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Basic Science and Clinical Aspects of Botulinum and Other Neurotoxins

Abstracts

Toxicon

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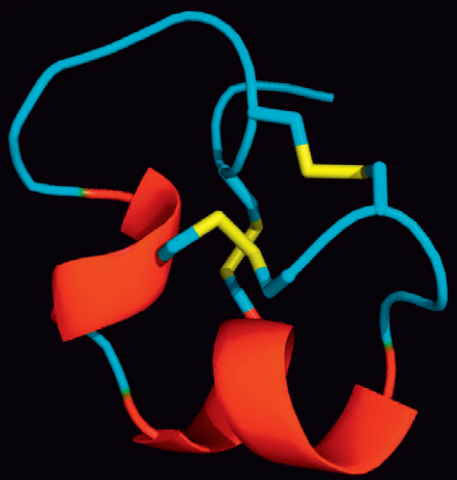

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Toxins 2022 6th International Conference

This special supplement of *Toxicon* contains the podium and poster abstracts accepted for TOXINS 2022 6th International Conference which will be held in New Orleans, Louisiana, July 27–30, 2022.

Since the last TOXINS conference, there have been important advances on the basic science and clinical research of botulinum neurotoxins (BoNTs) in a wide variety of established and emerging indications.

Topics that are addressed in the abstracts contained in this supplement include:

- Latest insights into the structure, pharmacology, and activity of BoNTs
- The scientific basis of, and latest clinical data on, the duration of action 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 established and emerging indications in adult and pediatric spasticity, dystonias and other movement disorders, headache and other pain syndromes, aesthetic and dermatologic applications, hypersecretory disorders, genitourinary disorders, and investigational applications
- New knowledge and experience regarding optimizing BoNT therapy (ie, injection protocols, dosing paradigms, and methods of localization) and evaluating treatment outcomes

We hope you find this supplement to be a useful resource in furthering your research or improving your clinical practice in the field of botulinum neurotoxins.

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Matteo Caleo: A brilliant experimentalist, a unique mentor, and above all, an upright man

Matteo Caleo passed away on April 12th, 2022, in his home near Pisa, Italy, after a long and resolute fight against an aggressive lung cancer that did not stop him from fully living his intense and fulfilling life. Armed with his contagious enthusiasm and energy, even during his illness, he continued to critically assess the scientific literature, assemble new grant proposals, and supervise PhD students, postdoctoral fellows, and young researchers. Until a few weeks before his death, he continued to plan new experiments and discuss scientific projects with his collaborators, with inspiring mental clarity and vision.

Matteo was born on May 10th, 1970, in La Spezia, where he attended a high school that required long commuting. Typical of Matteo, he accepted this challenge as a test of his convictions and priorities, something that boosted, rather than decreased, his enormous appetite for learning. He then passed a very difficult national examination to enter the biology course of the Scuola Normale Superiore, one of the most prestigious Italian higher educational institutions, founded by Napoleon in 1810, and attended courses at the University of Pisa. After graduation with top marks in biology, he was admitted to the PhD course in Neurobiology of the Scuola Normale to study the neurophysiology of vision under the supervision of Professor Lamberto Maffei, a world leader in this field.

Matteo studied the role of neurotrophic factors in the development and plasticity of the visual cortex. At the very beginning of his scientific research, he demonstrated that brain-derived neurotrophic factor (BDNF) produced in the retina can be anterogradely transported to the brain, thus altering the physiology and survival of postsynaptic target neurons. He developed an extraordinary expertise in the physiology of the visual system and exploited this knowledge to investigate the plasticity of neuronal connections occurring during brain pathologies, from epilepsy to brain tumours. His work was pivotal to redefining inter-hemispheric connections, unravelling their complex role in brain physiology and diseases, from development of visual function to ischemic insults, and highlighting the importance of balanced hemispheric communication for brain functionality.

These studies on the anterograde transport of BDNF in the optic nerve prompted two of us to contact Matteo and initiate a collaboration focussed on the spreading of BoNT/A in the CNS using a newly developed antibody specific for BoNT/A-cleaved SNAP-25. This approach provided an unequivocal demonstration that catalytically-active BoNT/A undergoes axonal transport and is capable of transcytosis into afferent synapses where it cleaves SNAP-25, thus blocking synaptic activity at distal sites. In addition, BoNT/A injected in the rat whisker pad was found to cleave SNAP-25 in the facial nucleus, which suggested that this toxin is capable of producing distal effects, a result crucial for the many clinical uses of BoNT/A.

Not surprisingly, this work generated shock waves in our community, and at the International Conference on Basic and Therapeutic Aspects of Botulinum and Tetanus Toxins in Baveno, Italy, in 2008, Matteo was challenged in an open session where every result of his work was deeply scrutinized and criticized. With typical kindness, intelligence, and clarity, he replied to every point raised by the audience, proving that the reservations voiced were misplaced and ultimately conveying the utmost support to the conclusions of this work. This meeting was very well attended and during subsequent discussions among Alberto Albanese, Mark Hallett, Joe Jankovic, Cesare Montecucco, and others, the concept of creating a novel society for the study of the basic science of neurotoxins and their therapeutic use was formulated, leading later to the foundation of the International Neurotoxin Association.

This breakthrough paper led to a series of subsequent investigations that clearly demonstrated a direct, central action of BoNT/A in motor neurons by its retrograde transport and transcytosis in second-order neurons. The central activity of BoNT/A has since become a well-established feature of this neurotoxin that prompted the reinterpretation of preclinical and clinical observations long known to neurophysiologists and generated new, exciting scientific questions. Matteo's initial finding was thus echoed by a series of follow-up studies from his and other international research teams and paved the way for a major rethinking of the mechanism of action of clostridial neurotoxins.

In 2018, Matteo was called on to fill the chair of Physiology in the Medical School of the University of Padova, where he became a member of the Department of Biomedical Sciences. Here he displayed a unique gift (one of his many!) that no one had even suspected before. He had a special talent for teaching and for inspiring students to study the complex, but extraordinary discipline of neuroscience. He spent extra time in organizing seminars and discussion groups with students who greatly appreciated his

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drive, competence, and dedication.

In a very short time, Matteo became an active part of the department's institutional activities and the coordinator of the Biomedical Sciences PhD Program. Everyone saw in him a positive, transparent, trustworthy person with a profound sense of integrity, always seeking the common good. Indeed, he devoted energy and time to creating new facilities for the in vivo study of animal physiology, pathology, and behaviour, a process that is now continuing, in the footsteps of the work he has begun. Matteo had a natural inclination to take the lead in the collective interest: he pursued an objective not just to satisfy his personal interests or test his hypotheses, but to facilitate the process of discovery and improve research culture for the benefit of everyone—students, early career scientists, and colleagues alike.

Matteo was a unique mentor, always supportive of his students and collaborators, encouraging them to fully enjoy life. He was indeed a man of many talents and passions. He could dedicate himself with the same vibrant enthusiasm to analyse an endless set of microscopy slides (especially on a Friday afternoon!), to work at the seaside on the next article, as well as to arrange to play tennis early in the morning with colleagues and friends, or to swim at sea—his beloved sea—in the late afternoon in the summertime. Matteo also had a passion (and talent) for football, and actively played *"o jogo bonito"* his entire life. Emblematic of the high esteem in which Matteo was held is an unprecedented tribute by the medical students of the University of Padova, who dedicated to him the annual football match, probably the most important non-academic event organized by the School of Medicine in Padova.

Despite his many career successes, Matteo was always open to new ideas and refinements of his scientific plans, with a unique humility and sense of purpose. We will miss him, his rare human qualities, and the harvest of results he was about to reap. We are left with the memory of the great, special person he was: a unique example of human integrity, a true upright man. This was Matteo.

Matteo was deeply Christian, full of an intelligent, inquisitive faith, which, together with the love for his family, may have eased the last difficult steps of his life. He never rebelled at the unexpected turns and tumbles that his illness reserved to him, which may seem utterly incomprehensible to all of us. Ultimately, he was a man of peace; and in peace and following his example, we shall remember him.

He is survived by his wife Laura and his two sons, Pietro and Marco.

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Abstracts of TOXINS 2022 6th International Conference

The Impact of Isokinetic Lower Limb Muscle Strengthening Combined With Focal Spasticity Treatment on Endurance, Strength, and Quality of Life in Multiple Sclerosis Patients

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Background: Several studies have demonstrated the benefit of isokinetic muscle strengthening (IMS) of the lower limbs on endurance, fatigue, and quality of life in multiple sclerosis (MS) patients.[1,2] However, no study has evaluated the effects of a program combining eccentric hamstring IMS and focal treatment of spasticity by intramuscular injection of botulinum toxin (BoNT) into the plantar flexors in MS patients with gait disorders and genu recurvatum.
Objective: The main aim of our study was to determine whether eccentric hamstring IMS combined with plantar flexor BoNT injections improved endurance, strength, and quality of life in MS patients.
Design: This study was an open-label, uncontrolled study carried out in the Department of Physical Medicine and Rehabilitation of the Sidi Bel Abbès University Hospital, Algeria.
Materials and methods: Thirty ambulatory spastic MS patients (15 women and 15 men, Expanded Disability Status Scale [EDSS] $\leq$ 6) completed a 12-session (3 times/week) program of eccentric hamstring strength deficit IMS 3 weeks after botulinum toxin injection using abobotulinumtoxinA (Dysport®; 500 Speywood units) into spastic plantar flexors causing gait disorders with genu recurvatum that were identified by electrical stimulation. Quality of life (evaluated using the SEP-59 questionnaire, endurance (on the 2-Minute Walk Test), walking speed (on the 10 Meter Walk Test), and isokinetic strength of the quadriceps and hamstrings (60°/s, 180°/s concentric, and 15°/s eccentric) were evaluated before and after the treatment program.
Results: Participants' mean age was 39.5 ±10 years (18; 60); median EDSS score: 4 (2; 6); mean time since diagnosis: 5.4± 5.6 years (range: 0-17 years); weight: 68.6± 13.4 kg (range: 45-96 kg); height: 170 ± 8.5 cm; and BMI: 23.5 ±4 kg/m^2. The types of MS included were: relapsing-remitting (21 patients), primary progressive (6 patients), and secondary progressive (3 patients). Median Modified Ashworth Scale score was 3 (1+; 3), and the mean total dose of BoNT used per patient was 463.54 ±101.97 units.
After the treatment program, spasticity decreased significantly (2.7 ± 0.6 to 0.5 ± 0.7, P<0.0001). Endurance and walking speed were improved by 14.2% and 17.8%, respectively, with better knee control during walking. Isokinetic muscle strength increased in the quadriceps and mainly deficient hamstrings at each speed. Quality of life was also enhanced in eleven domains.
Conclusion: Our study showed that eccentric isokinetic hamstring strengthening combined with BoNT injections of the plantar flexors in patients with MS is effective in improving the quality of walking, physical capacities, and quality of life in these patients.
Keywords: Botulinum toxin; Endurance; Isokinetic muscle strengthening; Multiple sclerosis; Quality of life; Spasticity
Disclosure: The authors have no conflicts of interest to declare.

