores (Table).

Table. SFGS scores.

Patient	Gender	Age	Etiology	SFGS pre-treatment	SFGS post-treatment
JL	F	33	Bell's Palsy	12	18
AJW	F	30	Bell's Palsy	70	72
DLA	F	36	Bell's Palsy	40	53
MAT	F	49	Bell's Palsy	28	37
LG	M	34	Bell's Palsy	43	47
PB	F	30	Bell's Palsy	44	48
PC	F	41	Bell's Palsy	25	31
JRL	M	56	Post-operative - Acoustic neuroma	27	33
VC	F	42	Bell's Palsy	63	73
AA	M	35	Cholesteatoma	25	34
DL	F	36	Bell's Palsy	28	37
VZB	M	36	Post-operative - Acoustic neuroma	56	65
RRM	M	43	Post-operative - Acoustic neuroma	33	41
VCC	F	43	Bell's Palsy	43	63
ES	F	44	Bell's Palsy	5	20
TS	F	45	Bell's Palsy	35	47
PB	F	34	Bell's Palsy	38	56
ES	F	63	Bell's Palsy	55	57
MAU	F	43	Bell's Palsy	24	31
AC	F	29	Post-operative - Acoustic neuroma	34	36
AJW	F	30	Bell's Palsy	43	44
SF	F	30	Bell's Palsy	49	52
AA	M	35	Cholesteatoma	24	27

Conclusions: IncobotulinumtoxinA injections, applied to the muscles responsible for synkinesis, and also in the non-paralyzed side of the face (to improve facial symmetry), are an effective alternative to the standard treatments of facial palsy sequelae. Use of physiotherapy as an adjunct to incobotulinumtoxinA injection improved the results and helped to improve motor control of facial expression. Structured services are needed to offer adequate treatment to these patients.
Keywords: Facial palsy; Paralysis; Sequelae; Synkinesis; IncobotulinumtoxinA

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EXERCISE-INDUCED SPREAD OF NEUROTOXIN FOLLOWING CHEMODENERVATION OF NECK MUSCLES. REPORT OF FOUR CASES

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The effects of exercise following cervical chemodenervation are unknown, while exercise after limb muscle injection is known to enhance the effects. We report four cases where exercise following cervical chemodenervation was associated with adverse events or no benefits.
The injections were under EMG guidance and dosing ranged from 70-175 units of onabotulinumtoxinA. Case 1: Patient worked for 6 hours salvaging computers, boxes, and other equipment from a flooded floor. Case 2: Patient worked for more than 4 hours lifting 65 ink containers (17-30 lbs) 130 times. Case 3: Patient did outdoor painting for several hours.
Case 4: Patient worked for 6 hours cleaning up the PTA room and lifting boxes. Afterwards, she applied a heating pad for more than a couple of hours. Adverse events ranged from dysphagia, dysarthria, blurred vision, muscle weakness, heavy head, and numbness, to no benefits. In conclusion, chemodenervation of neck muscles followed by exercise can exacerbate the spread of neurotoxin, limit the therapeutic effect, and cause adverse events.

EFFECTS OF RECOMBINANT BOTULINUM NEUROTOXIN TYPE A1 ON CFA-INDUCED MECHANICAL ALLODYNIA AND SENSORY NEURON RESPONSES TO MECHANICAL STIMULATION MONITORED WITH GCAMP FLUORESCENCE IN MICE

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Introduction: New analgesics are needed for the treatment of inflammatory and neuropathic pain. Botulinum neurotoxin (BoNT) has been successfully used in patients with neuromuscular dysfunction and has also become a third-line treatment for chronic migraine and neuropathic pain.[1,2] Clinical BoNT treatment is well tolerated with minimal adverse reactions. BoNT type A1 (BoNT/A) cleaves SNAP-25, necessary for the calcium-mediated vesicular release of neurotransmitters. Nonetheless, the efficacy of BoNT/A for the treatment of pain has not yet been fully elucidated. A study on cultured dorsal root ganglia (DRG) neurons indicates a reduction in slowly adapting currents upon mechanical stimulation in cells treated with BoNT/A.[3] In this study, we investigate the analgesic efficacy of intraplantarly administered recombinant BoNT/A1 in mice with complete Freund's adjuvant (CFA)-induced inflammatory pain by behavioural

experiments and in vivo imaging of calcium transients in the DRG using mechanical stimuli.
Methods: C57/BL6 mice received an intraplantar injection of CFA on day -2. Two days later (day 0) the animals were injected intraplantarly with rBoNT/A1 (5 pg/mouse in 20 μL vehicle) or vehicle only (gelatine phosphate buffer; GPB; n=10/group). Von Frey filaments were used to measure mechanical sensitivity (paw withdrawal threshold; PWT) on day -2 (baseline), day 0 (before rBoNT/A1 injection) and day 3. In a different group of C57/BL6 mice, postnatally injected with GCaMP6s-AAV9 into one hind paw, in vivo calcium imaging was conducted. In line with the behavioural test, as adults, the animals were treated intraplantarly with CFA (on day -2) followed by a single rBoNT/A1 (5 pg/animal) injection on day 0. In vivo imaging of L4-DRG neurons was performed under isoflurane anaesthesia on day 3. Peripheral stimulation of the glabrous paw skin with an electrically controlled mechanical probe was executed.
Results: On day 0, two days following CFA administration, a marked reduction of PWT, indicating mechanical allodynia, was measured. A single intraplantar rBoNT/A1 application resulted in significant increase in PWT ($P<0.01$) on day 3 in comparison to vehicle treatment.
Spontaneous and evoked calcium transients were recorded in L4-DRG neurons by in vivo microscopy in anaesthetised, GCaMP6s-transfected adult mice treated with CFA. Spontaneous calcium transients were less prominent in rBoNT/A1-treated animals. After baseline recording, peripheral mechanical stimulation of the inflamed glabrous paw skin induced a prompt calcium flux that triggered a long-lasting calcium discharge. The mechanically evoked signal remained unchanged in rBoNT/A1-treated mice but the discharge of calcium signals after mechanical stimulation was reduced.
Conclusions: Peripheral injection of CFA into the hindpaw of mice resulted in mechanical allodynia and evoked spontaneous calcium fluctuations in DRG neurons. rBoNT/A1 reduced the CFA-induced mechanical allodynia and altered discharges reported by calcium flux in DRG neurons. Thus, the peripheral sensory nervous system is important for the analgesic efficacy of rBoNT/A1 in inflammatory pain.
Conflict(s) of Interest: Ipsen provided the recombinant botulinum neurotoxin A (SXN102342).
Source(s) of Financial Support for the Project: The study was sponsored by IPSEN Innovation.
Keywords: Calcium signal; Inflammatory pain; In vivo imaging; Peripheral pain

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DOSING FROM A PHASE 3 PIVOTAL STUDY OF ABOBOTULINUMTOXINA INJECTION IN UPPER-LIMB MUSCLES IN PEDIATRIC PATIENTS WITH CEREBRAL PALSY

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Introduction: We report dosing data from a phase 3 pivotal study of repeat, upper limb abobotulinumtoxinA (aboBoNT-A) injections in children with cerebral palsy (CP).
Methods: This was a double-blind, repeat treatment (up to 4 cycles) study (NCT02106351). In the first cycle, children were randomized to aboBoNT-A 8 U/kg, 16 U/kg or 2 U/kg control dose groups. Doses were divided between the primary target muscle group and additional muscles tailored to clinical presentation.
Results: Two hundred twelve children were randomized, of which 210 received ≥1 aboBoNT-A injection. Per the protocol, the elbow and wrist flexors were the most commonly injected upper limb muscles. Across all 4 cycles, the brachialis was injected in 89.5% of children (dose range 0.8-6U/kg), the brachioradialis in 83.8% (0.4-3 U/kg), the flexor carpi ulnaris in 82.4% (0.5-3 U/kg), and the flexor carpi radialis in 79.5% (0.5-4 U/kg). The next most frequently injected muscle was the pronator teres, which was targeted in 70.0% of children (0.3-3 U/kg). Other frequently injected upper limb muscles were the adductor pollicis (54.3%, 0.3-1 U/kg), pronator quadratus (44.8%, 0.1-2 U/kg), flexor digitorum superficialis (39.0%, 0.5-4 U/kg), flexor digitorum profundus (28.6%, 0.5-2 U), flexor pollicis brevis/opponens pollicis (27.6%, 0.3-1 U/kg), and biceps (27.1%, 0.5-6U/kg). AboBoNT-A was generally well tolerated at these doses. Muscular weakness was reported in 4.3% of children in the 8-U/kg group and 5.7% in the 16-U/kg group.
Conclusions: These data provide information on the dose ranges used during this phase 3 study, which were well tolerated. In line with the protocol, most children received injections into the elbow and wrist flexors. However, there was a wide variety of other upper-limb muscles injected as physicians tailored injection patterns.
Funding: Ipsen
Keywords: AbobotulinumtoxinA; Dysport; Paediatric; Spasticity

THE SYSTEMATIC APPROACH TO DEVELOPING A CELL-FREE PLATFORM PROCESS FOR RECOMBINANT TOXIN PRODUCTION

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Introduction: The manufacture of highly potent biologics, such as toxins, has stringent safety requirements, which make the conventional, cell-based production process complex and challenging. The cell-free process platform developed here provides a viable alternative that makes toxin manufacture safer and decreases batch production times. A novel approach to scaling up was adopted to achieve productivity levels deemed viable for potential commercialization; the proof-of-concept was demonstrated in a production-scale vessel.
Methods: The Design of Experiment (DoE) methodology was used to discover new interrelationships amongst process parameters crucial to improving product titre and quality. This systematic optimization approach ensured that the increase in productivity seen during the small-scale development phase translated to the production-scale vessel with negligible loss in performance. Process development work was carried out in the Eppendorf DASBox parallel system with BioBLU 0.3f single-use vessels, and after that scaled up in the BioFlo® 320 reusable bioreactor.
Results: The scale-up criteria facilitated a similar external environment across scale, which ensured that productivity remained comparable. Also, factors such as pH, dissolved oxygen, temperature, and certain enzymes were identified as critical parameters for toxin production.
Conclusions: A commercially feasible, cell-free recombinant neurotoxin production process has been developed. The production platform designed here encourages a multiproduct approach to biologics manufacture, which further reduces cost and improves facility turnaround.
Funding: Innovate UK, project grant - 102610
Keywords: Cell-free bioprocess; Cell-free neurotoxin; Cell-free process

development; Neurotoxin production; Scale-up of toxin manufacture; Synthetic manufacture of biologics

RESULTS OF A THREE-YEAR STUDY OF THE USE OF BOTULINUM TOXIN IN PATIENTS WITH POSTHERPETIC NEURALGIA

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Introduction: Postherpetic neuralgia (PHN) is a painful condition that is defined as pain that persists for 90 days or more after reactivation of herpesvirus type 3. The development, occurrence, and suppression of neuropathic pain are associated with peripheral and central factors. Peripheral sensitization is caused by a prolonged flow of pain impulses that comes from the virus-damaged skin segment and adjacent nerves, with pain sensation then transmitted to the dorsal horn of the spinal cord, where neurons—the first integrative centers in the central nervous system—are located. As a result of excessive sensitization of the dorsal nuclei of the spinal cord, increased nociceptive impulses are transmitted to the central areas of the analgesic system, leading to a downregulation of inhibitory pain pathways in these structures. Thus, for the effective treatment of PHN, a pathogenetically appropriate therapeutic must not only impact the central pain mechanisms but also the source of peripheral pain sensitization. Pain in PHN is not relieved by conventional nonsteroidal analgesics. Currently, anticonvulsants and antidepressants that affect the central mechanisms of pain are the mainstay of PHN therapy. These medications are poorly tolerated, especially by the elderly. The need for therapies that can act on peripheral sensitization and modulate the pain in PHN is an urgent problem.

Materials and Methods: Based on the national and international experience of using botulinum toxin type A (BTA) for various types of pain and neuralgia, we conducted a 3-year study of the use of botulinum toxin in PHN. We proposed a hypothesis that the outcome of botulinum toxin treatment is dependent on the patient's age and the duration of PHN.

We found changes in the skin before and after treatment of herpes zoster. The influence of botulinum toxin on skin morphology in the area affected by PHN and on the unaffected side was evaluated. The study involved 32 patients with PHN, who had the area of PHN determined and the skin examined in ultraviolet light and using skin ultrasound. Patients were divided into two groups: group I, patients aged <73 years (n=14), and group II, those aged >73 years (n=18). We also categorized 2 groups based on the duration of PHN: group A, patients with PHN duration <1 year (n=18), and group B, patients with PHN duration >1 year (n=14). Patients were examined by a neurologist, and the boundaries of altered sensitivity were determined by the von Frey method. The intensity of pain before and after treatment was evaluated using a Visual Analog Scale (VAS); the nature of the pain was studied using the McGill Pain Questionnaire.

We observed the changes in the skin before and after treatment. To do this, we evaluated changes in the area and intensity of postherpetic hyperpigmentation in visible (normal) light and using ultraviolet light (Wood's lamp). Also, before and after treatment, an ultrasound (22 MHz) examination of the skin was performed, and changes in the thickness and density of the dermis were evaluated.

After the examination, intradermal and subcutaneous injections of botulinum toxin were performed using a standard solution (100 units of botulinum toxin type A reconstituted in 2 mL of 9% NaCl). We injected 1 unit at each injection site 1 cm apart, in the affected areas. We also identified areas with the most pronounced sensitivity disorders. In these zones, we kept a lesser distance between injection sites (0.5 cm). Cases of PHN involve the first ramus of the trigeminal nerve. To avoid asymmetry, botulinum toxin was injected symmetrically in the healthy side in the same doses. Results were evaluated on the tenth and thirtieth days after botulinum therapy.

Results:

1. The borders of the area with changed sensitivity indicated by the patients coincided almost exactly with the borders of changes in skin pigmentation that we see when examining the skin in ultraviolet light, and do not visualize in normal light.
2. Changes in the pigmentation that can be directly identified from a picture before treatment also approximate the changes of the border of the area with altered skin sensitivity. We can clearly see the reduction of intensity and area of pigmentation. This border also corresponds to changes in the patient's sensitivity in this area.
3. On high-frequency ultrasonography, there is an increase in the thickness and density of the dermis, as a result of an increase in the synthesis of fibrous components and the quantity of the matrix. The density of the plasma was 4.8%.
4. The VAS and McGill Pain Scale were used to explore changes in the intensity of pain, itching, and allodynia (on days 10 and 30). On average, in groups I and II, the score on the VAS was 5.8 points before treatment and 3.2 points 30 days after treatment. We conclude that the pain decreased more for younger patients (group I) with botulinum toxin therapy.
5. Patients in group A showed a more pronounced increase in dermal density than those in group B (6.2±1.5 versus 3.4±1.3), and the area of PHN decreased almost 2-fold more among group A patients than group B patients.

Keywords: Botulinum toxin; Pain; Postherpetic neuralgia

A CLINICAL CASE OF BOTULINUM TOXIN USE IN POSTHERPETIC NEURALGIA

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Introduction and Objectives: The most common complication of herpes zoster is postherpetic neuralgia (PHN). Unlike other pain syndromes, PHN pain is not relieved by classical analgesics. The treatment protocols include medications that have many restrictions on use. Choosing therapy is also complicated because PHN is more common among the elderly, who usually suffer from concomitant pathologies. Based on previous international and local experience, we decided to conduct a case study on the use of botulinum toxin to reduce neuropathic pain.

M., a 79-year-old male, complained of periodic allodynic pain, numbness, and increased sensitivity in the right lower back; sleep disorders; and depression.

History of disease: The patient began to complain of pain and rashes in the lumbar region from 3 October, 2017, and was prescribed valacyclovir. Herpes is associated with severe emotional stress and hypothermia. Due to the development of shortness of breath and aggravation of concomitant pathology, the patient was hospitalized. From 10 October, 2017 to 20 October 2017, he was treated in the infectious diseases department for a clinical diagnosis of herpetic infection: herpes zoster of moderate severity affecting the L2-3 spinal nerves.

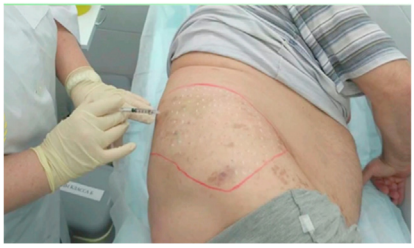
Concomitant conditions: The patient was diagnosed with ischemic heart disease, atherosclerotic cardiosclerosis, New York Heart Association Class

III heart failure, second-degree hypertension, chronic obstructive pulmonary disease, Type II respiratory failure, and secondary pulmonary embolism.

Treatments: Antiviral therapy (acyclovir 400 mg 5 times a day for 5 days), disinfection therapy, Ascorutin (Vitamin C [ascorbic acid]), carbamazepine, NSAIDs, and prednisolone were administered. The patient was discharged in a satisfactory condition: there were no new vesicles in the affected L2-3 area, but crusts and depigmented spots were detected. In January 2018, the patient visited a neurologist with complaints of pain, numbness, tingling, and hypersensitivity in the right lumbar region with consequent sleep deprivation and depression. He was diagnosed with PHN. Anticonvulsants (gabapentin) and antidepressants (amitriptyline) were prescribed. Due to the ineffectiveness of these treatments, inability to increase medication doses, and the presence of severe comorbidities, the patient was invited to participate in a study of botulinum toxin therapy for complications of herpes zoster.

We performed photofixation, measurement of the affected area, and ultrasound of the affected and healthy areas of skin. Assessment of pain and the boundaries of altered sensitivity was performed using the Von Frey method, Visual Analogue Scale, and the McGill Pain Questionnaire. The patient was treated with botulinum toxin type A (BTA) in a standard dilution at a dose of 1 unit per injection site 1 cm apart; the total dose was 198 units BTA (Pic. 1).

Pic. 1



Results: On the 10th day after treatment, the patient noted a decrease in night pain episodes, an increase in the intervals between pain attacks, and a decrease in pain intensity from 8 points to 6 (on the VAS). One month after therapy, there was a reduction in the area of sensitivity.

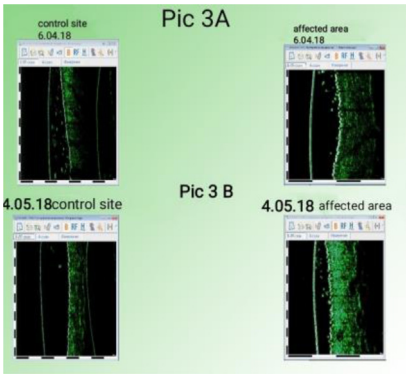
We compared the area of postherpetic hyperpigmentation before and after botulinum toxin therapy in ultraviolet light. We noted a decrease in the area of hyperpigmentation (Pic. 2A and 2B).

Skin ultrasound (22 Hz sensor) showed an increase in the thickness and uniformity of the dermis. (Pic. 3A and 3 B).

The patient became more comfortable socially and better adapted emotionally.

Conclusions: Botulinum toxin therapy has shown good efficacy and tolerability in treating PHN in this patient. Further study of the use of BTA in PHN is needed. If proven, the method can become an alternative treatment for neuropathic pain.

Keywords: Botulinum toxin; Pain; Postherpetic neuralgia



TREATMENT OF PERSISTENT NYSTAGMUS IN CHILDREN USING INCOBOTULINUMTOXINA

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Introduction: Nystagmus is a syndrome that manifests as spontaneous oscillatory movements of the eyes. The condition has been of interest in various fields of medicine for many years. Conservative methods of treatment are ineffective, and surgery is traumatic with the risk of postoperative complications. Herein, we consider a minimally invasive procedure with short postoperative recovery period and minimal risk of complications.

Objective: To investigate the effectiveness of incobotulinumtoxinA (incoBTA) treatment of nystagmus in children.

Methods: Twenty patients (40 eyes; 10 boys, 10 girls; average age: 2 years [y] and 9 months [mo]; range: 8 mo-8 y and 7 mo) were included in the study over a 12-month follow-up period. Children with permanent nystagmus were included, while those with temporary symptoms, younger than 6 months, or older than 18 years were excluded.

All patients were injected with incoBTA in the extraocular muscles at an average dose of 3.1 UI. The injections were performed under visual guidance using specially designed surgical tweezers to hold the oculomotor muscles in the operating room using mask induction of anesthesia with sevoflurane. The procedure lasted $\leq$1-2 minutes.

Results: A positive result was observed throughout the observation period in 55% of the patients treated with a single incoBTA injection. Forty-five percent of the group required repeat injections at intervals of 4 to 11 months. The course of treatment of this latter group of patients will be described in subsequent reports. No adverse events were observed after the injections.

Conclusions: A decrease in oscillatory movements was noted due to a lowering of muscle activity after the operation, which is associated with a reduction in the supply of motor impulses to the muscles. An improvement in visual function was also noted due to eye accommodation. Treatment of nystagmus using incoBTA injections into the extraocular muscles can be offered as an effective, safe, and minimally invasive approach.

Keywords: Botulinum toxin therapy; Chemodenervation; Incobotulinumtoxin A; Nystagmus; Nystagmus treatment; Surgical treatment of nystagmus

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MOLECULAR MECHANISMS OF INTERNEURONAL TRANSFER OF PATHOGENIC MOLECULES

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Introduction and Objectives: Tetanus neurotoxin (TeNT) and botulinum neurotoxin (BoNT) are clostridial neurotoxins, which show high specificity for the nervous system. They impair neurotransmitter release, causing spastic and flaccid paralysis, respectively. Clostridial neurotoxins are composed of two subunits: a heavy chain (H) containing the binding and membrane translocation domains, and a light chain (L) which is embedded with proteolytic activity.
This activity is specific for components of the synaptic SNARE complex, which is essential for neurotransmitter release at synaptic terminals. For a long time, it was believed that BoNT effects were restricted to the injection site. However, independent lines of evidence indicate that some of their therapeutic effects are due to their activity at distal loci. In the central nervous system, BoNTs show a preference for cholinergic terminals, but they can affect other neuronal synapses, such as GABAergic and glutamatergic synapses. However, the precise mechanism by which TeNT and BoNTs preferentially affect some nerve terminals is not yet completely understood. Our aim is to uncover this molecular mechanism using both in vitro and in vivo approaches.
Methods: In vivo: TeNT was injected into the whisker pad or in peripheral leg muscles, the tibialis anterior and gastrocnemius, of anaesthetised mice. A few days after the injection, brain or spinal cord different types of primary neurons in sections were collected. We determined which synaptic terminals in the brain are preferentially targeted by TeNT after retrograde axonal transport by immunohistochemistry.
In vitro: Different types of primary neurons were cultured in microfluidic chambers (MFCs) with two or three compartments connected by microgrooves. The fluidic isolation of the system allows us to differentially treat a selected compartment with molecules and pharmaceutical agents without affecting the others. Full-length toxins or the binding fragment of TeNT (HcT) conjugated with a fluorophore are applied in the distal compartment containing the axonal terminals.
Results: In vivo: We analysed the trafficking of TeNT in the central nervous system after injection of this neurotoxin into peripheral muscles. We found that TeNT shows a preference for GABAergic terminals, compared to cholinergic and glutamatergic synapses. We are now validating the results in spinal cord motor neurons.
In vitro: Since the routes of transport and spread exploited by neurotoxins might be hijacked by pathological protein aggregates, such as synuclein and tau, we are culturing different types of primary neurons in MFCs to understand the molecular mechanisms regulating toxin propagation. This system allows us to analyse the trans-synaptic transfer and action of both HcT and full-length toxins in a simplified neuronal circuitry.
Conclusions: TeNT and BoNTs are retrogradely transported in the central nervous system and they preferentially target specific neuronal subtypes. To enter and propagate through the nervous system, they exploit physiological routes and mechanisms that may be hijacked by other pathogenic agents. Using our approach, we identify these pathways, thus gaining a better understanding of how different pathogenic molecules can propagate in the nervous system.
Keywords: Alzheimer's disease; BoNT/A; Secretion; Transmission

ABOBOTULINUMTOXINA IN THE MANAGEMENT OF HALLUX VALGUS IN ADULT PATIENTS: RESULTS OF A RANDOMIZED AND PLACEBO-CONTROLLED PHASE II TRIAL

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Introduction: Hallux valgus (HV) affects ~25% of adults globally, causing pain and functional disability. This placebo-controlled study (NCT03569098) assessed the effect of abobotulinumtoxinA (aboBoNT-A) injections vs placebo (PBO) on pain and disability in HV.
Method: Patients received aboBoNT-A 300 U, 500 U, or PBO (1:1:1) in a ≥12-week double-blind phase followed by an open-label (OL) phase (OL Cycle 1, 300 U [all patients]; OL Cycle 2, 300 U, or 500 U [investigator judgement]). Patients: ≥18 years; HV angle 15-30°; numeric pain rating scale (NPRS) score of ≥4 (0=no pain; 10=worst possible pain). Primary endpoint: change from baseline (BL) in self-reported NPRS score (mean of 7 days prior to each timepoint) at Week 8. Post hoc endpoints: percentage of days patients' NPRS scores were: 1) lower than their lowest daily score at BL; and 2) ≥2 points lower than mean BL score. Adverse events (AEs) were recorded.
Results: Primary endpoint: no significant differences for aboBoNT-A 300 U (n=63) or 500 U (n=60) vs PBO (n=63, least squares mean [standard error]: -1.7 [0.3], -2.4 [0.3], vs -2.0 [0.3], respectively). Scores showed a trend towards reduced pain at Week 12 for aboBoNT-A 500 U (-2.4 [0.3]) vs PBO (-1.7 [0.3]; P=0.06), further reduced in OL Cycle 1. Post hoc analyses: significantly more days were spent with reduced pain at Week 8 with aboBoNT-A 500 U, vs PBO, respectively: analysis 1) 63% vs 38% (P<0.01); analysis 2) 55% vs 37% of days (P=0.06), with similar results at Week 12 (analysis 1: P<0.01; analysis 2: P=0.02). No new or unexpected AEs were reported.
Conclusions: The primary endpoint (Week 8) was not met. A trend towards significant pain reduction with aboBoNT-A 500 U vs PBO was reported at Week 12 and further reduced with repeat injection. Post hoc analyses showed a greater number of days spent with reduced pain with aboBoNT-A 500 U vs PBO, perhaps a more clinically relevant assessment of benefit than mean NPRS score. AEs aligned with the known profile of aboBoNT-A.
Funding: Ipsen
Keywords: AbobotulinumtoxinA; Botulinum toxin; Bunion; Clinical outcomes; Hallux valgus; Pain

INCOBOTULINUMTOXINA INJECTION FOR SEBUM CONTROL, FACE LIFTING, AND PORE SIZE IMPROVEMENT

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Introduction and Objectives: Previous studies have indicated intradermal injections of botulinum toxin products may improve sebum secretion, facial skin laxity, and facial pores.[1-6] However, incobotulinumtoxinA has not been studied for these indications. This study aimed to evaluate whether intradermal injections of incobotulinumtoxinA improve sebum secretion, face laxity, and facial pores.
Methods: This was a single-center, retrospective, clinical study. Patients treated with intradermal incobotulinumtoxinA for sebum control, face lifting, and pore size improvement were included if they had 3 follow ups over 12 weeks after the procedure. Under the protocol, injection points included the lateral face, anterior medial cheek, mandibular line, depressor anguli oris points, mid-glabella area, and chin. Assessment of sebum production was measured using a sebumeter. Mandibular length and facial pores were assessed using a 3-dimensional scanner. Facial laxity was assessed by facial laxity ratings and the global aesthetic improvement scale (GAIS).
Results: A total of 20 patients met the criteria for inclusion. All outcomes—sebum secretion, mandibular length, facial pores, and facial laxity ratings—were improved at 1 week, achieved maximum improvement after 4 weeks, and sustained improvements through 12 weeks.
All subjects scored grade 2 or more on the GAIS after 4 weeks. No adverse reactions or complications were reported during the 12-week follow up.
Conclusions: This is the first study to show that intradermal microdroplet injection with incobotulinumtoxinA improves facial laxity, sebum secretion, and facial pore count up to 12 weeks after injection.
Funding: Merz Pharmaceuticals GmbH
Keywords: Botulinum toxin; Facelift; Facial pore; IncobotulinumtoxinA; Sebum

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DEVELOPMENT OF THE HYGIENE EXTENSION LIMB POSITION PAIN (HELP) TOOL TO MONITOR WANING OF CLINICAL EFFICACY IN PATIENTS WITH SPASTICITY OR CERVICAL DYSTONIA TREATED WITH BOTULINUM TOXINS

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Introduction: The HELP Tool is a patient-reported outcome (PRO) instrument, designed to provide greater discernment of waning clinical efficacy post–botulinum neurotoxin type A (BoNT-A) injections before the next scheduled treatment in patients with upper and/or lower limb spasticity or adults with cervical dystonia (CD). The impact of spasticity or CD symptom recurrence will be better gauged with the HELP Tool, helping to determine appropriate treatment optimization. It is envisaged that the HELP Tool will be used by patients (as well as parents/caregivers) with upper and/or lower limb spasticity or CD.
Methods: Working with a consultant experienced in the development of PRO instruments, a panel of nine subject matter experts developed preliminary ~20-item questionnaires for potential use with patients with spasticity and CD and their parents/caregivers. Content validity will be assessed qualitatively (via patient feedback) and the questionnaire modified to provide a clinically useful screening tool to identify when treatment is waning prior to the next scheduled BoNT-A treatment and the effect on patients' quality of life. Psychometric properties of the new tool will be assessed quantitatively. Patient satisfaction with treatment relevant to symptoms will be evaluated. After the first 12 interviews of adult spasticity and CD patients for concept elicitation, a virtual advisory board with subject matter experts will develop a relevant, meaningful HELP Tool and ensure content validity. This will be followed by a second round of testing of the questionnaires via cognitive debriefing interviews in a larger patient and parent/caregiver cohort, with subsequent HELP Tool finalization.
Results: This study was conducted in the United States and Canada. The outcome of the HELP Tool development will be presented.
Conclusions: The development process of the HELP Tool has led to an easy-to-complete, short (5- to 10-minute) PRO instrument for clinical use .
Funding: Ipsen, Inc. (Cambridge, Massachusetts, USA)
Keywords: Spasticity; Cervical dystonia; Botulinum neurotoxin type A

THE ONE21 APPROACH COMPARED WITH STANDARD 5-POINT STRATEGY FOR TREATING GLABELLAR LINES WITH INCOBOTULINUMTOXINA

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Introduction and Objectives: Botulinum toxin type A improves dynamic glabellar lines.[1-3] Because there is individual variation in the muscle groups involved in the wrinkling of the glabella,[4] an individualized treatment approach could provide optimal results. This study examined whether a customized approach for treating glabellar lines with incobotulinumtoxinA (Merz Pharmaceuticals GmbH) improved outcomes.
Methods: In this single-center, therapeutic cohort study, 130 women with moderate or severe glabellar lines were treated with incobotulinumtoxinA using the standard 5-point approach to injection of the glabellar muscles (n = 65) or the ONE21 technique,[5] which involves an individualized assessment based on anatomical localization of contraction (n = 65). Standardized photographs were taken under maximum contraction before treatment (T0) and after four weeks (T28). Two blinded raters graded the glabellar lines based on the wrinkle-grading Merz Aesthetics Scales (MAS).[6] Efficacy was defined as a two-point or greater reduction in MAS score.