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How Prevalent and Relevant Are Adjuvant Therapies in Post-Stroke Spasticity Clinical Practice in the 20 Years' Experience of a Reference Spasticity Clinic?

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Introduction: Botulinum toxin type A (BoNT-A) is effective in the management of post-stroke spasticity (PSS).[1] Adjunct therapies such as physiotherapy, occupational therapy, and orthotics seem to be useful.[2] This study describes and analyzes real-world data, describing clinical practice combining adjuvant therapies with BoNT-A treatment over time in a reference spasticity treatment center with 20 years' experience.
Methods: This is a post hoc analysis of prospective observational data collected from a designated population of PSS patients treated with BoNT-A from 2001 to 2020. Patients were separated into two groups: G1 – those treated with BoNT-A injections alone and G2 – those treated with BoNT-A in combination with adjuvant therapies or other treatment strategies. For each group, we recorded the limbs injected, success rates, measured by percentage of Goal Attainment Scale (GAS) T-score $\geq$50 achieved, and change in GAS T-score over time. Statistical analysis used the Mann-Whitney U test and Chi-Square test, with a P value of $\leq$0.05 considered significant.
Results: A total of 288 patients and 2635 BoNT-A treatment sessions were included in the analysis. Mean age of patients at time of stroke was 54.3±12.5 years, and the majority were male (56.3%; n=162). Median time from stroke to first BONT-A treatment was 0.9 years (minimum [min]: 0.93; maximum [max]: 34.49 years), and the majority had an ischemic stroke (64.9%; n=187). The median number of treatment cycles per patient was 6 (min:1; max: 63), and the majority were treated in both limbs (62.0%; n=1632).

Most patients (90.0%) received adjuvant therapy post BoNT-A. Physiotherapy was administered in 81.7% (n=2153), occupational therapy in 29.7% (n=782), orthotics in 49.8% (n=1312), systemic medication in 12.4% (n=329), and transcutaneous electrical nerve stimulation (TENS) in 1.4% of patients; n=37). The majority of patients used one (30.7%; n=810), two (36.9%; n=971), or three (19.2%; n=505) adjuvant strategies. Due to missing data, 1230 treatment sessions were included in a subanalysis investigating the role of adjuvant therapies in combination with BoNT-A for treatment success. G1 included 101 injections and G2 1129. In both groups, most patients had an expected or greater than expected outcome regarding their GAS assessment (Table). However, a higher proportion was observed in G1 (71.9%) compared to G2 (64.4%). Mean GAS T-score was very good for both groups (G1: 48.3±5.1 vs G2: 48.7±4.8, P=0.263), as well as change in GAS T-score (G1: 11.5±5.1 vs G2: 11.7±4.9) with no statistical differences between the groups. Patients who had adjuvant therapies presented a higher success rate (achieved/overachieved goals) when treating only the upper (86.1% vs 73.1%) or only the lower limb (100% vs 66.7%). When both limbs were treated, success rates were not significantly different between groups.

Conclusions: In our 20 years of clinical practice in PSS management with BoNT-A, there is a very high percentage of patients using different modalities of adjuvant therapies, and treatment success rates tend to be higher in this group.

Table
GAS results according to group distribution.

		BoNT-A with Adjuvant Therapies (N=1129)	BoNT-A Monotherapy (N=101)
GAS	**T-Score Achieved**	48.7 ± 4.8	48.3 ± 5.1
	Goal ≥0	812 (71.9%)	65 (64.4%)
	Goal <0	317 (28.1%)	36 (35.6%)
	Change	11.7 ± 4.9	11.5 ± 5.1
	Change ≥10	840 (74.4%)	71 (70.4%)
	Change <10	289 (25.6%)	30 (29.6%)
GAS distribution with Injection site	**Upper Limb**	N=331	N=26
	Goal ≥0	285 (86.1%)	19 (73.1%)
	T-Score Achieved ≥50	257 (77.6%)	18 (69.2%)
	Change ≥10	257 (77.6%)	19 (73.1%)
	Lower Limb	N=96	N=9
	Goal ≥0	96 (100.0%)	6 (66.7%)
	T-Score Achieved ≥50	89 (92.7%)	6 (66.7%)
	Change ≥10	89 (92.7%)	6 (66.7%)
	Upper and Lower Limb	N=688	N=65
	Goal ≥0	568 (82.6%)	58 (89.2%)
	T-Score Achieved ≥50	466 (67.7%)	40 (61.5%)
	Change ≥10	494 (71.8%)	45 (69.2%)

Keywords: Adjuvant therapies; Botulinum toxin; Spasticity; Stroke; Treatment success

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Impact of Disease Severity on Presentation Subtype and OnabotulinumtoxinA Utilization in Patients With Cervical Dystonia: Results From the CD PROBE Completer Population

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Introduction: Cervical dystonia (CD) is a chronic condition in which the neck and upper shoulder muscles involuntarily contract, resulting in abnormal postures and/or movements. We aimed to examine the impact of cervical dystonia (CD) severity on presentation subtype and onabotulinumtoxinA utilization in the completer population from the CD PROBE study.

Methods: Multicenter, prospective, observational registry (NCT00836017). Patients with CD were treated with onabotulinumtoxinA according to the standard of care at each clinician's practice. OnabotulinumtoxinA utilization and safety data were collected at each session. Completers were defined as patients that completed all treatment sessions and had data for all outcome measures.

Results: Of 1046 patients enrolled in CD PROBE, 350 were categorized as completers. Completers were on average 57.3 years old, 74.9% female, 94.6% White, and 60.6% naïve to botulinum toxin. At injection 1, 54.3% had moderate severity with 32.6% mild and 13.1% severe. Regardless of severity, torticollis was the most common presentation subtype at injection 1 (mild: 44.7%, moderate: 55.8%, severe: 63.0%), followed by laterocollis (mild: 42.1%, moderate: 32.6%, severe: 26.1%). The median onabotulinumtoxinA dose to treat torticollis (injection 1: 160 U, injection 3: 200 U) and laterocollis (injection 1: 170 U, injection 3: 200 U) increased over time. For all severities, the median total dose increased from injection 1 to injection 3 (mild: 138 U to 165 U, moderate: 183 U to 200 U, severe: 200 U to 285 U, respectively). Eighty-one patients (23.1%) reported 139 treatment-related adverse events; there were no treatment-related serious adverse events. No new safety signals were identified.

Conclusions: CD severity impacted presentation subtype frequency and onabotulinumtoxinA utilization in CD PROBE, with higher and tailored dosing observed over time and with increasing disease severity.

Keywords: Cervical dystonia; Disease severity; OnabotulinumtoxinA

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Pinky Agarwal
Conflicts of Interest/Financial Disclosures: Served as a speaker/consultant for Acadia, Accorda, Adamas Pharmaceuticals, Amneal Pharmaceuticals, LLC, Kyowa Kirin, Sunovion, and US WorldMeds.

Richard Barbano
Conflicts of Interest/Financial Disclosures: Serves as an associate editor for *Neurology: Clinical Practice*; performs botulinum toxin injections at the University of Rochester (40% effort); serves/has served on scientific advisory board for Allergan, Ipsen, Merz, and Revance; receives research support from Vaccinex, Fox Foundation, Revance, and National Institutes of Health (via National Institute of Neurological Disorders and Stroke and the Office of Rare Diseases Research); Site PI: Dystonia Coalition Projects; Consultant for Oscine Corporation, AbbVie/Allergan; receives fees as

section editor and holds stock options in VisualDx; and has served as an expert witness in legal proceedings including malpractice, not involving commercial entities.

Henry Moore

Conflicts of Interest/Financial Disclosures: Served as consultant for TEVA Pharmaceuticals, Sunovion, Acadia, Lundbeck, LLC, Adamas Pharmaceuticals, Inc, Amneal Pharmaceuticals, LLC, Neurocrine Biosciences, UCB, US World Meds, and Merz.

Marc Schwartz

Conflicts of Interest/Financial Disclosures: Founder of MS Biostatistics, LLC, and was formerly an employee of MedNet Solutions Inc, which was contracted by Allergan (prior to its acquisition by AbbVie) to provide biostatistical services for the study.

Aleksej Zuzek

Conflicts of Interest/Financial Disclosures: Full-time employee of AbbVie.

Atul Patel

Conflicts of Interest/Financial Disclosures: Served as a consultant and speaker for Allergan, an AbbVie company, and Ipsen, and as a consultant for Revance. He has received research funding for clinical trials from Allergan, an AbbVie company, Ipsen, and Revance.

Muscle Weakness Duration Post-injection of a Single Dose of Botulinum Toxin into the Masseter Muscle Bilaterally: A One-Year Non-randomized Controlled Trial

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Introduction: Previous studies on the effect of botulinum neurotoxin (BoNT) on the masseter muscle have focused on aesthetics or pain management and have mainly assessed the reduction of muscle size through inspection of facial features or subjective difference in pain levels. A systematic review of studies utilizing objective measurements of bite force or electromyography (EMG) readings concluded that findings on the duration of effect of single BoNT injections into the masseter are inconclusive, with some studies recording effects exceeding 12 weeks, when the peripheral effect should have been worn out.[1]

Aim: To evaluate the duration of the reduction in maximal voluntary bite force and the power in the masseter muscle after BoNT injections.

Methods: Thirty-two individuals were included in the present study. Sample sizes of the groups were calculated using power analysis. Twenty individuals each received injections of 25 units (U) of incobotulinumtoxinA (Xeomin®) into the masseter muscle bilaterally (total dose: 50 U). The remaining participants (n=12) received no intervention and served as a control group. Subjects with temporomandibular disease or pain, having conditions or being on drugs affecting the central nervous system were excluded from the study. Maximum voluntary bite force was measured with a strain gauge meter at the incisors and the first molars bilaterally. The design of bite force recording in this study has been shown to be reliable with good reproducibility.[2] The intervention group was recruited from individuals seeking aesthetic treatment for masseter reduction, whereas the control group (n = 12) were volunteers recruited through advertising posters. Maximum voluntary bite force was measured at baseline, 4 weeks, 3 months, 6 months, and 1 year.

Results: All study subjects were women; there were no dropouts during the study. The control and intervention groups were similar in bite force and age at baseline. No significant change from baseline in the maximum voluntary bite force was seen in the control group, while significant reduction of bite force at all measurement points was seen at the 3-month reading in the intervention group. This difference was no longer significant when the intervention group was tested again 6 months after intervention. At the 1-year timepoint, the individuals in the intervention group showed a non-significant decrease in bite force compared to the 6-month results.

Conclusions: A single intervention using 50 U of botulinum neurotoxin produces a significant reversible reduction of bite force. At six months, the bite force is not significantly changed from baseline.

Funding: Funded through research funds from Umeå University. Merz Pharma GmbH & Co. KGaA provided the vial of BoNT.

Keywords: Bite force; Clinical trial; IncobotulinumtoxinA; Masseter muscle

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Real-World Safety and Efficacy of 156-195 U OnabotulinumtoxinA in Participants With Chronic Migraine: Results From the REPOSE Study

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Introduction: The safety and efficacy of 155-195 U onabotulinumtoxinA in individuals with chronic migraine (CM) was established in the phase 3 PREEMPT clinical trials and is the licensed dose in Canada and Europe. The objective of this analysis was to analyze efficacy and safety parameters of 156-195 U onabotulinumtoxinA in participants with CM from the real-world REPOSE study.

Methods: REPOSE (NCT01686581), a 2-year, prospective, noninterventional, observational, open-label study, described real-world use of onabotulinumtoxinA in adults with CM. Participants received onabotulinumtoxinA approximately every 12 weeks and were observed for 24 months after initiating treatment. Participant-estimated mean headache-day frequency in the last month (MHD), Migraine-Specific Quality-of-Life Questionnaire (MSQ) score, and adverse events (AEs) were collected at each treatment visit. In this analysis, participants from the safety analysis population ($\geq$1 onabotulinumtoxinA dose) were stratified by the dose received on $\geq$4 treatment visits into 155 U or 156-195 U groups.

Results: Of 641 enrolled participants, 633 received $\geq$1 onabotulinumtoxinA dose. On $\geq$4 treatment visits, 77 patients received 156-195 U and 218 received 155 U. Between-group baseline characteristics were similar. Treatment-emergent AEs (TEAEs) were reported in 10/77 participants in the 156-195 U group and 51/218 in the 155 U group; serious TEAEs were 1/77 and 3/218, respectively. Reductions from baseline in MHD frequency were observed at both doses (156-195 U range: -8.7 to - 17.3 MHDs; 155 U range: -8.2 to -13.2 MHDs). Mean change from baseline in MSQ domain scores improved across treatment-administration visits in a similar fashion with 155 U and 156-195 U.

Conclusions: Treatment with 156-195 U onabotulinumtoxinA was safe and generally well-tolerated in REPOSE participants, with no new safety signals identified. Between the two groups, numerically higher reductions in headache frequency and improvements in MSQ domain scores were observed with the 156-195 U dose. These real-world findings in the safety and efficacy of the 156-195 U onabotulinumtoxinA dose are consistent with data from the PREEMPT clinical trials as a treatment option for CM patients.

Keywords: Chronic migraine; Clinical setting; Effectiveness; Long-term; OnabotulinumtoxinA; Real-world; Safety

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Dipeptide Inhibitors as Potent Botulinum Neurotoxin Type A Light Chain Inhibitors

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Botulinum neurotoxin (BoNT), the potent agent that causes botulism, is the most lethal toxin known to man. The neurotoxin, which is composed of a heavy chain (HC) and a light chain (LC), enters neurons and cleaves soluble NSF (N-Ethylmaleimide-Sensitive Factor) Attachment Protein Receptor (SNARE) proteins, leading to flaccid paralysis, which, in severe cases, can result in death. There are seven BoNT serotypes, identified as BoNT/A-G, with serotype A being the most common cause of human botulism and the deadliest version. The LC, a zinc metalloprotease that directly cleaves SNARE proteins leading to muscle paralysis, is a therapeutic target for inhibition of BoNT intoxication.
We report dipeptides containing hydrophobic amino acids with a substituted aromatic ring connected to the N-terminus via a sulfonamide and a hydroxamic acid at the C-terminus as BoNT/A LC inhibitors. The dipeptides have been evaluated as inhibitors through a structure-activity relationship (SAR) study using a BoNT/A LC endopeptidase assay. The SAR study revealed amino acids with small hydrophobic side chains at the C-terminus and aromatic side chains for the N-terminal amino acid, resulting in potent inhibition. The study resulted in the design and synthesis of a dipeptide with an IC_{50} of 21 nM for the BoNT/A LC assessed using an endopeptidase assay. The BoNT/A LC was also crystallized with the dipeptides bound to the active site. Subsequent X-ray crystallography of the dipeptide bound to the BoNT/A LC revealed hydroxamate chelating to the zinc and numerous hydrophobic interactions within the active site. These interactions along with the SAR study provide a blueprint for the future design of dipeptides with high potency for BoNT/A LC inhibition. The physicochemical properties of the dipeptides will be further investigated to evaluate their therapeutic potential.
Keywords: Botulinum neurotoxin light chain; Drug discovery; Enzyme assay; Peptides; Small molecule inhibitors; X-ray crystallography

The Integration of the Hemiplegic Spastic Upper Limb Into Activities of Daily Living After Botulinum Neurotoxin Injection and Constraint-Induced Movement Therapy

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Introduction: In stroke patients, the activities of daily living are significantly affected by the paresis of the spastic upper limb.[1] Constraint-induced movement therapy, which counteracts the phenomenon of nonuse, combined with botulinum neurotoxin type A (BoNT-A) injections, seems to improve the integration of the affected upper limb into many activities in quality and quantity.[2,3]
Objectives: To assess the efficacy of combined constraint-induced movement therapy and BoNT-A injections on the integration of the affected upper limb into the activities of daily living.
Materials and methods: Eighteen stroke patients (9 women and 9 men) with spastic upper limb with some residual muscle power (minimum score of 2 on the Medical Research Council scale) were included in the study. They benefited from BoNT-A administered 3 weeks before constraint-induced movement therapy that was performed 4 hours per day, 5 days/week for 4 weeks. Outcomes were evaluated using the Modified Ashworth Scale (MAS) and the Motor Activity Log ([MAL] quality of movement scale Q1; amount of use scale, Q2) before and after the treatment protocol.
Results: Patients' mean age was 58.2 ±13.5 years (range: 38-75 years). Seventeen patients had spasticity due to an ischemic stroke and the other due to a hemorrhagic stroke, and the right side was affected in 10 patients. The MAS decreased significantly ($P<0.0001$) on the wrist (1.83±0.6 to 0.7±0.4) and on the fingers (1.72±0.5 to 0.5±0.5, $P<0.0001$). The quality of movement of the affected upper limb in daily activities (Q1 in the MAL) increased from 56.33±18 to 109.50± 27 ($P<0.003$), and the amount of use (Q2 in the MAL) increased from 54.18±18.3 to 109±29.5 ($P<0.005$). The satisfaction index exceeded 60% among all patients.
Conclusion: Constraint-induced movement therapy after BoNT-A injection for the hemiparetic spastic upper limb improved integration of the affected upper limb into activities of daily living (both quality of movement and amount of use), but further studies are needed to confirm these results.
Keywords: Botulinum neurotoxin type A; Constraint-induced movement therapy; Integration into daily activities; Spastic upper limb; Stroke

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Botulinum Toxin for Treatment of Somatosensory Tinnitus in Cervical Dystonia Patients

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Introduction: Tinnitus is the perception of sound without an auditory stimulus, and is itself a symptom of an underlying disease, injury, or medication side effect/toxicity. Somatosensory tinnitus changes (in pitch, volume, or localization) during a stimulation of the head or neck. This type of tinnitus seems to be secondary to a problem in the head or neck as opposed to the ear.[1] Studies by Levine and Ralli and colleagues showed an increase in loudness of tinnitus in 41-59% of patients with maneuvers of the head and neck.[2,3]
A study by Buergers et al showed that patients with temporomandibular

junction (TMJ) disorders are at an 8-fold higher risk of developing tinnitus, and that treatment of TMJ disorders in patients with tinnitus reduced tinnitus symptoms in 44% of participants.[4] Botulinum toxin was used to treat tinnitus in a study by Láinez and Piera.[5] In their study, patients were either injected with onabotulinumtoxinA or saline into three sites around the ear. The onabotulinumtoxinA arm noted significant reduction in tinnitus at 4 months compared to the saline arm.[5]

Case Presentation: A 75-year-old, right-handed male was seen in the movement disorders clinic for cervical dystonia associated with essential tremor for the previous 12 years. He was treated with onabotulinumtoxinA injections at 20 units in the left sternocleidomastoid, 20 units in the right splenius capitis, 20 units in the left levator scapulae, 10 units in the right levator scapulae, and 10 units in each thoracic paraspinal muscle using EMG guidance. At his next follow-up visit 4 months later he noted significant improvement in his tinnitus, which has continued for the next year.

Conclusions: Muscle disorders, including cervical dystonia, can cause or worsen tinnitus. Treatment of disorders such as TMJ disorder and cervical dystonia is likely to improve tinnitus. The underlying causes of tinnitus should always be thoroughly investigated in order to offer patients the best therapy, which can include onabotulinumtoxinA injection.

Keywords: OnabotulinumtoxinA; Somatosensory tinnitus

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Cell-Penetrating Peptides: Are They Useful Excipients in Botulinum Toxin Formulations?