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Results: There were no substantial differences between the ONE21 and 5-point groups in demographics or baseline MAS scores. At T28, 64 (98.5%) subjects from the ONE21 group and 52 (80%) from the 5-point group had at least a two-point reduction in MAS score ($P<0.01$). After adjustment for age and phototype, both groups had a reduced MAS score at T28 ($P< 0.01$), but the ONE21 group performed better ($P< 0.01$). Overall, 83.1% of the 5-point group utilized muscle groups other than the procerus and corrugator in glabellar wrinkle formation, and 24.6% presented with asymmetrical contraction. Patients from the 5-point group with glabellar asymmetry or who utilized the frontalis and orbicularis had worse performance ($P< 0.05$).
Conclusions: Compared with a standard 5-point treatment, a ONE21 approach provided better reduction of dynamic glabellar lines with incobotulinumtoxinA, especially for asymmetric lines of the glabella or in subjects with involvement of muscle groups other than the procerus and corrugator.
Funding/Disclosures: Carla Pecora, Gisele Jacobino de Barros Nunes, Maria Valéria Bussamara Pinheiro, and Karin Ventura Ferreira serve as speakers and medical consultants for Merz Farmacêutica, SP, Brazil. Hélio Miot serves as medical writer and consultant for Merz Farmacêutica, SP, Brazil.
Keywords: Botulinum toxin type A; Face; Glabellar lines; IncobotulinumtoxinA; Muscle contraction

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EVALUATION OF ONE21 TECHNIQUE WITH INCOBOTULINUMTOXINA FOR TREATMENT OF FOREHEAD WRINKLES

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Introduction and Objectives: Although treatment of upper facial lines is a frequent aesthetic procedure, there is limited research supporting systematic tailored approaches for the improvement of forehead wrinkles using botulinum toxin type A. This study evaluated the safety and efficacy of using the ONE21 technique[1] to treat forehead wrinkles with incobotulinumtoxinA (INCO; Merz Pharmaceuticals GmbH).
Methods: A total of 86 women with a baseline Merz Aesthetics Scales (MAS)[2] score $\geq$2 for dynamic forehead lines who had been treated with INCO using the ONE21 technique were included in this single-center, retrospective study. Standardized pictures were taken before (baseline) and 4 ($\pm$ 2) weeks after treatment. Two independent blinded raters assessed outcomes using MAS for forehead lines (dynamic and at rest) and eyebrow positioning. The primary efficacy outcome was the percentage of responders, defined by a MAS improvement $\geq$2 points for dynamic forehead lines at week 4 ($\pm$2 weeks). Secondary outcomes consisted of the treatment effect on MAS scores for resting forehead lines and eyebrow positioning.[3]
Results: The mean age of the subjects was 46.2 years. The mean total dose of INCO patients received was 20.3 U. A MAS improvement of $\geq$2 points for dynamic forehead lines at week 4 ($\pm$2) was reported in nearly all subjects (97.7%). Resting and dynamic lines and eyebrow positioning MAS scores significantly improved ($P<0.001$). The majority of subjects (56.9%) improved eyebrow positioning MAS score $\geq$1 point, and 39.5% maintained the same score. There were no occurrences of eyelid or brow ptosis, and 70 patients reported no adverse events. Seven patients reported pain during application, 8 reported ecchymosis, and 2 experienced headaches.
Conclusions: These results demonstrate INCO injection using the ONE21 technique is effective and safe for treatment of forehead wrinkles and yields a natural result with a customized treatment and a predictable eyebrow shape.
Funding/Disclosures: This work was supported by Merz Pharmaceuticals GmbH, Frankfurt am Main, Germany. CSP, MVBP, VF and RG have served as consultants and speakers for Merz Pharmaceuticals GmbH and received personal fees from Merz Pharmaceuticals GmbH outside the submitted work.
Keywords: Botulinum toxin; Forehead; IncobotulinumtoxinA; ONE21; Wrinkles

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TAILORED EYEBROW SHAPE AND POSITIONING USING INCOBOTULINUMTOXINA INJECTION IN THE UPPER FACE

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Eyebrow aesthetics vary by race, age, and gender. However, there is limited information in the published literature on how to develop a tailored approach to eyebrow shaping and positioning with botulinum toxin treatment. The aim of this retrospective study was to demonstrate use of the ONE21 customized technique to reshape and create a lifting effect of the eyebrow with botulinum toxin A injection.
A total of 86 women were treated at a single center in São Paulo, Brazil, between March 2018 to December 2019 with incobotulinumtoxinA in the frontalis and brow depressors using the ONE21 technique for the assessment of the forehead and glabella wrinkles. The treatment protocol considered clinical-anatomical patterns of the upper face, grading of wrinkles, strength of the muscle, and patient request for eyebrow shape and positioning. Treatment of periorbital wrinkles was based on classification of crow's feet patterns. Retrospective data that were collected included eyebrow positioning grading based on the Merz Aesthetics Scales (MAS), the dose used in each area, and points distribution. Photographs were taken before treatment at day 0 (visit 1) and 4 weeks ($\pm$ 2 weeks) after treatment (visit 2). Two independent raters assessed photographs in a blinded manner to determine the percentage of responders based on the eyebrow positioning MAS. Overall, 61% of the patients achieved a 1 point or greater improvement in MAS brow positioning, and 35% maintained the original shape and positioning. Of the 86 patients, 52 asked for a lateral arching effect of the eyebrow; 82.69% accomplished their desires. Adverse events included ecchymosis and headache, but there were no reports of brow or eyelid ptosis.
The results support the use of the ONE21 technique to facilitate a customized and predictable eyebrow reshape and positioning with

injection of incobotulinumtoxinA in the upper face.
Funding/Disclosures: Carla de Sanctis Pecora serves as a speaker and medical consultant for Merz Farmacêutica, SP, Brazil.
Keywords: Botulinum toxin; Eyebrow shaping; IncobotulinumtoxinA; ONE21 assessment

BOTULINUM NEUROTOXIN SEROTYPES A, B, C, E, AND F PREFERENTIALLY ENTER CULTURED HUMAN MOTOR NEURONS COMPARED TO OTHER CULTURED HUMAN NEURONAL POPULATIONS

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Introduction: Human-induced pluripotent stem cell (hiPSC)–derived neurons have been shown to be exquisitely sensitive to BoNTs, exceeding sensitivity of the mouse bioassay. Progress in stem cell technology now enables differentiation of hiPSCs into various defined neuronal subpopulations including cultures enriched in motor neurons, GABAergic neurons, glutamatergic neurons, and dopaminergic neurons. These cultures for the first time enabled in situ studies of human neuron–specific uptake of botulinum neurotoxins (BoNTs) (Pellett et al, 2019).
Methods: Four defined hiPSC-derived neuronal populations including primarily GABAergic, glutamatergic, dopaminergic, and motor neurons (all from Fujifilm Cellular Dynamics) were examined for BoNT/A, B, C, D, E, and F sensitivity.
Results: The data indicate that sensitivity varied markedly for the BoNTs tested. Motor neurons were significantly more sensitive than other neuron types for all BoNTs except for BoNT/D. Examination of SNARE protein levels and BoNT-specific cell surface protein receptors revealed few differences between the cell types except greater expression levels of SV2C and synapsin-IIa in motorneurons.
Conclusions: This study provides a model for determination of the mechanisms affecting BoNT sensitivity for different classes of neurons. The data presented show that both neuronal cell type–specific and BoNT serotype–specific properties affect BoNT potency in the four cell models. The motor neurons analyzed in this study were by far the most sensitive cell model for BoNTs A, B, C, E, and F, which is consistent with botulism symptoms observed in clinical human botulism cases. However, BoNT/D was significantly less potent in motor neurons than the other BoNTs and instead was more potent in the glutamatergic neuron cell model. The specific mechanisms of the neuro-specific sensitivity could not be explained by expression of cell surface protein receptors alone and remains to be shown. These data highlight the importance of considering both the cell source and characteristics, as well as the BoNT type analyzed in cell-based studies, and demonstrate the research potential of hiPSC-derived neuronal cell models.
Funding: This work was supported by the National Institute of Allergy and Infectious Diseases, R01 AI139306, R56 AI095274.
Keywords: Botulinum neurotoxin; Cell assay; Cell entry; Neuron; Motor neurons

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Pellett S, Tepp WH, Johnson EA. Botulinum neurotoxins A, B, C, E, and F preferentially enter cultured human motor neurons compared to other cultured human neuronal populations. *FEBS Lett.* 2019; 593(18):2675-2685.

AUTOMATED FERMENTATION PLATFORM FOR TOXIN-BASED THERAPEUTICS

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Demanding safety standards needed to protect personnel during botulinum neurotoxin (BoNT)-based pharmaceutical production can hinder development of these products, requiring extensive safety measures. However, speed-to-market is crucial to provide patients with new treatments without delay and reduce costs. We sought to accelerate BoNT drug development using a high-throughput approach. In upstream process (USP) development, this means using high-throughput parallel bioreactor systems. This study aimed to increase USP throughput and protect operators through minimal exposure.
Recombinant BoNT expression by *Escherichia coli* in an Eppendorf DASBox bioreactor system with BioBlu 0.3f vessels were compared. Conventional control of Eppendorf DASWare 5.0 software was compared to a scripted control (patent pending) in which dissolved oxygen (DO) levels and pH were more tightly regulated. The latter was achieved by script initiation with no further interventions. The standard approach required monitoring and manual input of >20 variables for each vessel for equivalent results.
The fermentation process compared employed a two-phase strategy in defined media,[1] with expression induced by isopropyl β-D-1-thio-galactopyranoside upon carbon depletion, detected by corresponding sharp DO increase ("spike").
The custom script automatically controls DO and pH, detects DO spike, switches phases, and controls feed and inducer addition. Compared to the conventional workflow, it brings: 1) instant setup of up to 24 bioreactors (subject to equipment capabilities), 2) rapid initiation of fermentations, 3) self-adapting algorithms for automated process control (Figure), 4) minimal operator input, 5) high reproducibility and robustness, 6) ease of USP transfer between laboratories, and 7) improved safety.
In conclusion, this scripted fermentation platform greatly improves speed, reproducibility, and robustness of USP without compromising personnel safety.
Funding: Ipsen
Keywords: Automation; Development; Fermentation; High-throughput; Process; Upstream

Reference

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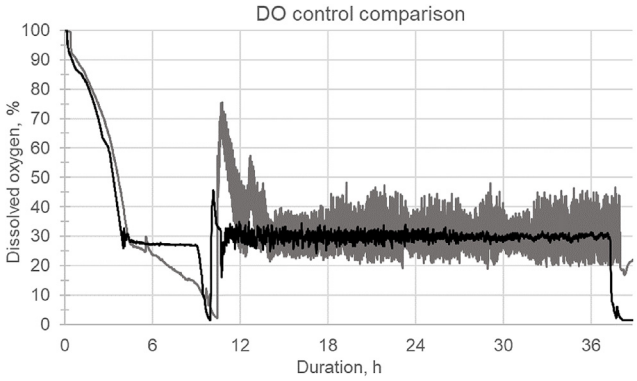

Figure. Dissolved oxygen (DO) control algorithms in 0.3-L bioreactor. Grey line is conventional DO control. Black line is script control. The DO was set to 30%. The process values deviate between 20% and 50% under conventional control and between 25% and 35% under script control.

DEVELOPMENT AND CHARACTERIZATION OF A CELL-BASED ASSAY FOR RELATIVE POTENCY TESTING OF BOTULINUM NEUROTOXIN SEROTYPE E

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Introduction: Current botulinum neurotoxin (BoNT)-based pharmaceuticals are limited to BoNT serotypes A and B, with serotype A (BoNT/A) accounting for the majority of the products currently on the market. In recent years, however, interest in the pharmacological properties of other BoNT serotypes has increased due to serotype-specific differences in therapeutic onset, cell targeting, and therapeutic duration that could increase the medical applications of BoNT-based drug products. One serotype, BoNT/E, is unique among the BoNT serotypes due to its quick onset but short duration (weeks rather than months as seen with BoNT/A) raising the possibility that a BoNT/E-based drug product could be developed for indications or medical procedures where short-term muscle paralysis is beneficial.
Commercial release of any BoNT-based drug product requires a means to determine drug product potency to ensure manufacturing consistency and patient safety. The mouse LD_{50} bioassay has historically been the standard method for testing BoNT-containing samples, including drug products. In recent years, however, any new BoNT-based drug product to be released in the US or EU should be supported by a cell-based assay (CBA) to meet government requirements for minimizing or eliminating the need for animal-based assays. A CBA's performance should also improve upon the LD_{50} bioassay's tendency towards a narrow linear range, low precision, and high assay failure rates. A well-developed CBA can eliminate the use of animal-based methods for BoNT detection while increasing throughput, accuracy, and precision. Here, we describe the development of such a CBA for the detection and relative potency testing of BoNT/E-containing samples.
Methods: We engineered a cell line to express a fluorescent reporter that enables the detection of BoNT/E and used the cell line to develop an assay commercially known as the BoCell® CBA. The BoCell CBA was specifically optimized for the detection of BoNT/E and was tested for stability indication capabilities. Finally, we qualified the CBA by testing its precision, accuracy, range, and linearity using BoNT/E-containing samples.
Conclusions: A CBA method for determining the relative potency of BoNT/E-containing samples was successfully developed. We demonstrate that conditions optimal for the detection of BoNT/E are different than those optimal for the detection of BoNT/A. The optimized method has the sensitivity required for determining the potency of BoNT/E-based drug products and is stability indicating. Further, the method offers excellent precision and accuracy over a range of sample potencies.
Keywords: Botulinum neurotoxin serotype E; Relative potency; Cell-based assay

BLOCKING TETANUS WITH PURIFIED HUMAN MONOCLONAL ANTIBODIES

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Tetanus neurotoxin (TeNT) is the causative agent of the spastic paralysis, induced by tetanus, and which is a life- threatening neuroparalytic syndrome of vertebrates, including humans. Tetanus is prevented by a very effective vaccine but still represents a major killer in countries with inefficient vaccination programs or in non-immunized, aged people. A common clinical practice in the emergency room after wounding is passive immunization via the administration of human anti-TeNT antisera, which is produced from the blood of hyperimmune individuals. Although effective in preventing tetanus, the use of hyperimmune human sera exposes patients to the possible effects of unknown viruses and other substances or to the development of anaphylactic reactions. The use of highly purified human monoclonal antibodies specific for TeNT (T-huMabs) would overcome these problems and be expected to be completely safe. In this work, we describe the functional test of a series of T-huMabs produced from immortalized human B memory cells isolated from human volunteers hyperimmunized with the tetanus toxoid vaccine. Among the many obtained, we have identified two T-huMabs that completely and very efficiently inhibit TeNT by blocking either the binding to the presynaptic membrane or the membrane translocation of the TeNT catalytic light chain into the cytosol. The combined use of these two T-huMabs completely prevents the development of tetanus in mice similar to that provided by a commercial human anti-tetanus antiserum.

MAGNETIC RESONANCE–GUIDED NAVIGATION OF BOTULINUM TOXIN INJECTION IN THE LATERAL PTERYGOID MUSCLE. FIRST RESULTS IN THE TREATMENT OF TEMPOROMANDIBULAR JOINT DISORDERS

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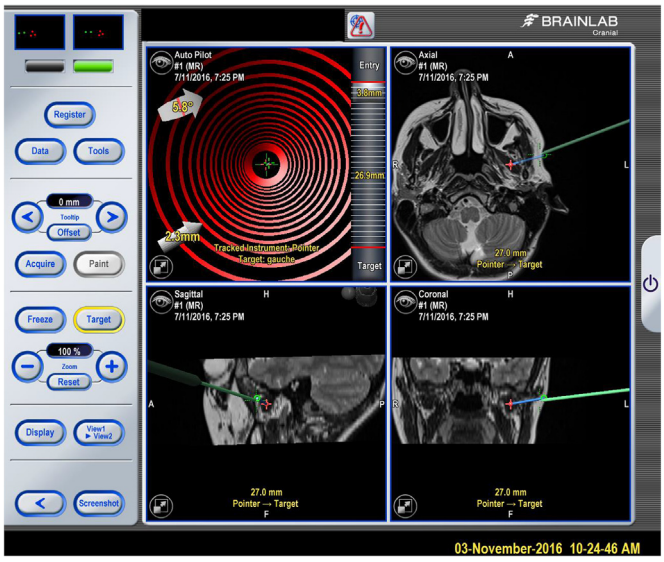
Introduction: Hyperactivity of the lateral pterygoid muscle (LPM) is one of the presumed mechanisms of temporomandibular disorders (TMD) and explains why intramuscular injection of botulinum toxin (BT) may be effective in treatment. Intramuscular injection of the LPM without guidance is difficult because of its deep location. The objective of this study was to determine the feasibility of magnetic resonance (MR)-guided navigation of BT injection in the LPM in TMD. We report our first results herein.
Patients and Methods: Six patients suffering from persistent myogenic TMD were enrolled in a prospective study and treated using intramuscular injection of botulinum toxin type A (BTA): 20 UI in each LPM with MR-guided navigation using the Brainlab platform (Kolibri®, Brainlab®, Munich, Germany). The center of the upper head was targeted, and 30 UI were injected in each masseter and 20 UI in each temporalis muscle with clinical guidance. The outcomes evaluated were pain intensity, maximum interincisal opening, and joint sounds.
Results: MR-guided navigation was used in all patients and the target was consistently attained. Pain improvement (mean reduction of 4.4 on a numeric scale ([P=0.0579]) was observed in 66.7% of the patients. Significant improvement of maximum interincisal opening was noted (P=0.0360) and joint sounds tended to disappear (P=0.5594).
Discussion: MR-guided navigation is an effective method for tracking the upper head of the LPM and enables precise injection of BT. Injection of BT in the upper head of the LPM, the masseter, and the temporalis muscles is effective in refractory TMD. A combination of morphologic guidance with electromyographic tracking will enable more accurate identification of the muscles to be injected.
Keywords: Temporomandibular disorders; Botulinum toxin; Lateral pterygoid muscle; Navigation