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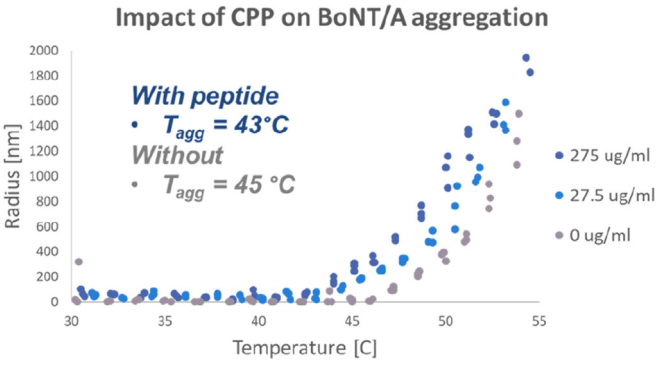
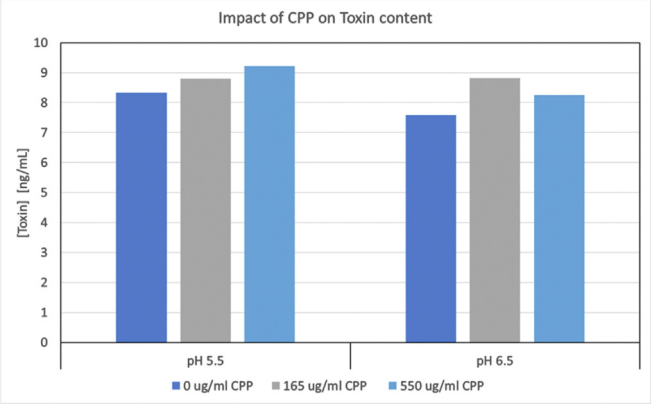
Introduction: The complexity and high potency of botulinum neurotoxins (BoNTs) represents a significant challenge in their formulation. Various excipients have been reported to prolong product shelf life or to affect BoNT/A duration of action: One class of excipients are cell-penetrating peptides (CPPs); these excipients are reported to stabilize BoNT/A against aggregation and adsorption, thereby indirectly increasing BoNT/A duration of action.

Methods: The study objective was to assess whether CPPs bound to purified 150-kDa BoNT/A protected the BoNT/A molecule against aggregation and adsorption. Bio-layer interferometry was used to determine a binding constant (K_d). Thermal ramp dynamic light scattering was used to determine T_{agg} (aggregation temperature). Adsorption studies were performed using enzyme-linked immunoassay (ELISA) to assess the impact of CPP presence on BoNT adsorption to Borosilicate glass. The studies were performed using formulations comprised of PS20-containing sugar solutions buffered to pH 5.5-6.5 with varying concentrations of CPP.

Results: The data reveal that cell-penetrating peptides do not stabilize BoNT/A against aggregation. Aggregation temperature (T_{agg}) with and without peptide was 43-46 °C. The addition of CPP does not reduce BoNT/A adsorption, relative to the control formulations without CPP (control 0 µg/mL). The control formulations in these experiments were PS20-containing sugar solutions buffered to pH 5.5-6.5.





Conclusions: No improvement in the adsorption or aggregation properties of BoNT/A was observed in formulations containing CPP. CPP binding to BoNT/A is very weak and it can be assumed that upon injection any molecular complex between CPP and BoNT/A will rapidly dissociate. Therefore, a mechanism by which CPP could affect BoNT/A duration of action is not apparent.

Keywords: Aggregation temperature; BoNT; Botulinum toxin; Cell-penetrating peptide; CPP; Dissociation constant; K_d; T_{agg}

Funding: This study was sponsored by Ipsen.

Modelling Long-Term Outcomes and Mortality Risk for Post-Stroke Spasticity Patients on AbobotulinumtoxinA Treatment and Rehabilitation Therapy

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Introduction: Stroke is associated with high risk of mortality and recurrent cardiovascular events, with risk increasing for people with comorbidities. Rehabilitation is critical for improving functional recovery, and was shown to reduce hospital readmissions, as well as all-cause and cardiovascular mortality and stroke recurrence (long-term outcomes), but spasticity (occurring in 30% of stroke survivors) may prevent effective recovery. AbobotulinumtoxinA (aboBoNT-A) is an established treatment

for post-stroke spasticity (PSS), but its impact on long-term outcomes is unknown. The goal was to model the clinical and economic effects of aboBoNT-A treatment on long-term outcomes in PSS.
Methods: Effects of aboBoNT-A on functional outcomes such as the Functional Independence Measure (FIM), and the impact of functional outcomes on long-term outcomes, were estimated from literature review and meta-analyses. A model was developed based on associations between aboBoNT-A, functional, and long-term outcomes. Cost-effectiveness analysis compared rehabilitation therapy (RT) with aboBoNT-A versus RT alone from the United Kingdom National Health Service perspective. Resource use and utilities were retrieved from the literature and modelled over a 10-year time horizon.
Results: Using the FIM, the model found a risk reduction of 8.8% for all-cause mortality and a risk reduction of 2.4% for cardiovascular events and recurrent stroke for RT + aboBoNT-A versus RT alone. In the base case, RT + aboBoNT-A led to an increase of 13% in life-years and 59% in quality-adjusted life years (QALYs [1.7 gained]). RT + aboBoNT-A was considered cost-effective versus RT alone (incremental cost-effectiveness ratio [ICER]: £24,602). Sensitivity analysis showed that healthcare resources, quality of life, relative risks for all-cause mortality, and effect of interventions had the most influence on the ICER. The ICER remained below the £30,000 threshold in a sensitivity analysis on the source of overall survival data (range: £24,602-£28,983).
Conclusion: In addition to being a cost-effective use of resources, treatment of PSS with aboBoNT-A and RT may lead to improved survival with considerable clinical and economic benefits.
Keywords: Botulinum neurotoxin type A; Cost-effectiveness; Economic outcomes; Functioning; Long-term outcomes; Mortality; Quality of life; Spasticity; Stroke
Funding: This study was sponsored by Ipsen.

Real-World Treatment Utilization and Effectiveness of OnabotulinumtoxinA in Multiple Sclerosis Patients Treated for Spasticity From the ASPIRE Study

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Introduction: OnabotulinumtoxinA (onabotA) treatment for spasticity is individualized and dependent on numerous factors. This analysis examines onabotA utilization and effectiveness to treat spasticity in multiple sclerosis (MS) patients.
Methods: International, multicenter, prospective, observational registry (NCT01930786) examining adults with spasticity treated with onabotA at the clinician's discretion. Assessments include onabotA utilization (each visit) and clinician (next visit)/patient (5±1 weeks post-treatment) satisfaction.
Results: Patients (N=731) were on average 54 years old, female (52%), and continuing botulinum toxins for spasticity (63%). Most had spasticity due to stroke (n=411;56%) or MS (n=119;16%). In MS patients (n=119), the most common upper limb presentation was flexed elbow (18%; 25-550 U). Muscles injected include: biceps brachii (100%), brachioradialis (54%), brachialis (46%), other (4%); anatomical localization (60%) was most often utilized. The most common lower limb presentation was equinovarus foot (61%; 15-875 U). Muscles injected included: gastrocnemius (79%), soleus (73%), tibialis posterior (46%), flexor digitorum longus (15%), other (11%), flexor hallucis longus (2%); EMG localization (57%) was most often utilized. Overall (N=731), ≥72% patients and ≥91% clinicians reported extreme satisfaction that onabotA helped patient's ability to participate in therapy/exercise, and 92% of patients and ≥98% of clinicians would definitely/probably continue treatment. Overall (N=731), 261 patients reported 831 adverse events (AEs); 23 AEs in 20 patients were considered treatment related. Ninety-four patients reported 195 serious AEs; 3 serious AEs in 2 patients were considered treatment related.
Conclusions: ASPIRE captured the individualized nature of onabotA use for spasticity in MS patients, while consistently demonstrating high satisfaction among patients and clinicians, with the majority indicating that onabotA helped patients participate in therapy/exercise.
Keywords: Multiple sclerosis; OnabotulinumtoxinA; Spasticity
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Daniel S. Bandari
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Treatment of Patients With Upper Limb and Lower Limb Spasticity With OnabotulinumtoxinA in the Adult Spasticity International Registry (ASPIRE)

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Introduction: OnabotulinumtoxinA utilization was examined in patients with upper (UL) and lower limb (LL) spasticity from the Adult Spasticity International Registry (ASPIRE) to gain insights into the real-world treatment of spastic hemiparesis.
Methods: In this 2-year, multicenter, observational registry, adults with spasticity were treated with onabotulinumtoxinA at clinician's discretion. Utilization and safety data were collected at each session. Patients with spastic hemiparesis were those who received ≥1 UL and ≥1 LL treatment.
Results: Of 730 patients, 275 were hemiparetic and treated for UL and LL at the same session. Hemiparetic patients were a mean of 53 years old, 51% male, 68% Caucasian, 39% botulinum toxin–naive for spasticity, and 73% post-stroke. Mean total onabotulinumtoxinA doses were 477 U for UL+LL,

257 U for UL, and 220 U for LL. Most hemiparetic patients had a 10-15–week treatment interval (56%), 5-15 injections/session (62%), and >5 muscles injected/session (82%). The most common UL presentation was clenched fist (n=219), with 55% of sessions for left side only. The most common LL presentation was equinovarus foot (n=238), with 52% of sessions for left side only. Two hundred ninety-three non-serious adverse events (AEs) were reported in 94 patients (34%); 9 AEs in 9 patients (3%) were considered treatment related. Eighty serious AEs were reported in 42 patients (15.3%); 3 serious AEs in 2 patients (0.7%) were considered treatment related.

Conclusions: This analysis of ASPIRE provides valuable real-world evidence on the utilization of onabotulinumtoxinA in spastic hemiparesis, with onabotulinumtoxinA most frequently used to treat clenched fist (UL) and equinovarus foot (LL). No new safety signals were identified.

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M. Schwartz served as a statistical consultant for Allergan, an AbbVie company.

A. Zuzek is a full-time employee of AbbVie.

Wolfgang H. Jost served as a speaker and consultant for Allergan, an AbbVie company, Ipsen, and Merz.