CERVICAL DYSTONIA MANAGEMENT WITH ABOBOTULINUMTOXINA USING PATIENT-CENTERED GOAL-SETTING AND GAS IN A YOUNG ADULT PATIENT WITH DYSKINETIC CEREBRAL PALSY

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Introduction: Cervical dystonia (CD) is a common cause of pain and dysfunction in dyskinetic cerebral palsy (D-CP). Botulinum neurotoxin type A (BoNT-A) injections into dystonic muscles is a well-established treatment. Nonetheless, a gap between the clinician's and patient's perceptions of treatment effect is not unusual. A patient-centered approach potentially enhances patient satisfaction and minimizes this gap.
Methods: Using an illustrative case of CD in a D-CP adult patient, the authors highlight the relevance of goal-setting and Goal Attainment Scaling (GAS) with BoNT-therapy.
Results: A 28-year-old man with D-CP and a history of CD with periodic BoNT-A injections and physical therapy since adolescence was referred to our outpatient department due to escalating neck pain and unsatisfactory upper limb functioning. He had severe right laterocollis, mild anterocollis, and cervical and upper limb dystonic impairment of voluntary movements. Over 17 months, he underwent 4 treatment cycles. Treatment goals were defined at each injection visit and GAS of previous goals was assessed. New goals were established according to the patient's status and expectations. Outcomes considered were magnitude and duration of effect, tolerability, and safety of treatment. Muscle selection, dosage, and treatment intervals were adjusted appropriately. The primary goals were improvement of head/neck posture and effective pain control during meals, sleep, and computer use. The most frequently injected muscles were the sternocleidomastoid, trapezius, scalenus, splenius capitis, and platysma. AbobotulinumtoxinA total dose injected was 240-450 U per cycle. Treatment intervals were flexible. Treatment goals were mostly achieved (mean GAS T-score: 49.1).
Conclusions: Clinical management of dystonia in D-CP is particularly challenging. Individual perception is critical in assessing treatment effect. Patient-centered goal-setting and evaluation using GAS allows clinicians to plan and monitor BoNT-A treatment in an individualized manner and may enhance treatment-related satisfaction.
Keywords: Botulinum neurotoxin type A; Cervical dystonia; Dyskinetic cerebral palsy; Goal-setting; Goal Attainment Scaling; Treatment-related satisfaction.

ICF REHABILITATION SET FOR MONITORING THE EFFICACY OF BOTULINUM TOXIN TYPE A IN POST-STROKE SUBJECTS WITH SPASTICITY: NEW PROSPECTS AND META-ANALYSIS OF THE FIRST RESULTS

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Introduction and Objectives: There are no reports in the literature of studies that have evaluated the efficacy of botulinum toxin type A (BoNTA) injections for rehabilitation,[1] using the World Health Organization's International Classification of Functioning, Disability and Health (ICF).[2] The aim of this study was to explore the feasibility of using the ICF2 to this end in post-stroke subjects treated with BoNTA.
Methods: We retrospectively analysed the record for each patient who received an individualized dose of BoNTA in the upper or lower limbs using palpation techniques, from September 2018 to September 2020. During the BoNTA injection period (phase T0), the rehabilitation team ascertained those goals that can be achieved with BoNTA injection according to the ICF2: 9 categories in Body Function and 21 categories in Activity and Participation. Each category has a qualifier: no, mild, moderate, severe, complete problem.[3] All patients received an individualized rehabilitation program following BoNTA injections and were followed up at 3 months after BoNTA injection (phase T1) to assess the achievement of the goals using the ICF.[1]
Results: One hundred eighty-six patients were included: 107 males and 79 females; ages: 42-85. During the injection period, the rehabilitation team selected 70% Activity and Participation goals and 30% Body Function goals ($P<0.05$). In the Activity and Participation category, the rehabilitation team assessed change in ability to change basic body position, maintain body position, transfer oneself, walking, toileting, dressing, recreation, and leisure. In the Body Function category, the team assessed change in pain and mobility of joints. In a comparison of phase T0 and T1, the patients had a significant reduction ($P<0.05$) in the qualifiers of the 7 Activity and Participation and 2 Body Function ICF categories selected by the rehabilitation team.
Conclusions: The ICF Rehabilitation Set is easy to use in clinical rehabilitation practice for evaluating the efficacy of BoNTA in post-stroke spasticity patients.
Keywords: BoNTA; ICF generic set; Post-stroke; Spasticity

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ANTIBODIES AND VACCINES AGAINST BOTULINUM TOXINS: AVAILABLE MEASURES AND NOVEL APPROACHES

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Background: Botulinum neurotoxin (BoNT) is produced by the anaerobic, Gram-positive bacterium *Clostridium botulinum*. As one of the most poisonous toxins known and a potential bioterrorism agent, BoNT is characterized by a complex mode of action comprising: internalization, translocation and proteolytic cleavage of a substrate, which inhibits synaptic transmitter release at neuromuscular nerve endings leading to peripheral neuroparalysis of the skeletal and autonomic nervous systems. There are eight major serologically distinct toxinotypes (A-G and X) of BoNT, which act on different substrates while the potential type H was identified as a BoNT/FA mosaic. Due to its extreme lethality and potential use as a biological weapon, botulism remains a global public health concern. Vaccination against BoNT, although an effective strategy, remains undesirable due to growing expectations around therapeutic use of BoNTs in various pathological conditions.

After summarizing the current therapeutic antibodies, this review focuses on botulism control by generation of animal hyperimmune anti-BoNT sera, anti-BoNT human immunoglobulins. It presents the current knowledge of the neutralizing epitopes in BoNTs, the generation of mouse, sheep, or humanized monoclonal antibodies and phage display technology, highlighting the future challenges. The clinical impact of subtype variants and BoNT sequences found in non-clostridial species remains to be elucidated.

Keywords: Antitoxin; Antibodies; Botulinum neurotoxins (BoNTs); BoNT variants; Vaccines

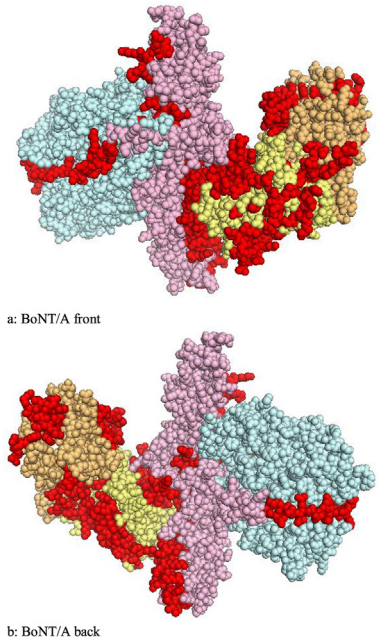

Fig. 1. Epitope mapping of neutralizing antibodies (red); blue: LC, pink: HN, yellow: HC_N, orange: HC_C. LC=light chain; HN=translocation domain; HC_N=N-terminal part of binding domain; HC_C=C-terminal part of binding domain.

IDIOPATHIC TOE GAIT: EFFICACY OF BOTULINUM TOXIN. DESCRIPTION OF A SERIES OF 134 PATIENTS

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Introduction and Objectives: Idiopathic toe gait is a benign condition of unknown origin. Children typically walk on their toes despite being able to do so in a heel-to-toe walking pattern. Neurological pathologies such as spasticity must be ruled out. Untreated, toe gait persists in 50% of patients, producing altered ankle mobility. Botulinum toxin injected into the gastrocnemius muscle has shown efficacy with few adverse events (AEs). We describe an investigation of the safety and efficacy of botulinum toxin treatment in a large series of patients.

Methods: This is an observational descriptive study. Data from 134 consecutive patients were analyzed. Botulinum toxin was injected into the gastrocnemius using anatomical localization. Doses were calculated based on body weight and the efficacy of prior doses. Patients were randomly assigned to receive consecutive abobotulinumtoxinA or onabotulinumtoxinA injections. Efficacy was defined as improvement in foot position during walking (Modified Physicians Rating Scale), in maximum ankle dorsiflexion angle, and in Achilles shortening. Additional treatments such as ankle orthoses, physiotherapy, and casting were frequently used but not systematically recorded, as many patients used them in an inconsistent manner.

Results: Toe gait was associated with mental retardation or autistic disorder in 28 patients (20%) and with family cases in 36 patients (27%). Mean age at which toe gait began was 25 months (range: 9 months to 9 years). The main symptom was toe gait itself, and, in 9 patients, it was accompanied by calf pain. Four patients had undergone Achilles tenotomy surgery prior to botulinum toxin injection, and 5 patients needed surgery after starting BoNT treatment. The mean number of injections was 4.2 (range: 1 to 21). Twenty-seven patients received only one injection, which resolved toe gait in 12 of them. Improvement in foot position during walking was achieved in 106 patients, with only 3 patients worsening after treatment. Improvement in ankle dorsiflexion was achieved in 81 patients, with worsening in only 1 patient. Fifty-two patients presented with shortening of the Achilles tendon, which resolved in 12 (23%) of them after treatment.

Fifty-one patients were treated with onabotulinumtoxinA, at an initial dose of 8 units/kg (mean; range: 3-11 units/kg), and a final dose of 9 units/kg (mean; range 3-19 units/kg). Eighty-three patients were treated with abobotulinumtoxinA, at an initial dose of 19 units/kg (mean; range: 8-33 units/kg), and a final dose of 20 units/kg (mean; range: 10-36 units/kg).

Fourteen patients experienced mild adverse events from any of the injections: 10 had local pain from day 1 to 2 weeks after injection, and 4 of them experienced tiredness from 3 to 7 days after injection.

Conclusions: Botulinum toxin injections into the gastrocnemius muscle are useful for treating idiopathic toe gait, showing high efficacy in improving foot position during walking and maximizing ankle dorsiflexion. AEs were mild and infrequent. More than one injection is usually needed. Based on our experience, we highly recommend treating idiopathic toe gait with consecutive botulinum toxin injections.

Keywords: Achilles shortening; Ankle dorsiflexion; Botulinum toxin; Idiopathic toe gait

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REAL-WORLD TREATMENT INTERVALS WITH BOTULINUM NEUROTOXIN TYPE A IN POSTSTROKE SPASTICITY MANAGEMENT—ARE DIFFERENT FORMULATIONS DIFFERENT?

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Introduction: The efficacy and safety of botulinum neurotoxin type A (BoNT-A) in the management of poststroke spasticity is established. This study aims to assess time intervals between the first 6 consecutive BoNT-A injections in patients with poststroke spasticity, as per routine practice of a reference spasticity clinic with 20 years of experience.
Methods: Data were prospectively collected from 2010 through 2020 and retrieved from clinical files of patients treated in a BoNT-A clinic. The analysis involved 216 patients. BoNT-A formulation, total doses, and injection intervals were recorded. The sample was split into 3 groups by BoNT-A formulation received: abobotulinumtoxinA (ABO), onabotulinumtoxinA (ONA), and incobotulinumtoxinA (INCO). We assessed the median time between all the treatments, including as many as 11 consecutive injections and searched for differences between formulations, but due to the small numbers of patients in the ONA and INCO groups, our analysis only went as far as the sixth injection. We measured the success rate of the treatments using the Goal Attainment Scale (success was considered scores 0, 1, and 2 = goal achieved/overachieved). For statistical analysis we used the SPSS tool.
Results: Overall (n=187), patients' assignment to groups was as follows: 64.70% ABO, 18.72% ONA, 16.58% INCO. Mean total dose expressed as a percentage of maximum dose per label showed no significant differences for the 3 formulations, and there was no increase in mean total dose over the 6 consecutive injections. The success rate expressed by achievement of individual goals (GAS scores 0, 1, and 2) was similar for the 3 groups. Likewise, there was no significant difference in median cumulative intervals between injection (days): ABO, 122.44; ONA, 117.63; INCO, 123.36.
Conclusions: Although several other factors may be involved, in our clinical practice, the 3 BoNT-A formulations seem to perform similarly in the management of our patients with poststroke spasticity, both in success rate and duration of benefit.

Keywords: Botulinum neurotoxin type A; Injections; Poststroke spasticity; Time intervals

PAIN IN CERVICAL DYSTONIA: A META-ANALYSIS OF OUTCOMES FOLLOWING TREATMENT WITH ABOBOTULINUMTOXINA IN RANDOMIZED, CONTROLLED CLINICAL STUDIES

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Introduction: Pain is a prominent, disabling feature of cervical dystonia (CD) that negatively impacts quality of life, and is one of the most common reasons for CD patients to present for treatment with botulinum toxin type A (BoNT-A) injections.
Methods: We present meta-analyses of patient-level data from 4 randomized, placebo-controlled studies[1-4] of abobotulinumtoxinA (AboBoNT-A) 500 U and 3 associated open-label extension studies. All studies assessed pain using the Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) pain subscale. The difference between AboBoNT-A and placebo was assessed using an ANCOVA with treatment as fixed effect, baseline values as covariates, and subject as random effect.
Results: In the randomized controlled studies, baseline TWSTRS pain scores were 10.5±4.0 in the AboBoNT-A group (n=340) and 10.8±4.3 in the placebo group (n=203). Treatment with AboBoNT-A significantly reduced Week 4 pain scores by (mean±SE) -3.2±0.2 points in the AboBoNT-A group vs -1.0±0.3 points in the placebo group (treatment difference: 2.2±0.4 points; $P<0.0001$). Statistical significance versus placebo was maintained at Week 12 (treatment difference: -1.3±0.4 points vs placebo; $P=0.0006$). In the open-label studies, reductions in Week 4 and Week 12 pain scores were maintained with repeat treatment with AboBoNT-A, ensuring relatively consistent pain control for patients across multiple injection cycles (Figure).
Conclusions: Treatment with AboBoNT-A significantly reduced pain in CD vs placebo at 4 weeks post-injection, and benefits persisted. Pain relief was maintained over time across multiple injection cycles.

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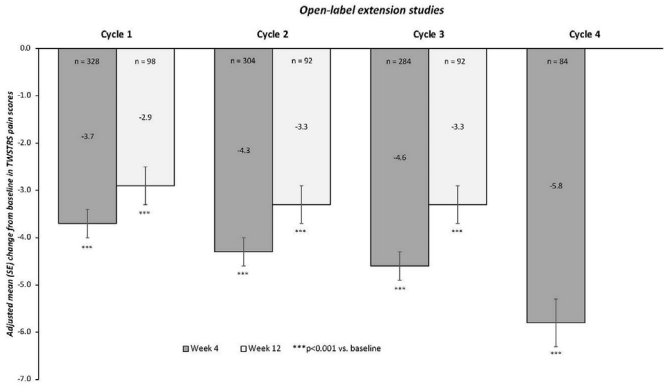

Fig. Reductions in Week 4 and Week 12 pain scores were maintained with repeat treatment with AboBoNT-A in open-label studies.

VOIDING DYSFUNCTION IN PATIENTS WITH HEREDITARY SPASTIC PARAPLEGIA

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Introduction: Hereditary spastic paraplegia (HSP) is known to cause voiding dysfunction. Prior studies have reported on clinical and urodynamic evaluation of smaller samples of patients with HSP.
Methods: One hundred patients suffering from HSP were included in the study. The presence of urinary symptoms was determined by assessment using the Urinary Symptom Profile (USP) questionnaire. Depending on the clinical results, urodynamic evaluation was performed. Urodynamic assessment consisted of uroflowmetry and measurement of postvoid residual urine volume. Urethrocystomanometry was usually performed after uroflowmetry.
Results: Eighty-seven patients suffered from lower urinary tract symptoms (LUTS), mostly overactive bladder (OAB) syndrome (85 patients). Dysuria was present in 45 patients.
Ano-rectal disorder was frequently seen (41 patients). Total USP score was correlated with the age of the patient (P= 0.0001). Moreover, this correlation was even stronger on the OAB sub score (P=1.9, 10 E-6). Urodynamic analysis was performed for 40 patients. It showed detrusor overactivity in 19 cases, with high detrusor pressure of 7. Dyssynergia was present for 9 patients. Complications such as urinary infections, urolithiasis, and vesicoureteral reflux, occurred in 14 patients. In earlier series, complication rates varied between 6.9%[1] and 54.5%.[2]
Conclusions: The present study confirms the occurrence of LUTS in patients suffering from HSP. OAB syndrome seems to be more frequent with increasing age. Detrusor overactivity was the most frequent urodynamic abnormality.
Keywords: Hereditary spastic paraplegia; Lower urinary tract symptoms; Urodynamics

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RESULTS OF BOTULINUM TOXIN THERAPY FOR SPASTICITY IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Introduction: Spasticity is a frequent and disabling symptom in patients suffering from amyotrophic lateral sclerosis (ALS).[1] Standard treatment includes physiotherapy and oral drugs such as baclofen but these treatments may lack efficacy and tolerability. Studies of botulinum toxin (BTX) therapy for ALS patients are sparse.[2,3] We conducted a study to examine the safety and efficacy of BTX therapy in the management of spasticity in these patients.
Methods: One hundred three ALS patients referred to a physical medicine and rehabilitation department between 2006 and 2018 were included. Of these, 91 received BTX injections. The medical charts of these patients were retrospectively analysed to determine the efficacy and tolerability of this treatment.
Results: Of the 91 patients receiving BTX injections, 83 were injected in the lower limbs, 4 in the upper limbs, and 4 in both upper and lower limbs. Seventy-six patients were still able to walk. For these patients, the goal of treatment was functional improvement for 71, and improved comfort for 5. Fifteen patients were unable to walk. Of these, 8 underwent BTX therapy to improve their transfer capabilities and 7 to improve comfort. For 28 patients, an additional goal of treatment was reducing cramps and pain due to spasticity. Fifty-one patients expressed satisfaction with treatment, and the injections were repeated. Some patients continued the treatment through several reinjections, up to 16. The main reason for stopping injections was a worsening of the disease.
Of the 234 injections performed in ALS patients, there were only 10 reports of adverse events (AEs) with 2 cases of transient respiratory degradation. This AE is rare, but it requires a cautious use of BTX in ALS patients, especially regarding the level of toxin doses administered.
Conclusions: More than a half of the patients had a satisfactory reduction of their spasticity after BTX injections, and this treatment can be proposed for patients with ALS and spasticity. Nevertheless, because of the fragility of these patients, especially regarding respiratory function, we advise injection of lower doses than are administered to patients with spasticity due to other neurological diseases.
Keywords: Amyotrophic lateral sclerosis; Botulinum toxin; Spasticity

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RESULTS OF BOTULINUM TOXIN INJECTIONS FOR THE TREATMENT OF SPASTICITY IN PATIENTS WITH PRIMARY LATERAL SCLEROSIS

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Introduction: Primary lateral sclerosis (PLS) is a rare motoneuron pathology resulting in pyramidal and bulbar syndromes. The clinical pattern mimics lateral amyotrophic sclerosis (ALS). Nevertheless, the course of PLS resembles hereditary spastic paraplegia and most patients have a good life expectancy. Spasticity is a disabling symptom of the disease (Le Forestier, Meninger, 2009). Our team treated a large number of patients suffering from PLS relative to the rarity of this pathology. This study describes our approach to treating spasticity in patients with PLS using botulinum toxin injections.
Methods: Fifty-five patients suffering from PLS with either paraparesia or tetraparesia were referred between 2009 and 2018 to a dedicated clinic in the Physical Medicine and Rehabilitation Department in Pitié-Salpêtrière Hospital for management of their spasticity. Their medical charts were retrospectively analyzed to determine the outcomes of botulinum toxin therapy.
Results: Of the 55 patients, 44 received botulinum toxin injections, preceded by neuromotor block for 16 patients. Injections were mostly performed in the lower limbs in order to improve comfort or ambulation. The treatment was uncomplicated. Of the 44 patients treated with botulinum toxin injections only, 28 (63.6%) were satisfied with the treatment. Twenty-two patients (50%) underwent botulinum toxin therapy for at least a year, 6 (13.6%) for more than 3 years.
Conclusions: This study confirms the effectiveness of botulinum toxin for the treatment of spasticity in patients with PLS, as it provided clinical relief of spasticity in almost two-thirds of our patients. As PLS progresses slowly, this treatment may be repeated in a long-term approach.
Keywords: Botulinum toxin; Primary lateral sclerosis; Spasticity

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GREATER BIOLOGICAL ACTIVITY AND NON-INTERCHANGEABILITY OF ONABOTULINUMTOXINA COMPARED WITH VACUUM-DRIED PRABOTULINUMTOXINA

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Introduction: PrabotulinumtoxinA (prabotA) is a botulinum toxin type A1 formulation originally manufactured in Korea as a lyophilized product. The manufacturing process for prabotA has been converted to a vacuum-drying process for distribution in the United States, Europe, and Canada. To understand how the biological activity of the 100 U vacuum-dried product (prabotA-VD) compares with onabotulinumtoxinA (onabotA), multiple preclinical assays were utilized.
Methods: PrabotA-VD and onabotA (100 U vials) were reconstituted and tested at equal product label activity units in 3 distinct assays: the plate capture light chain activity (PC LCA) assay, the cell-based potency assay (CBPA), and the mouse digit abduction score (DAS). The PC LCA provides a measurement of light chain activity after toxin is recovered from reconstituted product using an antibody directed to the heavy chain portion of the toxin molecule. The CBPA measures 3 steps of toxin intoxication: binding, translocation, and light chain enzymatic activity (SNAP25 cleavage). DAS is a preclinical assay for evaluating motor neuron intoxication/muscle paresis. Three separate lots of prabotA-VD and onabotA were evaluated on more than 1 test date using each assay.
Results: Multiple orthogonal assays established that the biological activity of Daewoong prabotA-VD units is less than that of Allergan onabotA units. OnabotA displayed 1.51 ± 0.14−fold (mean ± standard deviation; $P<0.05$) and 1.3 ± 0.1−fold (mean ± standard error of the mean; $P<0.05$) higher activity than prabotA-VD in the PC LCA and CBPA assays, respectively. This finding was supported by DAS data, in which onabotA showed 1.4 ± 0.1−fold (mean ± SEM) higher potency than prabotA-VD.
Conclusions: Data from several preclinical assays have established that, on a unit-to-unit basis, onabotA displays greater biological activity than prabotA-VD and confirm that the units of prabotA-VD and onabotA are not interchangeable.
Funding: Allergan Aesthetics, an AbbVie Company.
Keywords: Botulinum toxin type A; OnabotulinumtoxinA; CBPA; DAS; Light chain activity; Vacuum

A CELL-PENETRATING PEPTIDE (CPP) DID NOT DECREASE 150-KDA BONT/A TOXIN ADSORPTION TO SURFACES OR INCREASE TOXIN POTENCY OR DURATION IN A PROTOTYPE FORMULATION

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Introduction: Unit potencies of botulinum neurotoxin type A (BoNT/A) drugs are not interchangeable due in part to unique manufacturing processes and drug formulations. Various excipients are used to stabilize BoNT/A and prevent adsorption to surfaces. We evaluated the effect of a cell-penetrating peptide (CPP) in a prototype formulation using toxin adsorption, in vivo potency, and duration analyses.
Methods: A CPP with the sequence RKKRRQRRRG-[K]15-GRKKRRQRRR (Malmirchegini R, et al, 2018) was synthesized. Size-exclusion chromatography assessed adsorption of 1 μg/mL 150-kDa BoNT/A toxin to glass vials (borosilicate, Type 1). Samples were analyzed in 50 mM potassium phosphate/150 mM NaCl buffer containing 0.05% polysorbate 20 with 23.5 μg/mL CPP, polysorbate 20 alone, CPP alone or 50 μg/mL human serum albumin (HSA). Buffer alone was used as a negative control. Mouse digit abduction score (DAS) testing compared the potency and duration of 150-kDa BoNT/A toxin at an approximate ED_{50} dose formulated in 20 mM histidine buffer 2% trehalose, pH 6.0 buffer containing 0.05% polysorbate 20, buffer containing 0.235 μg CPP/unit BoNT/A with or without 0.05% polysorbate 20, or in 0.5% bovine serum albumin (BSA)/0.9% NaCl.
Results: Toxin adsorption to the glass surface occurred in buffer control and CPP-only solutions at 7 hours, both with toxin recovery of <64%. At 14 and 21 hours, buffer control and CPP-alone samples had decreased toxin recoveries of <42%; samples containing HSA or polysorbate 20 continued to display toxin recoveries of >95%. In the DAS assay, BoNT/A prepared in buffered polysorbate 20 with CPP, or without CPP, or in BSA/0.9% NaCl exhibited mean peak DAS responses of 2.17 ± 0.07, 2.57 ± 0.14, and 2.27 ± 0.10, respectively, and these were not significantly different from one another. Likewise, duration of action profiles for BoNT/A in these formulations were similar. In contrast, BoNT/A formulated in CPP alone, without polysorbate 20, demonstrated decreased potency (mean peak DAS=0.17 ± 0.09) and negligible duration.
Conclusions: Inclusion of a CPP in a BoNT/A formulation neither prevents toxin adsorption nor increases potency or duration.
Funding: Allergan Aesthetics, an AbbVie Company
Keywords: Adsorption; Botulinum toxin type A; Excipients; Formulations; Neurotoxins

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BOTULAX DISPLAYS LOWER ENZYMATIC ACTIVITY WHEN COMPARED TO ONABOTULINUMTOXINA IN A LIGHT CHAIN ACTIVITY ASSAY

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Introduction: Botulax* 100 U is a lyophilized 900-kDa botulinum toxin type A product formulated with 0.5 mg human serum albumin and 0.9 mg sodium chloride (Botulax 100 U packaging, Hugel). The manufacturer of Botulax claims similar or better efficacy compared with onabotulinumtoxinA (onabotA) at a 1:1 dose ratio for the treatment of cosmetic indications. This study compared the enzymatic activities of 100 U vials of onabotA and Botulax using the light chain activity high-performance liquid chromatography (LCA-HPLC) assay.
Methods: Two lots of onabotA and 2 lots of Botulax were evaluated on 2 separate test dates. Different lots of onabotA and Botulax were evaluated on each test date (3 vials/lot). Product vials were reconstituted with digestion buffer (50 mM HEPES, 0.5 mM zinc acetate, 2 mM dithiothreitol, 0.05% Tween 20). Samples were reduced for 30 minutes at 37°C and then reacted with a commercially available substrate (SNAPtide® 520). Sample aliquots, incubated at 30°C, were analyzed at 6, 12, and 18 hours. Cleavage products were chromatographically separated and quantitated using fluorescence detection.
Results: In both studies, Botulax displayed lower enzymatic activity. In the first study, Botulax displayed 84.9%, 84.6%, and 83.6% of the light chain activity of onabotA at 6, 12, and 18 hours, respectively. In the second study, Botulax displayed 75.1%, 74.6%, and 72.5% of the light chain activity of onabotA at 6, 12, and 18 hours, respectively. One-way ANOVA followed by post hoc Tukey's multiple comparison test showed that the differences between onabotA and Botulax were statistically significant for both studies at all time points tested ($P<0.001$).
Conclusions: Test results indicate that Botulax displayed lower enzymatic activity when compared with onabotA in the LCA-HPLC assay. Differences in the assay results do not support dose interchangeability between the 2 products.
*The Republic of Korea, where Botulax is manufactured, does not recognize nonproprietary/generic names for botulinum toxin products.
Funding: Allergan Aesthetics, an AbbVie Company
Keywords: Aesthetic; Biologic assay; Botulinum toxin, type A; Excipients; Interchangeability; OnabotulinumtoxinA

ABOBOTULINUMTOXINA IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS WITH SIALORRHEA

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Introduction: Sialorrhea is a frequent symptom of amyotrophic lateral sclerosis (ALS). It exacerbates the overall physical and psychosocial burden on patients, causing social embarrassment and significant risk of choking and pneumonia.
Objective: The aim of our study was to evaluate the safety and efficacy of botulinum neurotoxin type-A (BoNT-A) in ALS patients with sialorrhea.
Materials and methods: Seven ALS patients with moderate and severe drooling were selected (6 female and 1 male), with a median age of 62 (range: 55-68) years. Speech, swallowing, and salivation were evaluated using the Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS) with gradation from 4 points, normal to 0 points, severe disorders (eg, anarthria, significant drooling). Self-reports of the severity of sialorrhea were obtained using a visual analogue scale (VAS). Each rating was obtained at baseline and 4 weeks post-treatment.
We used a quantitative determination of the unstimulated saliva flow rate (normal: 0.3 mL/minute [min]) before and after BoNT-A injection. All patients received abobotulinumtoxinA (Dysport®) injections at total doses ranging from 150 U (75 U for each gland) in moderate cases to 250 U (125 U for each gland) in severe cases of drooling.
Results: The median score on the ALSFRS for salivation was 0 (range: 0-1) points; for speech, 2(1-2); and for swallowing 3(2-3) points. Self-evaluation of sialorrhea by VAS before treatment varied from 7 to 9 points with a median rating of 8 (7-8). The median unstimulated saliva flow rate before BoNT-A injections was 5.2 (4.3-5.7) mL/min. Reduction of sialorrhea was reported by all patients: 5 patients noted a reduction to the level of "light but significant excess of saliva in the mouth", and 2, a decrease in severity to moderate from significant drooling. Differences in baseline and 4 weeks post-treatment evaluations of sialorrhea were significant. By the ALSFRS, the score for salivation was 2(2-3) ($P=0.018$), with no change in speech and swallowing; by VAS, sialorrhea decreased to 3(2-3) ($P=0.023$); and the median unstimulated saliva flow rate was 2.7 (2.1-3.2) mL/min ($P=0.023$).
Conclusions: BoNT-A injections are an effective and safe treatment for sialorrhea, providing improvements in patient quality of life while avoiding side effects such as are associated with the use of tricyclic antidepressants and anticholinergic drugs in ALS patients.
Keywords: AbobotulinumtoxinA; Sialorrhea; ALS

SPASTICITY AND SURGERY: FIRST YEAR OF A COLLABORATIVE APPROACH TO COMPLEX UPPER LIMB MANAGEMENT

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A collaborative Upper Limb Surgical Assessment Clinic was developed at the end of 2017 to meet the needs of patients who had complex hand, wrist, or elbow problems due to spasticity and/or contracture as a result of a neurological diagnosis, and who had limited improvements with standard interventions. A Multidisciplinary Team including an Occupational Therapist, Consultant in Rehabilitation Medicine, and a Consultant Hand and Plastic Surgeon working in the Northumberland, Tyne and Wear and Newcastle upon Tyne Hospitals National Health Service Foundation Trusts carried out assessments and provided interventions and follow up.
Clinical notes for the period December 2017 through December 2018 were reviewed retrospectively. Also, a questionnaire was sent to all patients addressing: information provided, assessment process, decisions made, personal outcomes, and how they would improve the service. A follow-up focus group was set up to explore these issues in more detail and to review possible outcome measures.
A total of 21 patients were seen in the clinic: seven proceeded with surgery, two chose no surgery, and two were still undecided at the time of the review. Surgery was performed approximately 4½ months after the initial assessment and included: tendon transfers, tendon lengthening, hyperselective neurectomy, and joint stabilisation/release.
In their own words, patients identified some of the post-surgery benefits as:
"Looks more 'normal'"
"Easier to get hand open and cleaned"
"Easier to cut nails"
"Less pain"
"Easier getting dressed"
"Not as self-conscious"
"Able to get wedding ring on"
"Easier to position"
The service evaluation also highlighted several actions to continue to develop the service:

- Provision of information via multimedia, including short videos by

the staff and by patients who have undergone surgery

- Use of the Arm Activity measure and goal satisfaction scales as outcome measures as chosen by the patients
- Providing training in neurological issues to staff on the surgical wards to enable them to address physical, communication, and cognitive needs

There is a need for an increase in the number of clinics equipped to meet the demand for the service; development of a research protocol; and review of the clinic referral and action pathway.