Keywords: Lower-limb spasticity; OnabotulinumtoxinA; Spastic hemiparesis; Upper limb spasticity

Development of a Patient Journey Map for People Living With Cervical Dystonia

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Introduction: Patient journey maps are increasingly used as a tool that enables healthcare providers to refine their service provision to best meet patient needs. We developed a cervical dystonia patient journey map (CDPJM) that describes the holistic patient experience from pre-diagnosis through to long-term treatment.

Methods: The CDPJM was developed in 2 stages: a patient survey (open, multichoice questions) of 15 patients with CD was conducted to inform the design of the CDPJM, which was then refined and validated by an expert-patient focus group.

Results: Prior to diagnosis, most patients (n=12, 80%) reported abnormal head and/or neck positions as their first CD symptom. The other 3 patients reported tremor (n=2) and pain (n=3) as their earliest symptoms. Patients described making multiple visits to their family doctors who prescribed strong pain killers and muscle relaxants and referred their patients to up to 10 different specialists for diagnosis. Over half (53.3%) of patients received ≥1 misdiagnosis. Patients reported relief at having a diagnosis but a lack of understanding of the prognosis and treatment options; 46.7% said their neurologist did not spend enough time addressing their concerns. Patients reported that botulinum toxin (BoNT) was presented as the main treatment option. While some neurologists mentioned physiotherapy, counselling, and other complementary approaches, patients were often left to seek such services themselves. Patients reported a "rollercoaster" of relief with BoNT treatment, with symptoms (and subsequent impact on daily life) returning towards the end of an injection cycle. *"When BoNT works well I can return to an almost normal life … when the injections stop working so well, I have to rest more and avoid going to work and experience life restrictions."*

Conclusions: We present the first patient journey map for CD that can be used to guide local service mapping and to compare current provision with what patients say they want and need.

Funding: Ipsen

Keywords: Cervical dystonia; Diagnosis; Map; Patient journey; Treatment

Biochemical Stability and Microbial Control of Reconstituted DaxibotulinumtoxinA for Injection (DAXI)

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Introduction: DAXI is a lyophilized product that requires reconstitution with saline prior to administration. The objective was to evaluate reconstituted DAXI stored under refrigerated (2-8°C) conditions in vials for biochemical stability, microbial control, and pathogen proliferation.

Methods: The stability of reconstituted DAXI was evaluated in 2 studies under 2-8°C storage conditions. Study 1 compared DAXI 50 U/vial reconstituted with unpreserved and preserved saline. Up to 14 days, samples were analyzed for maintenance of biological activity by a validated mouse median lethal dose (LD_{50}) assay and pH stability. To assess control of microbial growth, vials reconstituted in unpreserved or preserved saline were individually inoculated with ~100 colony-forming units (CFU) of 5 challenge organisms (*Aspergillus brasiliensis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa,* and *Staphylococcus aureus*). Up to 36 days, these inoculated samples were cultured in appropriate growth media and assessed for pathogen proliferation using the $\log_{10}$ values. Study 2 evaluated DAXI 50 and 100 U/vials after reconstitution with unpreserved saline stored at 2-8°C. Up to 6 days, samples were analyzed for 150-kDa neurotoxin content by enzyme-linked immunosorbent assay (ELISA) and bioburden, in addition to pH and mouse LD_{50}. Study 2 also assessed pathogen proliferation with the 5 challenge organisms up to 7 days.

Results: In Study 1 with 50 U/vial, the pH range was stable at 5.5 from Time 0 to 14 days after reconstitution in unpreserved or preserved saline. The LD_{50} ranged from 52-57 U/vial and 52-56 U/vial for unpreserved and preserved saline, respectively. Growth proliferation of inoculated challenge organism was not observed in the reconstituted product, with a decrease in $\log_{10}$ values from Time 0 to 36 days of ≥0.2 and ≥0.1 in unpreserved and preserved saline, respectively. In Study 2 using unpreserved saline, the pH remained stable at 5.6 in reconstituted 50 and 100 U/vials, from Time 0 to 6 days. The LD_{50} ranged from 50-56 U/vial and 97-112 U/vial for reconstituted 50 and 100 U/vials, respectively. The measured content of 150-kDa neurotoxin ranged from 0.23-0.27 ng/vial and 0.47-0.52 ng/vial for reconstituted 50 and 100 U/vials, respectively. Bioburden was maintained at <1 CFU/mL in the reconstituted product. Growth proliferation of inoculated challenge organism was not observed in the reconstituted product, with a decrease in $\log_{10}$ values from Time 0 to 7 days of ≥0.1 and ≥0.6 in reconstituted 50 and 100 U/vials, respectively.

Conclusions: Taken together, the 2 studies show that reconstituted DAXI 50 and 100 U/vials in either unpreserved or preserved saline are biochemically stable in refrigerated (2-8°C) storage for up to 6 days with no observable changes in pH stability, 150-kDa neurotoxin content by ELISA,

and maintenance of biological activity by mouse LD_{50} assay. Additionally, the reconstituted product did not support microbial growth, with no observable bioburden and no pathogen proliferation over the duration of the studies.

Funding: The study was funded by Revance Therapeutics, Inc.
Keywords: Botulinum toxin type A; DaxibotulinumtoxinA; Microbe; Pathogen; Reconstitution

Clinical and Economic Outcomes of Adult Limb Spasticity Treatment with Botulinum Toxin Type A Products: A Single-Center, Non-Interventional Study

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Introduction: An effective limb spasticity (LS) treatment approach combines physiotherapy and injections of botulinum neurotoxin type A (BoNT-A).[1,2] The North Staffordshire Rehabilitation Centre (Midlands Partnership NHS Foundation Trust) initiated LS patients on onabotulinumtoxinA (onaBoNT-A) prior to 2017; after 2017, patients were initiated on abobotulinumtoxinA (aboBoNT-A). This study describes real-world outcomes of BoNT-A treatment, pre- and post-2017.
Methods: A single center, non-interventional, retrospective study assessed treatment outcomes with aboBoNT-A vs onaBoNT-A in toxin-naïve adult LS patients. Assessments consisted of patient's routine visits at 6 weeks ±2 weeks and at 12 weeks ±4 weeks after the date of the first injection. Outcomes included Goal Attainment Scaling (GAS), patient satisfaction recorded on a Likert scale, and cost per responder (based on response rate, average dose, and UK drug unit costs). Treatment response was defined as achievement or overachievement of all therapeutic goals.
Results: The treatment groups were comparable on demographic and clinical characteristics. In the aboBoNT-A group (n=54), 55.6% patients had lower limb (LL) spasticity, 35.2% had upper limb (UL) spasticity, and 9.3% had lower limb and upper limb (LL+UL) spasticity. In the onaBoNT-A group (n=60), the split was 50.0%, 38.3%, and 11.7%, respectively. Mean doses of aboBoNT-A and onaBoNT-A were 718.8±410.7 U and 194.8±87.9 U in UL, and 689.7±449.9 U and 186.4±97.9U in LL, respectively. In the overall population, the doses of aboBoNT-A and onaBoNT-A were 753.7±457.3 U and 206.0±98.8 U, respectively. At Week 6, the proportions of patients who achieved or overachieved all therapeutic goals based on GAS were 56.4% (40.8%-72.0%) on aboBoNT-A and 37.5% (22.5%-52.5%) on onaBoNT-A. At Week 12, those proportions were 40.9% (20.4%-61.5%) and 33.3% (17.9%-48.7%). Patient satisfaction level at Week 6 was reported as "much better" by 44.1% (27.4%-60.8%) and 29.3% (15.3%-43.2%) of patients on aboBoNT-A and onaBoNT-A, respectively. AboBoNT-A safety was consistent with the regulatory label. At Week 6, total costs per responder, estimated on the overall study cohort on aboBoNT-A vs onaBoNT-A, were £411.60 vs £759.18 at Week 6 and totalled £567.58 vs £854.93 at Week 12.
Conclusions: These real-life data suggest switching a hospital practice from onaBoNT-A to aboBoNT-A could be beneficial in terms of clinical outcomes and patient satisfaction, and efficient as illustrated by the cost-per-responder analysis.
Keywords: Botulinum neurotoxin type A; Economic analysis; Patient satisfaction; Spasticity costs; Therapy switch; Treatment response
Funding: This study was sponsored by Ipsen.

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Healthcare Resource Utilization and Costs Among Patients With Stroke-Related Spasticity Before and After Treatment With OnabotulinumtoxinA

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Introduction: Stroke is the most common cause of upper and lower limb spasticity, for which onabotulinumtoxinA (onabotA) is an approved treatment in adults. This study addresses the paucity of data regarding the impact of onabotA on real-world healthcare resource utilization (HCRU) and costs among those with stroke-related spasticity.
Methods: Retrospective claims analysis used IBM MarketScan® Commercial data and Medicare Supplemental Databases. Eligible adults had ≥1 onabotA claim for stroke-related spasticity from 1/1/2010 to 06/30/2018 and continuous enrollment for ≥12 months pre- and post-index date (first onabotA claim). Pre- and post-index differences in all-cause and spasticity-related HCRU and costs were compared. Effect of stroke diagnosis time (±180 or ± 365 days) to index date on pre- and post-index period differences was assessed.
Results: Seven hundred thirty-five patients met criteria and were included in the study. Compared with the pre-index period, a smaller number of patients had ≥1 all-cause Emergency Department visit and ≥1 all-cause hospitalization admission in the post-index period ($P<0.0001$). Among those who had a hospital admission, fewer experienced all-cause hospitalizations in the post-index period ($P\leq0.0011$). Similar reductions in spasticity-related HCRU were also observed between pre- and post-index periods. All-cause costs decreased in the post-index period by 65% (pre: $140,947; post: $48,553) ($P<0.001$), mainly driven by reduction in inpatient costs (pre: $95,268; post: $9,752). Patients receiving onabotA saw reductions in all-cause costs in the post-index period ($P<0.0001$) regardless of stroke diagnosis timing; greatest cost reductions were observed among patients who received onabotA earlier (ie, ≤180 days; ≤365 days vs >180 days; >365 days). A similar trend was observed for spasticity-related costs.
Conclusions: All-cause and spasticity-related HCRU and costs decreased in 12 months after onabotA initiation; onabotA may help alleviate large economic burdens associated with stroke-related spasticity.
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Lisa Bloudek, Kristen Migliaccio-Walle, and David Oliveri are consultants to Abbvie.
Keywords: Healthcare resource utilization; OnabotulinumtoxinA; Stroke-related spasticity