CLINICAL VALIDITY AND BENEFITS OF USING BOTULINUM TOXIN IN THE PREVENTIVE TREATMENT OF REFRACTORY MIGRAINE HEADACHES

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Introduction: The high prevalence of migraine and the significant difficulties associated with its treatment result in a considerable need for more effective therapies to improve the course of the disorder by decreasing the frequency and intensity of headaches, lengthening remission periods, reducing maladjustment of patients, and controlling refractory migraine attacks.
Objective: The aim of the study was to investigate the efficacy of botulinum toxin type A (BoNT-A) in the preventive treatment of refractory migraine headache symptoms.
Methods: An open-label, non-controlled, single-center study was performed. Seventy-two patients with refractory migraine, consisting of 33 (45.8%) with status migrainosus (SM) and 39 (54.2%) with chronic migraine (ChM), participated in the study and received BoNT-A injections. Ethics approval was obtained from the Pharmacological Committee and the Ministry of health of the Republic of Uzbekistan. Written informed consent was obtained from each study participant.
BoNT-A blocks the release of acetylcholine from the presynaptic neuromuscular terminal by cleaving synaptosomal-associated protein of 25 kDa (SNAP-25). This relieves muscle spasms and disrupts pain neurotransmission via afferent pathways and the peripheral nociceptive system, thereby exerting an analgesic effect.
BoNT-A (Dysport® [abobotulinumtoxinA]) was injected into the glabellar region, temporalis, frontalis, trapezius, occipitalis, and posterior muscles of the neck.
Three to five units of BoNT-A were injected per injection site, for a total amount of 125-155 units per patient. The main outcome measures were duration, frequency, and intensity of migraine attacks. Outcomes were assessed using the ID-migraine questionnaire, MIDAS (Migraine Disability Assessment) scale, three-dimensional Psychological Pain Scale (TDPPS), electronic version of the Numeric Rating Scale (NRS), Verbal Rating Scale (VRS), Visual Analog Scale (VAS), and the FACES Pain Scale (FPS).
Fourteen patients with SM and 7 patients with ChM were followed for 5 years. The severity of the clinical course of migraine and the duration of the disease correlated with the frequency of attacks and the resistance of migraine episodes to basic therapy. The ChM attacks lasted for as long as 12 days, SM headaches up to 72 hours with severe recurrent episodes of vomiting. Migraine attacks in the ChM group occurred on average 4.1±0.09 times every 3 months, and in the SM group 3.5±0.08 times. Over the past 3 years, 88.9% of study patients needed to take analgesics every day. Both subgroups experienced a severe clinical course, with a higher incidence of multiple occurrences of vomiting in patients with SM (P<0.01), and a higher incidence of single vomiting episodes (P<0.01) with phonophobia (P<0.05) in patients with ChM.
Severe migraine attacks led to the loss of 6.9±0.82 working days in ChM patients and 4.2 ± 0.61 days in SM patients. Patients experienced depression and anxiety, which exacerbated the clinical course and further reduced their ability to work. In the ChM subgroup, depression was more often observed (P <0.01). In contrast, in the SM subgroup, anxiety was more often detected (P <0.01). However, in both groups, maladjustment was noted in 94.4% of patients.
Results: In ChM patients, after three injections, headaches were of moderate intensity (-4.7±1.2 points on the TDPPS and 2.3±0.8 points on the FPS). After the fifth injection, recurrent headaches were of low intensity (2.7±0.9 and 1.8±0.3 points) in these patients. In patients with SM, even after 5 BoNT-A injections, migraine episodes recurred with moderate intensity (4.3±1.3 on the TDPPS and 2.8±0.7 on the FPS).
After regular use of BoNT-A for 2.5 years, the duration of migraine attacks decreased to 68% in patients with ChM and 52% in patients with SM; the frequency of attacks decreased by 37% and 49%, respectively. Over the 5 years of follow up, the duration of the attacks decreased by 97%, and the frequency of the attacks by 88%. Based on the Results of the ID-migraine questionnaire, the general condition of the patients has improved: the amount of vomiting has decreased and their ability to perform work has been restored. On the TDPPS and FPS, the intensity of headaches decreased by 2.5-5 times and recurrent migraine attacks were better tolerated (Figure 1). The patients improved their daily activity and began to adapt to social life.
Conclusion: BoNT-A impacts the most important peripheral cause of headache — muscle spasms — with the complete elimination of migraine attacks, a significant decrease in their frequency, duration, and intensity without the use of analgesics.
Keywords: Refractory migraine; Three-Dimensional Psychological Pain Scale; Facial pain scale; ID-migraine questionnaire; Botulinum toxin; Dysport

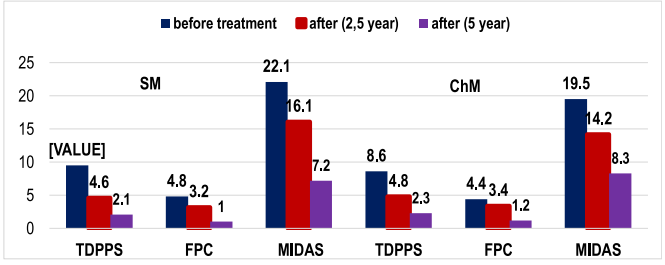

Fig. 1. Dynamics of treatment of refractory migraine for 5 years (in points).

SUSTAINED EFFICACY OF INCOBOTULINUMTOXINA OVER 6 INJECTION CYCLES FOR THE TREATMENT OF LOWER-LIMB SPASTICITY IN CHILDREN AND ADOLESCENTS WITH CEREBRAL PALSY

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Introduction: Two Phase 3 studies assessed the efficacy and safety of incobotulinumtoxinA for the multilevel, multipattern treatment of spasticity in children and adolescents with cerebral palsy (CP). Here we report the efficacy and safety of repeated lower-limb (LL) treatment over 6 injection cycles (ICs) in patients who took part in both studies.
Methods: Ambulant and non-ambulant patients (2-17 years of age; uni- or bilateral CP; Ashworth Scale [AS] plantar flexor score ≥2; clinical need for treatment) were enrolled in these studies. In TIM (NCT01893411), patients

were randomized (2:1:1) to 3 incobotulinumtoxinA dose groups: 16, 12, 4 U/kg body weight (BW, maximum 400, 300, 100 U) for treatment of 2 LL clinical patterns in 2 ICs. In the open-label TIMO study (NCT01905683), TIM completers received a further 4 ICs with 16 U/kg (maximum 400 U) for LL treatment. Changes from TIM study baseline in AS scores and Global Impression of Change Scale (GICS) scores at Week 4 of all 6 ICs were assessed. The incidence of adverse events (AEs) by IC is reported.

Results: In total, 124 patients (54.8% male, mean [SD] age 6.3 [4.0], BW 21.7 [11.9] kg, 28.2% GMFCS IV–V) completed 2 ICs in TIM and entered TIMO. Of these, 107 (86.3%) patients completed TIMO and received a total of 6 ICs. AS scores for the plantar flexors, knee flexors, and thigh adductors showed sustained and cumulative improvements from study baseline across all ICs (Table). Investigator's, child/adolescent's and parent/caregiver's GICS scores confirmed improvement in LL spasticity at Week 4 of each IC. The incidence of AEs per IC ranged from 9.2% (IC5) to 25.8% (IC2) of patients. There was no evidence of increasing incidence of AEs with increasing IC. No AEs of special interest or fatal AEs occurred.

Conclusions: IncobotulinumtoxinA showed sustained efficacy with cumulative improvements and was well tolerated over up to 6 ICs for LL spasticity treatment in patients with CP.

Funding: Merz Pharmaceuticals GmbH, Frankfurt am Main, Germany

Keywords: Cerebral palsy; IncobotulinumtoxinA; Lower-limb spasticity; Multipattern; Pediatric; Repeated injections

Table
Change in AS score for the main clinical patterns treated in the lower limbs from study baseline to Week 4 in each IC.

	Plantar flexors		Knee flexors		Thigh adductors	
	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
IC 1	124	−0.7 (0.7)	20	−0.5 (0.6)	8	−1.0 (0.5)
IC 2	123	−0.9 (0.6)	20	−0.8 (0.6)	8	−1.1 (0.6)
IC 3	123	−1.1 (0.7)	20	−0.8 (0.7)	8	−1.5 (0.5)
IC 4	112	−1.3 (0 8)	16	−0.9 (0.7)	8	−1.6 (0.7)
IC 5	108	−1.5 (0.8)	15	−0.9 (0.7)	8	−1.6 (0.5)
IC 6	107	−1.4 (0.8)	14	−0.9 (0.7)	8	−1.8 (0.5)

AS, Ashworth scale; IC, injection cycle; n, number of non-missing observations; SD, standard deviation.

UTILIZATION OF A PHENOTYPIC MICROARRAY FOR SCREENING OF *CLOSTRIDIUM BOTULINUM* GROWTH-ENHANCING SUBSTRATES

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The feasibility of a commercial phenotypic microarray to study the impact of different carbon sources on growth and therefore potentially botulinum neurotoxin (BoNT) production of *Clostridium botulinum* was tested.

A Group I *C. botulinum* type A strain was grown in the Biolog PM1 MicroPlate™ Carbon Sources (Biolog, Hayward, CA, USA) for 3 days at 37 °C under shaking in a multiwall plate reader placed in an anaerobic box. Each well of the MicroPlate™ contained a dried supplement serving as carbon source after dilution into the culture medium post-inoculation. Bacterial cell density was recorded as optical density at 595 nm. The experiment was repeated three times. The supplier's protocol was modified as follows: the inoculation medium was replaced by casamino acid–based semi-synthetic medium CDM-1 (modified from (1)), the plate was anaerobically pre-incubated with 50 μL per well of CDM-1 for 2 days at 4°C prior to inoculation to improve solubility of the substrates and then inoculated with 50 μL per well of 1:20 diluted overnight culture grown in CDM-1.

Modification of the protocol yielded growth in all tested substrates and led to higher reproducibility of growth compared to the supplier's protocol. The growth curves acquired allowed us to distinguish between substrates having no or little effect on growth of the test strain and substrates enhancing its growth. The growth-enhancing substrates led to higher maximum cell density, prolonged stationary phase or a combination of both. Amongst the growth-enhancing substrates, we found carbohydrates as well as amino acids and nucleosides. Specifically, high cell densities were achieved with trehalose, glucose, maltotriose, and maltose.

The phenotypic microarray using an in-house modified protocol proved to be a valuable screening tool for comparing carbon substrates effecting growth of *C. botulinum*. However, the small culture volume and the low cell mass gained might limit downstream applications, such as BoNT ELISA (enzyme-linked immunosorbent assay).

Keywords: *Clostridium botulinum*; Growth; Phenotypic microarray; Screening tool

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A NOVEL QUANTIFICATION METHOD FOR MEASURING NEUTRALIZING ANTIBODIES AGAINST BOTULINUM TOXINS IN SERA OF TOXIN – NON-RESPONSIVE PATIENTS

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Introduction and Objectives: Botulinum neurotoxins (BoNTs) are the most toxic substances in nature and act by preventing the release of acetylcholine at the neuromuscular junction.[1]

Among botulinum neurotoxin serotypes, type A (BoNT/A) and B (BoNT/B) are commonly used for therapeutic and cosmetic applications.[2] Repeat injections of BoNTs are a common clinical practice that can cause therapeutic failure of BoNTs.[3] The most common cause of treatment failures is production of neutralizing antibodies (NAbs) against the BoNTs.[4,5] There are several assays to detect and quantify the NAbs in patient sera, such as the mouse lethality assay (MLA),[6] mouse phrenic nerve hemidiaphragm assay (MPN)[7], and enzyme-linked immunosorbent assay (ELISA).[8] The mouse bioassay and MPN are expensive, labor intensive and raise ethical concerns. Although the ELISA is an easily accessible method, it detects not only NAbs but also toxin-binding proteins. For these reasons, novel assay methods that mimic the physiological mechanisms of interaction between BoNTs and NAbs are required.

Methods: We have designed a cell-based neutralizing antibody assay (CBNA) to assess the amount of NAbs in human serum. Previously, we developed the toxin-sensitive cells containing luciferase reporter gene for quantifying the potency of BoNT/A.[9]

Results: CBNA results showed a consistent dose-response relationship ($R^2 \geq 0.99$) between luciferase activity and titers of BoNT/A antitoxins supplied by the National Institute for Biological Standards and Control (NIBSC). Under optimized conditions, CBNA was able to detect 0.4 m IU/mL of NAbs from patient sera. We recruited 4 patients categorized as "complete non-responders" to BoNT/A by clinicians. Using CBNA, we could detect and titrate the NAbs (range 4-12 m IU) from sera of all 4 patients. We also confirmed that the assay results from CBNA correlated with the results of the mouse bioassay.

Conclusions: We have developed a highly sensitive quantification method for measuring the anti-drug antibody to botulinum toxin products. The easy, rapid, and sensitive nature of this assay will make it a very useful

method for monitoring NAbs from non-responders in clinical practice.

Keywords: Botulinum toxin type A; Cell-based assay; Neutralizing antibody

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PATIENT SATISFACTION WITH ABOBOTULINUMTOXINA FOR AESTHETIC USE IN THE UPPER FACE: A SYSTEMATIC LITERATURE REVIEW AND POST HOC ANALYSIS OF THE APPEAL STUDY

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Introduction: Patient satisfaction with aesthetic treatment is of utmost importance for determining treatment success. We aimed to analyze current literature on patient satisfaction with abobotulinumtoxinA (aboBoNT-A) for upper facial aesthetic indications.
Methods: Systematic review of literature databases to identify English language publications on patients receiving aboBoNT-A for upper facial aesthetic indications (e.g., glabellar lines, lateral canthal lines) that assessed patient and/or physician satisfaction with treatment. Structured data extraction enabled inter-study analysis. Post hoc analysis assessed patient satisfaction by gender and age, using data from the non-interventional APPEAL study (NCT02353897) of aboBoNT-A in glabellar lines (Gubanova E, et al, 2018).
Results: Overall, 22 original research papers were identified. Patient satisfaction rates for aboBoNT-A treatment were significantly higher versus placebo from 2 weeks up to 3-5 months post-injection across studies. At 2-3 weeks post-injection, patient satisfaction rates were 52-99% across studies. In studies with later time points, patient satisfaction rates were 85-87% at 5 months and 30-100% at 6 months post-injection. Physician satisfaction was also high (97-100%, across three treatment cycles). In the APPEAL post hoc analysis, patient satisfaction was similar when assessed by gender, although few male patients were enrolled (Table). Patient satisfaction was also similar across age groups (Table), with the highest and lowest proportions of "very satisfied" patients among those aged 18-30 years (80%) and >60 years (67%), respectively. Attributes associated with patient satisfaction were largely similar by both gender and age (Table).
Conclusions: This review suggests high patient satisfaction rates have been achieved with aboBoNT-A in upper facial aesthetic indications. Despite the current recommended injection interval of ≥12 weeks, satisfaction is still evident for up to 6 months in some patients.
Funding: Ipsen
Keywords: AbobotulinumtoxinA; Botulinum toxin; Glabellar lines; Lateral canthal lines; Crow's feet; Patient satisfaction

Table 1
Outcomes of patient satisfaction evaluation by gender and age group after three aboBoNT-A treatment cycles in the APPEAL study, reported at 3 weeks ± 7 days following each injection

Satisfaction questionnaire attributes*	All patients n (%) (N=135/150)	Gender, n (%)		Age group, n (%)				
		Men (N=9/13)	Women (N=126/137)	18–30 years (N=10/13)	31–40 years (N=25/32)	41–50 years (N=57/58)	51–60 years (N=31/34)	>60 years (N=12/13)
Satisfaction†								
'Very satisfied'	100 (74.1)	6 (66.7)	94 (74.6)	8 (80.0)	19 (76.0)	42 (73.7)	23 (74.2)	8 (66.7)
'Satisfied'	34 (25.2)	2 (22.2)	32 (25.4)	2 (20.0)	6 (24.0)	15 (26.3)	7 (22.6)	4 (33.3)
'Neutral'‡	1 (0.7)	1 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.2)	0 (0.0)
'Dissatisfied'	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
'Very dissatisfied'	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
'Happy to receive treatment again' (Yes)	133 (98.5)	8 (88.9)	125 (99.2)	10 (100.0)	24 (96.0)	57 (100.0)	30 (96.8)	12 (100.0)
'Recommend treatment to family or friends' (Yes)	134 (99.3)	8 (88.9)	126 (100.0)	10 (100.0)	25 (100.0)	57 (100.0)	30 (96.8)	12 (100.0)
'Perception of age' (looking younger)§	124 (91.9)	7 (77.8)	117 (92.9)	8 (80.0)	23 (92.0)	56 (98.2)	27 (87.1)	10 (83.3)
'Result of treatment looks natural' (Yes)	135 (100.0)	9 (100.0)	126 (100.0)	10 (100.0)	25 (100.0)	57 (100.0)	31 (100.0)	12 (100.0)
'Meets or surpasses expectations' (Yes)	132 (97.8)	8 (88.9)	124 (98.4)	10 (100.0)	23 (92.0)	57 (100.0)	30 (96.8)	12 (100.0)
'Feeling about yourself' (feeling better)‖	128 (94.8)	8 (88.9)	120 (95.2)	9 (90.0)	22 (88.0)	56 (98.2)	30 (96.8)	11 (91.7)
'Aesthetic outcome within 3 weeks after injection' (satisfaction)[a]	134 (99.3)	8 (88.9)	126 (100.0)	10 (100.0)	25 (100.0)	57 (100.0)	30 (96.8)	12 (100.0)

* Percentages are based on the number of patients with evaluable data (N), missing data: 15/150 [men, n=4; women, n=11];
‡ Neither satisfied nor dissatisfied; Satisfaction was indicated by the following responses on the questionnaire:
† 'very satisfied' or 'satisfied';
§ 'much younger' or 'a little younger';
‖ 'a lot better', 'much better' or 'a little better';
[a] 'very satisfied' or 'satisfied'. AboBoNT-A=abobotulinumtoxinA.