Phage-Assisted Evolution of Botulinum Neurotoxin Proteases With Reprogrammed Specificity

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Introduction: This research program examined the capacity of a phage-based directed evolution platform (PACE) to redirect botulinum neurotoxin (BoNT) light-chain proteases to selectively cleave new intracellular protein targets.
Methods: Phage-assisted continuous evolution (PACE) selections were developed to facilitate the evolution of novel proteases with high selectivity for their intended targets. The biochemical properties of the evolved proteases were assessed using bacterial and protease kinetic assays. Functional properties of the evolved proteases were assessed in mammalian tissue culture models.
Results: We evolved BoNT/X protease into separate variants that preferentially cleave vesicle-associated membrane protein 4 (VAMP4) and Ykt6, evolved BoNT/F protease to selectively cleave the non-native substrate VAMP7, and evolved BoNT/E protease to cleave PTEN but not any natural BoNT protease substrate in neurons. The evolved proteases display large changes in specificity (218- to >11,000,000-fold) and can retain their ability to form holotoxins that self-deliver into primary neurons.
Conclusions: These findings demonstrate that advanced directed evolution methodologies can effectively retarget BoNT light-chain proteases to selectively cleave new targets of therapeutic interest.
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Keywords: Botulinum neurotoxins; Directed evolution; Protease reprogramming

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DaxibotulinumtoxinA for Injection Demonstrates Consistent Safety and Efficacy in African American Subjects: Subgroup Analysis From a Large, Open-Label, Repeat-Dose Study

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Introduction: African American (AA) individuals account for 9% of cosmetic procedures in the USA (ASAPS, 2018). DaxibotulinumtoxinA for Injection (DAXI) is a novel botulinum toxin type A in clinical development for moderate-to-severe glabellar lines (GL). We compare the safety and efficacy of DAXI in AA vs Caucasian subjects.
Methods: SAKURA 3 was a multicenter, 84-week, prospective, open-label, repeat-dose study in subjects with moderate-to-severe GL. The study included rollover subjects (both active and placebo) from the preceding double-blind studies (SAKURA 1 and 2) as well as de novo subjects. Subjects received ≤3 treatments with DAXI 40 U.
Results: The study enrolled 2691 subjects (477 from SAKURA 1/2; 2214 de novo). In treatment cycle 1 (n=2380), 119 (5%) AA subjects and 2130 (89.5%) Caucasian subjects received DAXI, and 98.3% of AA vs 95.7% of Caucasian subjects achieved none/mild GL (investigator assessment) at Week 4. Median time to loss of none/mild GL (investigator and subject assessment) for AA and Caucasian subjects was 25.7 and 24.0 weeks, respectively, and median time to return to baseline GL status (investigator and subject assessment) was 28.3 and 28.0 weeks, respectively. Common treatment-related adverse events in AA subjects were injection site pain, headache, facial paresis (reported as "forehead muscle weakness"), erythema, and edema. There were no treatment-related cases of brow ptosis or dyschromia. DAXI demonstrated a high degree of efficacy and a duration of effect in AA subjects similar to that observed in Caucasian subjects. No new safety signals were identified.
Conclusions: These data demonstrate consistent safety and efficacy of DAXI in AA subjects.
Funding: The study was funded by Revance Therapeutics, Inc.
Keywords: Aesthetics; Botulinum toxin type A; DaxibotulinumtoxinA; Glabellar lines; Race

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Immunogenicity of Botulinum Toxin Formulations: Potential Therapeutic Implications

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Botulinum neurotoxins (BoNTs) are proteins produced by bacteria of the Clostridium family. Upon oral ingestion, BoNT causes the neuroparalytic syndrome botulism. There are seven serotypes of BoNT (serotypes A-G); BoNT-A and BoNT-B are the botulinum toxin serotypes utilized for therapeutic applications. Treatment with BoNT injections is used to manage chronic medical conditions across multiple indications. As with other biologic drugs, immunogenicity after long-term treatment with BoNT formulations may occur, and repeated use can elicit antibody formation leading to clinical nonresponsiveness. Thus, approaching BoNT treatment of chronic conditions with therapeutic formulations that minimize stimulating the host immune response while balancing patient responsiveness to therapy is ideal. Immunogenicity is a clinical limitation in many settings that use biologic drugs for treatment, and clinically relevant immunogenicity reduction has been achieved through engineering smaller protein constructs and reducing unnecessary formulation components. A similar approach has influenced the evolution of BoNT formulations. Three BoNT-A products and one BoNT-B product have been approved by the US Food

and Drug Administration for therapeutic use: onabotulinumtoxinA, abobotulinumtoxinA, incobotulinumtoxinA, and rimabotulinumtoxinB; a fourth BoNT-A product, daxibotulinumtoxinA, is currently under regulatory review. Additionally, prabotulinumtoxinA is a BoNT-A product that has been approved for aesthetic indications but not therapeutic use. Here, we describe the basic science of immunogenicity as a potential clinical barrier to the efficacy of biologic therapies and its effect on the evolution of BoNT formulations. We summarize available nonclinical and clinical evidence of immunogenicity and clinical nonresponsiveness associated with different BoNT formulations and discuss whether there is a lower risk of immunogenicity with a second-generation BoNT formulation, incobotulinumtoxinA. Finally, we provide an immunological perspective for considering immunogenicity as a factor in choosing a BoNT formulation.

Keywords: AbobotulinumtoxinA; Clinical response; IncobotulinumtoxinA; Neutralizing antibodies; OnabotulinumtoxinA; Second generation

Safety and Efficacy of OnabotulinumtoxinA for Treatment of Masseter Muscle Hypertrophy: Results from a Phase 2 Study

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Introduction: Benign bilateral masseter muscle hypertrophy (MMH) is characterized by protrusion of the masseter near the angle of the mandible, which can be prominent enough to negatively impact facial aesthetics. Current effective treatment for MMH is predominantly surgical reduction. In this Phase 2 study, the safety and efficacy of onabotulinumtoxinA (onabotA) versus placebo treatment of MMH was assessed for reduction in lower face volume.

Methods: This was a multicenter, randomized, double-blind, placebo-controlled, dose-escalation study. Eligible participants were 18-50 years old with symmetrical bilateral "Marked" (Grade 4) or "Very Marked" (Grade 5) MMH, investigator-assessed using the 5-grade Masseter Muscle Prominence Scale (MMPS). Participants were randomized to receive onabotA or placebo in a 4:1 ratio in dose-escalating groups (24 U, 48 U, 72 U, and 96 U). On Day 1, treatment was administered as 6 injections (3 injections per masseter). Participants were followed monthly for 1 year (Day 90, primary time point; Day 360, exit visit). Qualifying participants assessed as Grade 4 or 5 on the MMPS received retreatment at Day 180. The primary efficacy measure was change in lower face volume calculated from VECTRA M3 photography. Secondary efficacy was investigator-assessed MMH using the MMPS. Participant perspective was gained using the Lower Facial Shape Questionnaire (LFSQ). Safety assessments included adverse event (AE) surveillance throughout, with computed tomography (CT) scans of the mandible and dental exams at Screening/Day 1, 90, and 360 after first treatment.

Results: A total of 188 participants (mean age 35; 82% female; 80% Asian) with "Marked" (80%) or "Very Marked" (20%) MMH were enrolled and assigned to 4 onabotA dose groups (24 U, N=37; 48 U, N=37; 72 U, N=38; 96 U, N=38) or placebo (n=37). Lower facial volume was significantly reduced for all dose groups vs placebo ($P<.001$) at Day 90 with significant volume reduction ($P<.05$) maintained for up to 6 months after each of two treatments with 48 U, 72 U, or 96 U. The proportion of responders achieving MMPS Grade 3 (clinically relevant) and >2-grade change (more stringent) at Day 90 was significant for all dose groups vs placebo ($P<.001$). Significant difference ($P<.05$) was maintained at all time points through Day 180 with 48 U, 72 U, or 96 U doses, compared with placebo. Reduction of MMH severity was sustained for approximately 9 months after a single dose of 72 U and 96 U. Key items of the LFSQ indicated a trend toward greater satisfaction and reduced impact/symptoms in all dose groups at Day 90. The most frequent treatment-related AE was mastication disorder (onabotA 5.3%, placebo 2.7%), mainly reported as mild chewing weakness. Overall, 167 (89.3%) participants completed the study with no treatment-related discontinuations. CT evaluation of changes in masseter volume confirmed the primary endpoint results, while no adverse events were reported from CT scans or dental exams.

Conclusions: OnabotA significantly reduced masseter volume and MMH severity at Day 90 for all dose groups. Volume reduction was maintained for up to 6 months after each of two treatments with 48 U, 72 U, or 96 U. Treatments were well tolerated with no clinically-relevant correlations between AE incidence and dose.

Disclosure(s) of interest:
J Carruthers serves as consultant and investigator for Allergan plc.
S Liew serves as an investigator, speaker, and consultant for Allergan plc.
SG Chen has no conflicts of interest to report.
J Rivers and S Humphrey are consultants, speakers, and investigators for Allergan, plc.
B Bowen, E Lee, and MF Brin are employees of Allergan plc and may own stock/stock options in the company.
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Keywords: Aesthetic; Computed tomography; Masseter muscle hypertrophy; OnabotulinumtoxinA; Photography

Applicability of Botulinum Toxin in Treatment of Holmes Tremor: Our Cases and Detailed Review of Current Literature

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Introduction: Holmes tremor is defined as a 2-4 Hz high-amplitude tremor present both at rest and with movement, with a possible postural component.[1] It is seen primarily in the upper limbs and can develop from 1 to 24 months after brain injury due to stroke, multiple sclerosis, or other causes.[2] No validated treatment currently exists for Holmes tremor.