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ONABOTULINUMTOXINA EXHIBITS GREATER EFFICACY COMPARED TO PURIFIED BOTULINUM NEUROTOXIN TYPE A (BONT/A-150 KDA) IN PERIPHERAL PAIN MODELS

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Introduction: OnabotulinumtoxinA (onabotA), a botulinum neurotoxin type A (BoNT/A) protein complex containing non-toxic neurotoxin-associated proteins (NAPs), is approved for migraine and has shown anti-inflammatory and analgesic properties in numerous animal pain models and clinical pain disorders.[1,2] Herein, we compared the anti-inflammatory and analgesic efficacy of onabotA to purified 150-kDa BoNT/A toxin (BoNT/A-150) in several pain models.
Methods: Maximum equi-efficacious doses of onabotA and BoNT/A-150 on a motor indication were initially determined using the rat Digit Abduction Score (DAS) assay.[3] We then evaluated the efficacy of the toxins in 3 inflammatory pain rat models: formalin-induced nociceptive behavior, Complete Freund's Adjuvant (CFA)-induced acute paw inflammation, and monoiodoacetate (MIA)-induced chronic joint inflammation, and 1 neuropathic pain model: spared nerve injury (SNI).
Results: Maximum equi-efficacious doses of onabotA and BoNT/A-150 in the DAS assay were determined as 15 U/kg and 0.1 ng/kg ($\approx$17 U/kg), respectively, and these doses were used for testing in the pain models. In the formalin-induced pain model, onabotA displayed a marked 47% reduction in nociceptive behavior, while BoNT/A-150 showed a 35% decrease. In the CFA pain model, both onabotA and BoNT/A reduced CFA-induced paw inflammation equally. In the MIA-induced pain model, onabotA significantly diminished knee joint inflammation, whereas BoNT/A-150 showed only a trend in reduction. OnabotA and BoNT/A-150 improved MIA-induced dynamic weight-bearing deficit by 50% and 38%, respectively. In the SNI neuropathic pain model, only onabotA evoked a significant reduction in mechanical allodynia.
Conclusions: OnabotA displayed greater anti-inflammatory and analgesic efficacy compared to purified BoNT/A-150 in models of inflammatory and neuropathic pain, suggesting that the NAPs present in onabotA may play a vital role in enhancing the antinociceptive properties of this toxin.
Funding: Allergan Aesthetics, an AbbVie Company
Keywords: Animal pain model**;** Botulinum neurotoxin type A; Inflammation; Neuropathic pain

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RELABOTULINUM TOXIN – A NOVEL, HIGH-PURITY BONT-A1 IN LIQUID FORMULATION

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Introduction and Objectives: Botulinum toxin has been used clinically for 40 years and is commonly used for aesthetic purposes. The global botulinum toxin market is currently dominated by lyophilized products that need to be reconstituted in saline before clinical use. Using a liquid formulation would facilitate ease of use and circumvent potential dosing errors from faulty reconstitution. Although attempts have been made, so far, very few liquid products have entered the market. The only approved liquid BoNT-A product on the market to date is Innotox® from Medytox, which to our knowledge is only sold in South Korea.
The goal of Galderma has been to develop a high quality BoNT-A1 toxin, designed to be stable in a liquid formulation. The aim was to produce a complex-free BoNT-A1 toxin with high purity and activity, using a gentle and highly efficient manufacturing process, in a state-of-the art manufacturing facility.
Methods: Manufacturing of Drug Substance (DS) is done using an in-house isolated natural anaerobic *Clostridium botulinum* type A1 strain. The manufacturing process is designed to be gentle and robust, in order to produce highly purified and fully potent BoNT-A1 toxin, free of complexing proteins. The DS process uses modern fermentation, filtration, and chromatography methods in combination with the latest single-use technology, thereby avoiding harsh process steps and open handling of highly potent solutions. The production process of the Drug Product (DP) also uses the latest single-use technology and is performed in a newly built, dedicated facility. The DP is a stable and animal origin–free toxin formulation. The formulation is a key achievement in the development of the liquid toxin product.
DS was analysed for protein purity using Ultra-High–Performance Liquid Chromatography–Size Exclusion Chromatography (UPLC-SEC) and SDS-PAGE (sodium dodecyl sulphate–polyacrylamide gel electrophoresis) . In the UPLC-SEC analysis, DS was separated on a Waters BEH™ SEC column, and analysed using absorbance (A_{280}). SDS-PAGE was run on both reduced and unreduced material and was stained with Colloid Coomassie to enable quantification of impurities. The specific activity was determined using mouse LD_{50} potency data in combination with total protein analysis using the μBCA method.
Results: Production of Relabotulinum toxin was achieved using the latest single-use technology. Results show that BoNT-A1 of high purity was produced. UPLC-SEC analytical results show over 98% purity (main peak). SDS-PAGE results confirmed the high purity, with only expected toxin bands detected on the gel. High specific activity data (~2.0×10^8 U/mg total protein), confirm that the highly purified Relabotulinum toxin is fully active. The high-quality DS was a prerequisite for the development of a stable liquid DP. The stability of DP as a liquid formulation was further achieved by the unique DP formulation that is using polysorbate 80 in combination with tryptophan as stabilizing agents.
Conclusions: Relabotulinum toxin in a stable liquid formulation, has been produced by Galderma in a newly built, dedicated facility, using state-of-the-art, single-use technology and processes designed to be gentle and robust. Results show that Relabotulinum toxin is a highly purified, complex-free BoNT-A1 toxin with high specific activity.
Keywords: Botulinum toxin; BoNT-A1; Liquid formulation; Relabotulinum toxin; Single-use technology

LOW-DOSE BOTULINUM NEUROTOXIN AND ALCOHOL IN POST-STROKE SPASTICITY: THE 25-YEAR EXPERIENCE

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Introduction and Objective: Spasticity manifests as an increase in stretch reflexes, producing tendon jerks and resistance appearing as muscle tone, caused by damage to central motor pathways that control voluntary movement.[1] Botulinum neurotoxin type A (BoNT/A) injections have been used to block the final common pathway at the neuromuscular junction. There is level A evidence for BoNT/A therapy in various movement disorders, including spasticity.[1,2] BoNT/A is better tolerated than alcohol as it is a relatively safe medication and has few serious side effects. However, its high cost limits its use. Both BoNT/A and alcohol have dose-ceiling limitations.[3] Restoration of neurological function is the long-term aim of spasticity treatment.[1-3] Low-dose BoNT/A therapy for Thai patients and patients in hot climate counties/or during hot season optimized outcomes and improved function.[3-9] This study aimed to analyze 25 years' experience with the use of low-dose BoNT/A and alcohol in treating adult focal spasticity.
Methods: A retrospective study of spasticity patients at the Spasticity and Dystonia Clinic in the Faculty of Medicine, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Bangkok, Thailand, who were treated with BoNT/A and/or alcohol, was carried out. Data studied included baseline patient demographics, evaluation of spasticity, BoNT/A injection and/or alcohol neurolysis protocols and dosages, injection guidance techniques, number of sessions, intervals between treatments, additional treatments, outcomes, and assessment of patients' satisfaction with treatments.
Results: A total of 4315 patients and 28995 injections for all indications were studied (see Figure). There were 385 (8.92%) post-stroke spasticity patients who received a total of 2745 (9.47%) injections. For post-stroke spasticity, 845 injections (2.91% from all indications and 30.78% from post-stroke spasticity) were with BoNT/A plus alcohol, 315 (1.09% and 11.48%) with BoNT/A alone, and 1585 (5.47% and 57.74%) with alcohol alone. BoNT/A dosages used were 20 and 50 units (U) and 40 and 75 U of onabotulinumtoxin A (Botox®) for small and large muscles, respectively in patients with Modified Ashworth Scale (MAS) scores of 3 and 4. The ratio of onabotulinumtoxinA (Botox®) and abobotulinumtoxin A (Dysport ®) was 1:3. The injection guidance techniques used for BoNT/A were: ultrasound in 658 (23.97%) and electromyography in 416 (15.15%) patients.
The injection guidance techniques used for alcohol injections were guidance using compound motor action potentials for 1205 (43.90%), electrical stimulation for 371 (13.52%), and anatomic localization for 429 (15.63%) patients. The onset of BoNT/A effect was 3-7 days, with peak effect at 2-4 weeks, and duration of action was 3-6 months. The number of treatment sessions was 3 per year in the first year and one each year afterwards. The patients rated treatment as good to excellent. All patients received stretching and strengthening exercises, balance and gait training, and upper limb training after injections.
Conclusion: My 25-year experience with use of low-dose BoNT/A and alcohol neurolysis in treatment of adult focal spasticity shows that optimal results were obtained, and treatment was helpful in improving function. Additional rehabilitation programs can extend the relaxation of spastic muscles and increase patients' abilities in walking and activities of daily living, as well as decrease spastic pain and improve sleep quality and overall quality of life.
Keywords: Alcohol neurolysis; 25-Year experience; Botulinum neurotoxin type A (BoNT/A); Low dose; Spasticity

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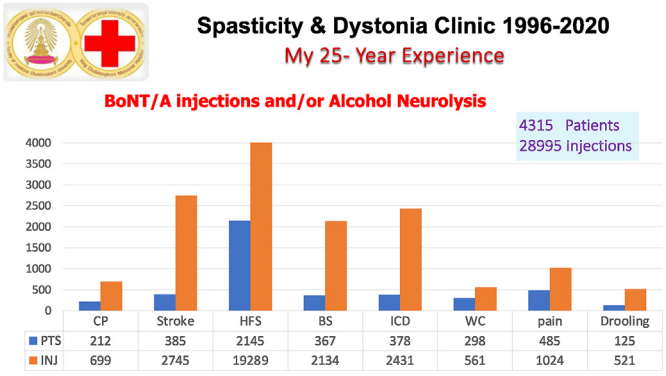

Fig. Number of patients and injections of BoNT/A and alcohol in each indication. CP=cerebral palsy; HFS=hemifacial spasm; BS=blepharospasm; ICD=idiopathic cervical dystonia; WC= writer's cramp; PTS=number of patients; INJ=number of injections.

FUNCTIONAL ANALYSIS OF THE TYROSINE PHOSPHATASE LAR IN THE ENDOCYTOSIS OF THE TETANUS TOXIN

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Introduction: Tetanus is a life-threatening disease caused by tetanus neurotoxin (TeNT) and is a major cause of death in non-vaccinated areas. Intoxication by TeNT causes spastic paralysis by blocking neurotransmitter release in spinal cord inhibitory neurons.[1] Uptake of TeNT is facilitated by its interaction with the extracellular matrix proteins nidogen-1 and-2 at the mammalian neuromuscular junction (NMJ), both of which are sorted into signalling endosomes and retrogradely transported towards the neuronal soma.[2] However, the key molecules enabling the uptake of the nidogen-TeNT complex into motor neurons are currently unknown. A proteomic screen aimed at describing the molecular composition of TeNT-carrying signalling endosomes in motor neurons identified the tyrosine phosphatase leukocyte antigen–related protein (LAR) as a component of these endosomes.[3] Additionally, LAR has been shown to interact with the laminin-nidogen complex.[4,5] In this context, we are examining the role of the LAR family of phosphatases in the internalisation of the tetanus toxin in motor neurons.

Methods: Our combined approach includes: i) immunoprecipitation and western blotting; ii) lentivirus-mediated protein knockdown; iii) immunocytochemistry; and, iv) quantitative, high-resolution imaging.
Results: Our studies have shown that LAR interacts with nidogens in the presence of the TeNT non-toxic binding fragment HcT, and that this binding is governed by alternatively splicing LAR. Importantly, knockdown of LAR leads to a decrease in the uptake of HcT in motor neurons, which is independent of its reported role as a modulator of tyrosine receptor kinase B (TrkB) activity.
Conclusions: Our data indicates that LAR is a component of the TeNT receptor complex, thus identifying a key molecule involved in TeNT intoxication. Furthermore, it uncovers a crucial link between the TeNT and neurotrophin pathways and presents LAR as a potential therapeutic target in tetanus.
Keywords: Neuromuscular junction; Neuron; Nidogen; LAR; Tetanus neurotoxin; Tyrosine phosphatase

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APPLICATION OF SINGLE-PHOTON EMISSION COMPUTED TOMOGRAPHY IN CERVICAL DYSTONIA: A DOUBLE-BLIND, RANDOMIZED STUDY

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Objectives: Focal intramuscular botulinum toxin type A (BTX-A) injection is the first-line treatment for cervical dystonia (CD). The key requirement for a better outcome is a correct screening of dystonic muscles. The present study assessed the usefulness of single-photon emission computed tomography (SPECT) in guiding BTX-A injection in CD.
Methods: This was a single-center, randomized, double-blind study. Patients were randomized into the study group (candidate muscles were selected by clinical evaluation and SPECT) and a control group (candidate muscles were selected by clinical evaluation alone). In both groups, electromyography was performed for all selected candidate muscles. Follow-up was conducted at the end of the 2nd week and the 1st, 3rd, and 6th months. The Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) and Tsui score were used to assess the efficacy of the treatment.
The primary outcomes were the reduction rates in TWSTRS and Tsui scores at the 1st month after injection.
Results: A total of 122 patients were enrolled, and 108 completed the study. The reduction rate in the TWSTRS score at the end of the 1st month was significantly higher in the study group compared to the control group (54.8 ± 22.9% versus 44.5 ± 24.3%, P=0.026). However, the reduction rate in the Tsui score did not differ significantly between the two groups (60.0 ± 23.5% in the study group versus 53.0 ± 21.2% in the control group, P=0.107). The reduction rates in the TWSTRS and Tsui scores at the end of the 2nd week and the 3rd month were significantly higher in the study group than in the control group. However, at the end of the 6th month, the reduction rate did not differ between the two groups for either score. Within six months, the re-injection interval was significantly longer in the study group vs the control group (159.1 ± 28.6 and 141.8 ± 51.0 days, respectively; P=0.032). The duration of maximum symptom relief was also longer in the study group vs the control group (98.8 ± 19.9 and 88.4 ± 17.5 days, respectively; P=0.005). In the study group, more injections were administered to the sternocleidomastoid, levator scapulae, obliquus capitis inferior, semispinalis capitis, semispinalis cervicis, longissimus capitis, and posterior scalene than in the control group.
Conclusion: SPECT imaging could be a useful tool to identify dystonic muscles in CD patients before BTX-A injection. It can improve the efficacy of BTX-A injections by increasing the detection and treatment of deep dystonic muscles.
Keywords: Single-photon emission computed tomography; Cervical dystonia; Botulinum toxin; ^{99m} Technetium-sestamibi

COVALENT INHIBITION OF BOTULINUM NEUROTOXIN A – EXPLORATION OF WARHEAD REACTIVITY AND FUNCTION USING A BIFUNCTIONAL APPROACH

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Introduction and Objectives: Botulinum neurotoxin type A (BoNT/A) is extremely toxic, possessing an estimated intravenous LD_{50} of 1-2 ng/kg and as such has been designated a category A bioterrorism agent.[1,2] BoNT/A also possesses an extremely long half-life and persists within muscle neurons for months to >1 year.[3] Because of BoNT/A longevity, we have utilized covalent inhibition as a means to abrogate BoNT/A's toxicity. To this end, we describe an approach to designing inhibitors that possess both electrophilic warheads and metal-binding groups for the bifunctional inhibition of BoNT/A.
Methods: Small molecule inhibitors that possessed electrophilic moieties were designed, using X-ray crystallography as guidance, to target both the zinc metal-binding region and Cys165 within the active site of BoNT/A. Synthesized compounds were evaluated for covalent inhibition using a continuous SNAPtide FRET assay[4], and exhaustive dialysis. Compounds were also evaluated against a C165A variant. Compound reactivity, stability, MMP selectivity, and cellular efficacy/toxicity were also evaluated.
Results: Several electrophilic warhead types were confirmed to inhibit BoNT/A LC covalently with substantial differences in time-dependent inhibition between the WT and C165A variant. A trend in warhead reactivity was reflected in inhibitor stability and toxicity. Compounds exhibited moderate potency in a BoNT/A neuronal cellular assay but were not further explored due to undesirable therapeutic potential.
Conclusions: A fundamental framework for the bifunctional covalent inhibition of BoNT/A LC has been established. This approach has potential to be translated to other small molecule metal-binding inhibitors of BoNT/A LC with the vision that different pharmacophores, possessing improved physicochemical properties, will address BoNT/A's toxicity and longevity within cells.
Funding: This work was funded by NIH grants R01 AI153298 and R21

AI137709 and F32; the Fulbright Scholar Program; the Natural Sciences and Engineering Research Council of Canada PGSD3-502274; and the Skaggs Institute for Chemical Biology.
Keywords: Bifunctional; Botulinum neurotoxin A; Covalent; Reactivity

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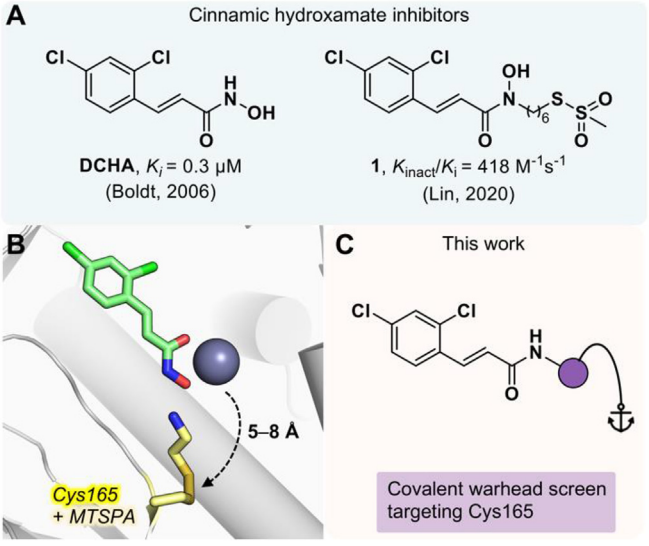

Fig. 1(A). BoNT/A inhibitors based on the cinnamic hydroxamate scaffold. (B) X-ray co-crystal structures outlining the basis for a bifunctional approach, overlaying DCHA (green; PDB 2IMA) and MTSEA (yellow; PDB 4ELC) bound to Cys165 near the active site Zn^{2+} (purple sphere). (C) Overview of research explored.

ECONOMIC OUTCOMES IN REAL-WORLD USE OF BOTULINUM TOXIN-A PRODUCTS FOR ADULT PATIENTS WITH UPPER LIMB SPASTICITY: A UK PERSPECTIVE

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Introduction: Clinical trials show that botulinum toxin A (BoNT-A) can be effective in the treatment of upper limb spasticity (ULS). However, comparative data on effectiveness and economic outcomes are limited. Based on real-world data from an observational study, this analysis aimed to compare outcomes of treatment with abobotulinumtoxinA (aboBoNT-A), onabotulinumtoxinA (onaBoNT-A) and incobotulinumtoxinA (incoBoNT-A), from a UK perspective.
Methods: ULIS III (NCT02454803) is an international, multicenter, non-interventional, prospective, longitudinal (2-year) study of adult patients with ULS treated with aboBoNT-A, onaBoNT-A, or incoBoNT-A. Patients were excluded from analysis of response and injection interval if they changed BoNT-A during follow-up. Response was defined as a ≥10-point increase in the cumulated Goal Attainment Scaling (GAS) T score, vs baseline. Toxin costs were estimated by applying unit costs (from the British National Formulary) to the annualized mean dose for each product.
Results: Overall, 832 patients contributed data for injection interval (N=555 for aboBoNT-A, 198 for onaBoNT-A, and 79 for incoBoNT-A), and 830 for response. Response rates for the three BoNT-As were 78.2%, 61.6%, and 75.6%, respectively. The mean (SD) dose per injection was 843 (353), 256 (136), and 278 (129) units, with mean (SD) days between injections of 222.8 (167.2), 204.4 (173.6), and 166.9 (105.1). This corresponds to annualized toxin costs of £464 (aboBoNT-A), £707 (onaBoNT-A), and £872 (incoBoNT-A).
Conclusion: These real-life data indicate that aboBoNT-A injections may be a cost-effective treatment for ULS, due to lower injection costs and longer intervals between treatments. However, additional comparative data from larger patient cohorts would be valuable to confirm these findings.
Funding: This study was funded by Ipsen.
Keywords: Botulinum neurotoxin type A; Cost-effectiveness analysis; Quality of life; Spasticity costs

THE SPASTICITY-RELATED QUALITY OF LIFE 6-DIMENSIONS TOOL (SQOL-6D) IN UPPER LIMB SPASTICITY: A FIRST PSYCHOMETRIC EVALUATION

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Introduction: To describe the psychometric properties (validity, reliability, responsiveness) of SQoL-6D, a tool designed to assess disease burden and change after focal upper limb spasticity (ULS) therapy.
Methods: Multicentre prospective study in adults (aged ≥18 years) with ULS. Patients completed the SQoL-6D at enrolment (Visit [V]1), 8 (±2) weeks (V2) and 1–4 days after V2 (re-test). SQoL-6D covers 6 dimensions (pain/discomfort, involuntary movements/spasms, restricted range of movement, caring for the affected limb, using the affected limb, mobility) each rated from 0-4 (4=worse outcome) and a total score (TS) calculated from 0-100 (100=better outcome). Standard ULS measures (Modified Ashworth Scale, Arm Activity Measure, Goal Attainment Scaling, EuroQoL 5 Dimensions 5 Levels questionnaire, global assessment of benefit) were also recorded.
Results: The SQoL-6D was shown to be unidimensional and demonstrated adequate construct validity. The internal reliability of the SQoL-6D was supported by Cronbach's alpha (>0.70), while the intraclass correlation coefficient supported the test-retest reliability (0.82). Correlation coefficients with established instruments supported convergent validity, while significant differences between known-groups (of differing clinical severity of weakness) in SQoL-6D TS confirmed its sensitivity. Significant

differences in mean SQoL-6D TS change and effect sizes across patients rating "some benefit" and "great benefit" (0.51 and 0.88 respectively, Table) supported its sensitivity to clinical change.
Conclusions: SQoL-6D is a promising new measure of patient-reported disease burden and health status in ULS.
Funding: Ipsen
Keywords: Patient-reported disease burden; Spasticity, Quality of life; Spasticity-related Quality of Life 6-Dimensions tool; SQoL-6D

Table. Change in SQoL-6D Total Score Between V1 and V2 by Subgroups of Clinical Benefit*.

Subgroups of clinical benefit	N	Change in SQoL-6D total score	
		Mean (95%CI)	Effect size
Patient global assessment of benefit scale			
No benefit	5	2.5 (-10.5, 15.5)	0.19
Some benefit	51	9.5 (6.6, 12.4)	0.51
Great benefit	31	17.6 (13.1, 22.1)	0.88
Clinician global assessment of benefit scale			
No benefit	5	-0.8 (-14.2, 12.6)	-0.04
Some benefit	56	10.1 (7.1, 13.1)	0.52
Great benefit	29	17.5 (13.3, 21.8)	0.98

* Defined by change in clinical severity by Visit 2. CI, confidence interval; SQoL-6D, spasticity-related quality of life 6-dimensions; V, visit.

LONGITUDINAL GOAL ATTAINMENT WITH INTEGRATED UPPER LIMB SPASTICITY MANAGEMENT INCLUDING BOTULINUM TOXIN A: PRIMARY RESULTS FROM THE ULIS-III STUDY

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Introduction: The primary aim of the ULIS-III study was to assess the longitudinal effects of integrated spasticity management incorporating repeated cycles of botulinum toxin A (BoNT-A) over 2 years.
Methods: ULIS-III (NCT02454803) was a prospective, observational study following adult (≥18 years) patients living with spasticity over 2 years of integrated upper limb spasticity (ULS) management including repeat BoNT-A treatment. The study was the first to use the Upper Limb Spasticity Index (ULS Index), an assessment battery including a structured approach to goal attainment scaling (GAS) alongside a set of standardized measures. Participants continued with their usual concomitant therapies, which were recorded in the Upper Limb Focal Spasticity Therapy Recording Schedule (ULSTR) to document the number/duration/type of therapies related to specific goals.
Results: A total of 1004 participants from 14 countries were enrolled, of which 953 underwent ≥1 BoNT-A injection cycle and had ≥1 GAS assessment. Overall, participants underwent a median [range] of 4 [1-9] BoNT-A injection cycles. A majority of participants (55.9-64.6% across cycles 1-6) saw a therapist after BoNT-A treatment; the most frequent therapy intervention was passive stretch (70.1-79.8% across cycles 1-6). Patients achieved their goals as expected over repeated cycles; mean [95% CI] GAS T scores at baseline were 36.7 [36.5, 36.9] and mean cumulated GAS T scores at 2 years was 49.5 [49.1, 49.9]. Higher rates of goal achievement were seen for primary goals related to passive vs. active function (86.6% vs 71.4% achievement) [Figure]. Standardised measures of spasticity, pain, involuntary movements, active and passive function improved over each treatment cycle.
Conclusions: This large, international study provides evidence for the benefit of repeated cycles of BoNT-A, sustained over 2 years and captured through goal attainment scaling and standardised measures.
Keywords: Botulinum toxin; Goal attainment scaling; Observational study; Spasticity; Upper-limb

Fig. Percentage achievement of primary goal set by area.

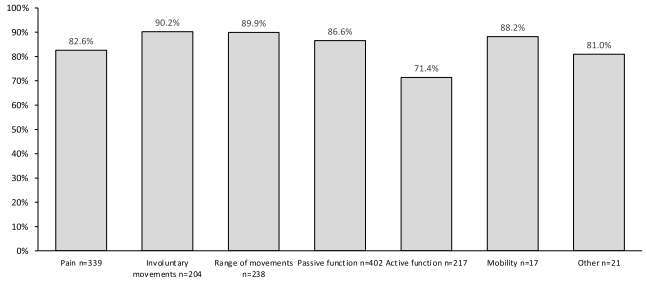

Fig. Percentage achievement of primary goal by goal area.

NOVEL SECRETION INHIBITORS OF PAIN-RELATED MEDIATORS WITH POTENTIAL FOR RELIEVING CHRONIC PAIN

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Targeted delivery of a potent inhibitor of pain-related mediators into inflammatory or pain-sensing cells is a promising avenue for treating chronic pain, a major, world-wide healthcare problem. There is an unmet need for a specific and effective delivery strategy. Herein, we developed a novel approach using a sortase to site-specifically ligate a non-toxic botulinum neurotoxin type D (BoNT/D) core therapeutic (synaptobrevin-cleaving protease and translocation domains) to cell-specific targeting ligands. A recombinantly-engineered core therapeutic was efficiently ligated to IL-1β ligand within minutes. The resultant conjugate specifically entered into cultured murine primary macrophages, cleaved synaptobrevin isoform 3 and inhibited LPS/IFN-γ–evoked IL-6 release. Likewise, a CGRP receptor antagonist ligand delivered BoNT/D protease into sensory neurons and inhibited K^+-evoked substance P release. As cytokines and neuropeptides are major regulators of inflammation and pain, blocking their release using novel engineered inhibitors highlights the therapeutic potential of these inhibitors. Our study provides a new platform with broad applicability for developing targeted biotherapeutics for safer treatments of chronic diseases.
Funding: This research was funded by Science Foundation Ireland through a Career Development Award (13/CDA/2093), a SFI Technology Innovation Development Award (17/TIDA/4977), and a Starting Investigator Research Grant (15/SIRG/3508).
Keywords: Cytokine; Neuropeptides; Neurotoxin; Protein conjugation; Targeting; Therapeutics

CHARACTERIZATION OF THE PROTOSPACER TARGETS OF *Clostridium botulinum* CRISPR-CAS SPACER ARRAYS

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Introduction and Objectives: The botulinum neurotoxin (*bont)* genes are horizontally distributed across multiple species of *Clostridium.* While recombination has occurred across *bont* genes and gene clusters, the primary serotypes have significantly diverged from each other over time. Comprised of a Cas nuclease system and CRISPR spacer arrays, CRISPR-Cas systems (CCS) enable sequence-specific targeting of invading foreign plasmid/phage-borne DNA via protospacer complementation. CCS represent a restrictive barrier to the horizontal movement of DNA across individual bacteria. We identified spacer-protospacer matches between CRISPR/Cas^{+} *C. botulinum* strains and plasmid/phage to further explore how endogenous CCS restrict and regulate the horizontal gene transfer across *Clostridia*.
Methods: We conducted a bioinformatic investigation of endogenous CRISPR spacer arrays and *cas* gene clusters from a selection of *C. botulinum* genomes containing conserved CCS. Spacer arrays were matched to complementary protospacers via homology inference and high-quality matches were analyzed and mapped onto an interaction network.
Results: Types I and II *cas* gene families are prevalent throughout *C. botulinum* strains. In most strains, matched protospacers were identified both on plasmids or phage uniquely targeted by that strain, and those targeted by other analyzed strains. In particular, we found that the large, ≥100 kbp, and often BoNT encoding plasmids were frequently the mutual target of CRISPR spacers in *C. botulinum* strains carrying well-conserved CCS.
Conclusions: Our analysis of endogenous CCS begins to reveal the scope of the restriction network on the horizontal flow of genetic material across *C. botulinum* strains, with implications for the horizontal trafficking of *bont* genes. Alongside restriction-modification systems and other barriers to the uptake and transfer of horizontally acquired material, CCS may have contributed to the evolutionary divergence of the BoNT serotypes.
Funding: This work was supported in part by a grant from the Food Research Institute, University of Wisconsin-Madison and by a cooperative agreement between the University of Maryland, Joint Institute for Food Safety and Applied Nutrition, and the Food and Drug Administration.
Keywords: Botulinum neurotoxin; *Clostridium botulinum*; CRISPR; CRISPR-Cas; Plasmid; Toxin

BONT INTOXICATION: FUNCTIONAL GENOMICS REVEALS AN UNEXPECTED TRAFFICKING ROUTE

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Introduction: Botulinum neurotoxins (BoNTs) exert their paralytic effects by cleaving soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins, blocking neurotransmission. To exert this function, the catalytic light chain unit of the toxin must first be translocated into the neuronal cytosol. While it is proposed that translocation occurs in endosomes shortly after internalization, the mechanisms remain poorly understood.
Methods: An image-based, genome-wide RNA interference screen assay was employed to explore how BoNT type A1 (BoNT-A1) intoxicates neurons. The assay is based on the degradation of a fluorescence reporter upon BoNT-mediated SNAP-25 (synaptosomal-associated protein 25) cleavage in a differentiated human neural stem cell line (Red SNAPR). In brief, cells were differentiated for 2 weeks and treated with small interfering (si)RNA for 3 days before BoNT intoxication for 48 hours. siRNA against thioredoxin reductase was used as a positive assay control. Genome-wide data analysis was carried out using ScreenSifter, PANTHER gene ontology, and STRINGS.
Results: The screen revealed a wide array of genes (>300 hits) with a significant effect.
Gene ontology and protein network analysis revealed numerous genes in host pathways known to be required for BoNT intoxication, including endosomal acidification and thioredoxin reduction. We also identified a distinct repertoire of genes associated with membrane trafficking, in particular genes involved in trafficking of the BoNT receptor, SV2. Interestingly, several hits are required for endosome to Golgi retrograde traffic, suggesting a role for this transport step in BoNT intoxication.
Conclusions: Our results strongly suggest that BoNT relies on a complex trafficking pathway and involvement of host cell machinery for cytosolic translocation of the catalytic light chain, thus shedding a new light on the mechanism of BoNT intoxication.
Funding: This work was jointly funded by the Institute of Molecular and Cell Biology A*Star Research Entities, and Ipsen.
Keywords: BoNT-A; High-content screen; Neuronal assay; siRNA knockdown; SNAP-25