Methods: We conducted a PubMed literature review for all publications on the therapeutic options used for Holmes tremor within the last 10 years. A total of 64 articles were found, focused on the etiology, neuroanatomical mechanisms, or management of Holmes tremor. Levodopa was the first-line medication, with one case series showing benefit in 54% of patients.[3] Surgical methods such as ventral intermediate nucleus thalamotomy or deep brain stimulation have shown benefit, but due to the invasive nature of these therapies that remains the last resort for many patients. Onabotulinumtoxin A (ona-BoNT-A) is a less invasive intervention, but its effectiveness needs to be further studied.

Results: We present videos and tremor physiology study data on two patients with Holmes tremor: one as a result of an acute posterior circular ischemic stroke and another due to demyelinating lesions involving the pons and superior cerebellar peduncles. Both patients had previously failed several pharmacological interventions, including carbidopa-levodopa, clonazepam, primidone, beta-blockers (ie, propranolol, and metoprolol), and gabapentin. Botulinum toxin was offered to them, and, in both patients, electromyography (EMG) was used for better localization of the active muscle groups thought to be involved in their tremor, and standard formulations of botulinum neurotoxin type A (BoNT-A) were injected. BoNT-A injections significantly reduced tremor intensity in both patients and improved their ability to perform activities of daily living.

Conclusions: Taken together, our clinical experience and review of the literature suggest that BoNT-A is a viable minimally invasive treatment for symptom control in Holmes tremor. Future prospective randomized clinical trials are indicated to further assess the efficacy of BoNT-A in relation to the current standard of care involving levodopa and surgical methods.

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Keywords: Botulinum; Brain injury rehabilitation; Holmes tremor

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Millennial Subject Satisfaction With Two Treatments of AbobotulinumtoxinA Per Year

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Background: Millennials are increasingly becoming a larger part of the aesthetic market. However, expectations are high, particularly with their first aesthetic treatment, which data shows is likely to be botulinum toxins. They also represent a subset that may not be as open to frequent aesthetic treatments.
Methods: A post hoc analysis compared subject satisfaction by age in a 12-month, open-label, multicenter study (NCT03687736) of two glabellar line injections of 50 U abobotulinumtoxinA (at baseline and 6 months). Age groups were segmented by decade, from 20-61+ years old, with millennials defined as age 21-40 years. Subject satisfaction and subject-reported FACE-Q scores were evaluated 6 months after each treatment.
Results: While the overall satisfaction rate was high across all age groups (≥86%), a higher proportion of millennials was highly satisfied (21-40 years: 75%; 50-60+: 49%). Millennials were happier with their glabellar lines at baseline, but all age groups reported large improvements 6 months after the second treatment (mean Rasch score increase: 10.1-28.2). The youngest and oldest groups had larger improvements in psychological wellness (mean Rasch Rating Scale score increase 6 months after each treatment: 21-30 years: 4.1-8.9, >60 years: 8.6-15.7). At study end, 100% of millennials stated they would like to receive the treatment again.
Conclusions: While all age groups were satisfied with and experienced psychological benefits from treatment, millennials appeared to be particularly happy with two treatments per year of abobotulinumtoxinA.

Funding: Research was funded by Galderma.
Keywords: AbobotulinumtoxinA; Aesthetic/cosmetic treatment; Glabellar lines; Subject satisfaction

Subject Satisfaction With Two Treatments Per Year (Every Six Months) of AbobotulinumtoxinA Is High Among Both Previously Treated and Toxin-Naïve Cohorts

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Background: Patients who have previous experience with and those naïve to aesthetic botulinum toxin treatments may have different expectations, leading to differences in satisfaction. Furthermore, some have suggested onset and duration of effect may differ. Therefore, a post hoc analysis of a study of twice-yearly treatment with abobotulinumtoxinA was done to compare outcome by prior treatment experience.
Methods: A post hoc analysis of toxin-naïve subjects vs previously treated was performed of an open-label, multicenter study evaluating subject satisfaction after glabellar line (GL) injections of 50 U abobotulinumtoxinA at baseline and retreatment at 6 months (NCT03687736). Follow up at Months 1, 3, 6, 7, 9, and 12 assessed subject satisfaction, subject-reported FACE-Q, and Glabellar Line Severity Scale (GLSS) score.
Results: Subject satisfaction rates were high at Month 12 for both previously treated (93%) and toxin-naïve (97%) subjects, though toxin-naïve subjects were more likely to be highly satisfied (66% vs 57%). Moreover, toxin-naïve subjects reported feeling better about themselves, more satisfaction with their appearance, and feeling more attractive 6 months after each treatment. Previously treated subjects reported earlier onset of effect (24 hours: 35-37% vs 14-24% toxin-naive). Subject-assessed GLSS responder rates were higher for toxin-naïve subjects, while investigators found higher rates in previously treated subjects.
Conclusions: Subject satisfaction with two treatments per year (every 6 months) was high, whether they had experience with previous toxin treatments or not. Clinical efficacy did not appear to differ, though toxin-naïve subjects were more likely to report high overall satisfaction.

Funding: Research was funded by Galderma.
Keywords: AbobotulinumtoxinA; ABO; Glabellar lines; Subject satisfaction

Theoretical, Methodological, and Statistical Issues With Claims about the Effect of Glabellar-Region Botulinum Toxin Injections on Depression

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Some researchers have proposed that blocking facial feedback via glabellar-region botulinum toxin injections (GBTX) can reduce depression.[1] However, recent reviews suggest that there are several issues with this claim.[2,3]

Theoretically, the proposed mechanisms are not well established. Proponents suggest that: (1) sensorimotor feedback from facial expressions impact emotion; (2) blocking feedback from negative facial expressions via GBTX reduces negative emotions; (3) decreases in negative emotions translate to improvements in depression. Ample evidence supports proposed causal effect #1, but not #2 or #3 (for a review, see reference 2).

Methodologically, existing studies have not ruled out placebo effects. Researchers often assign participants to receive either GBTX or saline injections. However, participants can easily infer their condition because only GBTX affects facial muscle mobility and appearance. Ineffective blinding could lead to the placebo response being amplified in the GBTX group and attenuated in the saline-injection group, leading to upward-biased effect size estimates and an increased Type I error rate. Similarly, existing studies have not ruled out the role of improvements in quality of life and social treatment.[4]

Statistically, the size of the observed overall effect of GBTX on depression is unusually large (twice as large as the average effect of FDA-approved antidepressants). This anomaly can be indicative of problems with research design, proposed mechanisms, and/or the analysis and reporting of data.[5] Furthermore, a large proportion of effect size estimates are missing from the scientific record, and there is some evidence of publication bias.

Taken together, the proposed effect of GBTX on depression is not yet

supported by a credible balance of evidence.
Funding: The authors have not received funding from any external source.
Keywords: Botulinum toxin; Depression; Facial feedback hypothesis; Meta-analysis; Methodology

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Injections of IncobotulinumtoxinA at Intervals Less Than 10 weeks Are Effective and Safe for Cervical Dystonia Patients With Inadequate Benefit From Standard Injection Intervals

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Introduction: In clinical practice, some cervical dystonia (CD) patients (pts) receiving long-term botulinum toxin (BoNT) therapy report early waning of treatment benefit (even after favorable peak response) before a typical 3-month reinjection interval. This study addresses the safety and efficacy of incobotulinumtoxinA injection intervals <10 weeks to meet the needs of such patients. The objective was to compare efficacy and safety of two injection schedules of incobotulinumtoxinA for treating CD.
Methods: CD Flex (NCT01486264) was a phase IV, open-label, randomized, noninferiority study comparing 2 incobotulinumtoxinA injection schedules (short-flex: 8±2 weeks; long-flex: 14±2 weeks) in CD subjects. BoNT-responsive subjects (≥2 prior successful injections) reporting acceptable clinical benefit lasting <10 weeks were recruited. Efficacy and safety were evaluated after 8 injection cycles. The primary endpoint was the change in the Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) severity subscale (blinded rater) 4 weeks after injection 8. Secondary endpoints included TWSTRS total and other subscale scores. Immunogenicity was assessed in a subset of patients at baseline/post-injection 8. Safety was monitored throughout the study.
Results: Two hundred eighty-two CD subjects were randomized (short-flex, n=142; long-flex, n=140), and 207 completed the study. Mean dosing was similar in the short-flex (272 U) and long-flex (268 U) groups; mean intervals were 54 days (short-flex) and 86 days (long-flex). Significant improvements in TWSTRS-severity from study baseline to 4 weeks after cycle 8 were observed in both the short-flex (4.1 pts; $P<0.0001$) and long-flex (2.4 pts; $P=0.002$) groups, and the short-flex was noninferior to the long-flex group (LS mean difference=1.4 pts; $P=0.0693$). Responder rates (≥20% improvement in TWSTRS-severity) after injection 8 did not differ significantly between groups. Adverse events (AEs) were comparable between groups. There was no secondary loss of treatment effect due to neutralizing antibodies after 8 cycles among those tested.
Conclusions: Injection cycles <10 weeks for incobotulinumtoxinA are effective (and noninferior to longer intervals) for treating CD patients with early waning of clinical benefit. Shorter intervals did not increase AEs or lead to loss of treatment effect due to neutralizing antibodies.
Funding: This study was sponsored by Merz Pharmaceuticals, LLC.
Keywords: Botulinum toxin; Cervical dystonia; IncobotulinumtoxinA; Movement disorders

The Effect of Neuromodulators on Proprioception

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Introduction: A retrospective review of patients who switched from onabotulinumtoxinA and/or abobotulinumtoxinA to incobotulinumtoxinA detected a difference in "feel" not described previously. We wished to understand these patient-reported symptoms as they may impact toxin selection and treatment satisfaction.
The four FDA-approved botulinum neurotoxin type A (BoNT-A) preparations derive from the same Hall strain but are manufactured by different proprietary means. Despite similar clinical efficacies in aesthetic indications, physicians have reported some clinical differences between the preparations. These include a difference in spread, immunogenicity, and time of onset. Some patients who switched from onabotulinumtoxinA and/or abobotulinumtoxinA to incobotulinumtoxinA reported that incobotulinumtoxinA felt "lighter" while other toxins led to treatment-associated headaches or tightness.
Methods: Patients who had prior treatments with more than one BoNT-A preparation for facial aesthetic indications completed a questionnaire. The questions covered the BoNT-A formulations and incidence with which the following symptoms occurred: muscular weakness, tightness, stiffness, headache, heaviness, and "frozen" sensation in the targeted area. The results were collated in a spreadsheet for tabulation.
Results: The questionnaire was completed by 79 patients. Symptoms were reported by 69.6% of patients, 54% of whom observed differences in symptoms between formulations. The commonest symptom was tightness (33%), followed by headache (29%), heaviness (27%), "frozen sensation" (20%), stiffness (14%), and muscular weakening in the targeted area (14%). Symptoms were experienced by 68% of onabotulinumtoxinA recipients, 38% of abobotulinumtoxinA recipients, and 11% of incobotulinumtoxinA recipients.
Conclusions: Our observations of differences in sensory symptoms with the different preparations may be linked to previously unknown mechanisms affecting the trigeminal branches. The occurrence and incidence of the observed symptoms occurred differently among the formulations, confirming that some patients can detect sensory symptoms, and that there is a perceptible difference between formulations. This difference in symptoms must be considered when switching patients from one formulation to another as patients may assume that a toxin is ineffective or non-durable even if a motor effect is present.
Keywords: Botulinum neurotoxin type A; Motor effect; Proprioception; Sensory symptoms; Toxin selection; Treatment satisfaction

How Different or Similar Is Management of Post-Stroke Spasticity With Botulinum Toxin When Pain Is Present?

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Introduction: Botulinum toxin type A (BoNTA) is an effective therapy for post-stroke spasticity[1] and pain management. This study aims to characterize the success rate and doses used in post-stroke spasticity (PSS)-related pain.
Methods: We performed a post hoc analysis of prospective, observational data collected from a designated population of patients with PSS, treated with BoNTA (2001-2021). Patients were divided into two groups: G1− pain was one goal (primary and/or secondary); G2− other treatment goals except pain. The treatment success rate was mapped to the International Classification of Functioning, Disability, and Health (ICF),[2] and doses used were compared between the groups. Statistical analysis was performed using the Mann-Whitney U test with $P \leq 0.05$ considered significant.
Results: Two hundred eighty-eight patients, who received a total of 2635 injections were included in the analysis. The mean age was 63.6±12.8 years (yrs), and age at time of stroke was 54.3±12.5 yrs. Ischemic stroke was the predominant type (64.9%; n=187), 56.3% of patients were male, and 98.6% had hemiparesis. The median time between stroke and first BoNTA injection was 0.93 yrs (minimum [min]: 0.09 yrs, maximum [max]: 34.49 yrs). The median number of treatment sessions per patient was 6 (min 1; max 63) and treatment targeted: both limbs (62.0%; n=1632); only upper limb (UL) (27%; n=710); only lower limb (LL) (11%; n=209). Regarding formulations, 65.8% of the injections (n=1734) used abobotulinumtoxinA (1113.1±376.1 U), 17.9% onabotulinumtoxinA (n=472) (410.6±183.9 U), and 16.3% incobotulinumtoxinA (n=429). Due to missing data, only 1230 treatment sessions were finally included in the subanalysis (Table). A total of 366 injections were administered to G1 (n=228, pain as primary goal; n=165, pain as one secondary goal); G2 received 864 injections. Regarding doses**,** greater doses were used in G1 when treating UL (719.11±354.47 U vs 615.69±338.05 U; $P=0.021$). Lower doses were administered in G1 for LL than in G2 (464.71±237.49 U vs 526.52±276.88 U; $P<0.001$). Doses were similar when both limbs were treated (G1: 990.58±457.50 U; G2: 998.95±457.72 U). Most treatment sessions resulted in expected or better than expected outcomes as measured by Goal Attainment Scale (GAS): G1: 70.22%; G2: 71.76%) with no statistical significance for the differences between the two groups ($P=0.738$). GAS T-score and change in GAS T-scores were similar between the groups: G1: mean T-score 48.67±5.02, change in GAS score: 11.78±5.17, change in GAS score ≥10: 10 72.40%; G2: mean T-score 48.72±4.75, change in GAS score: 11.60±4.85, change in GAS score ≥10: 74.77%. Success was also high, regardless of whether pain was a primary (mean T-score: 48.51±5.02, change in GAS score: 11.50±5.11) or a secondary goal (mean T-score: 49.30±5.34, change in GAS score: 12.71±5.5).
Conclusions: Success of botulinum toxin treatment, measured by goal achievement, was similar in PSS patients with and without pain, as well as when pain was a primary or secondary treatment goal. The doses used to treat the UL were significantly higher, but were lower for the LL, when pain was a treatment goal. Pain management with BoNTA in PSS patients should be considered as an effective therapy option.

Table
Doses and GAS Results for G1, G2, and Global.

		G1: Pain Goal (N=366)	G2: No Pain Goal (N=864)	Global (N=1230)
Doses	**Upper Limb**	123 (33.6%)	245 (28.4%)	368 (29.9%)
	BoNTA Units	719.11±354.47	615.69±338.05	650.0±347.07
	Lower Limb	17 (4.6%)	92 (10.6%)	105 (8.5%)
	BoNTA Units	464.71±367.49	526.52±276.88	516.88±272.04
	Both Limbs	225 (61.5%)	527 (61.0%)	752 (61.1%)
	BoNTA Units	990.59±457.50	998.95±457.72	996.44±457.67
GAS	**T-Score Achieved**	48.67±5.02	48.72±4.75	48.70±4.84
	T-Score ≥50 Achieved	257 (70.2%)	620 (71.8%)	877 (71.3%)
	Change in GAS score	11.78±5.17	11.60±4.85	11.65±4.95
	Change in GAS Score ≥10	265 (72.4%)	646 (74.8%)	911 (74.01%)

Keywords: Botulinum toxin; Pain; Spasticity; Stroke; Treatment Success

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What Categories of Goals Are More Impacted by Adjuvant Therapies in Post-Stroke Spasticity Management With Botulinum Toxin Type A? An Analysis of 20 Years of Real-World Data

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Introduction: Botulinum toxin type A (BoNTA) is effective in the management of post-stroke spasticity (PSS).[1] Adjunct therapies such as physiotherapy, occupational therapy and orthotics seem to be useful.[2] This study aims to describe the differences between groups regarding percentage of goals achieved/overachieved for patients who received adjuvant therapies after BoNTA injections and those who did not.
Methods: This is a post hoc analysis of prospective, observational data collected from a designated population of PSS patients treated with BoNTA (2001-2021). We separated patients into two groups: G1 − those treated with BoNTA injections alone and G2 − those treated with BoNTA and adjuvant therapies or other treatment strategies. For each group, we report the percentage of goals achieved/overachieved per goal category.
Results: Two hundred eighty-eight patients, who had a total of 2635 injections were included in the analysis. The mean age was 63.6±12.8 years (yrs) and age at time of stroke was 54.3±12.5 yrs. Ischemic stroke was the predominant (64.9%; n=187) type, 56.3% of patients were male and 98.6% had hemiparesis. The median time between stroke and first BoNTA injection was 0.93 yrs (minimum [min]: 0.09 yrs, maximum [max]: 34.49 yrs). The median number of treatment sessions per patient was 6 (min: 1, max: 63) and treatment targeted: both limbs (62.0%, n=1632); only upper limb (UL) (27%, n=710); only lower limb (LL) (11%, n=209). Most patients (90.0%) had adjuvant therapy post BoNTA. Physiotherapy was administered in 81.7% (n=2153), occupational therapy in 29.7% (n=782), orthotics

in 49.8% (n=1312), systemic medication in 12.4% (n=329), and transcutaneous electrical nerve stimulation (TENS) in 1.4% (n=37) of patients. Most patients used one (30.7%, n=810), two (36.9%, n=971), or three (19.2%; n=505) adjuvant strategies. Due to missing data, 1230 treatment sessions were included in a subanalysis investigating the role of adjuvant therapies to BoNTA for treatment success.
Patients in the G1 group received 101, and those in G2, 1129 injections. In both groups most patients had an expected or greater than expected outcome on their Goal Attainment Scaling (GAS) assessment (Table). Analysis by goal category showed higher success rates for the G2 group in all goal categories except for involuntary movements, where the G1 group scored higher (91.2% vs 79.2%, respectively). The G2 group advantage was more evident in goals related to pain (88.6% vs 76.5%) and range of motion (88.8% vs 80.0%), suggesting the relevance of a multimodal approach to pain management and the prominent role of stretching techniques for maintenance of range of motion (ROM).
Conclusions: In our 20 years of clinical practice in PSS management with BoNTA, there is a very high percentage of patients using different adjuvant therapies and treatment success rates tend to be higher in this group, except for goals related to involuntary movements.

Table
GAS Results According to Group Distribution.

		Treatment with BoNTA and Adjuvant Therapies (N=1129)	Treatment With BoNTA Monotherapy (N=101)
GAS	**T-Score Achieved**	48.7 ± 4.8	48.3 ± 5.1
	Goal ≥0	812 (71.9%)	65 (64.4%)
	Goal <0	317 (28.1%)	36 (35.6%)
	Change	11.7 ± 4.9	11.5 ± 5.1
	Change in GAS score ≥10	840 (74.4%)	71 (70.4%)
	Change in GAS score <10	289 (25.6%)	30 (29.6%)
Goals with GAS score ≥0	**ROM maintenance**	103 (88.8%)	4 (80.0%)
	Pain	187 (88.6%)	13 (76.5%)
	Involuntary movement	251 (79.2%)	31 (91.2%)
	Active function	92 (82.1%)	14 (77.7%)
	Passive function	118 (89.46%)	5 (83.3%)
	Mobility	194 (81.6%)	17 (81.0%)
	Treatment facilitation	4 (100.0%)	0 (0.0%)

Keywords: Adjuvant therapies; Botulinum toxin; Spasticity; Stroke; Treatment success

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Study Design of LONG RUN: A LONGitudinal Evaluation and Real-World Evidence of Uniquely Purified IncobotulinumtoxinA in Treatment-Naïve Participants

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Introduction: IncobotulinumtoxinA (INCO) is approved for the temporary improvement of moderate-to-severe upper facial lines (UFL), in the North American, Latin American, European, and Asia-Pacific regions (specific indications vary with country). Though widely used since 2010, clinical data on the long-term effectiveness, safety, and participant satisfaction with INCO in aesthetic management is limited. Here, we describe the design of an open-label, prospective, observational study, aimed at capturing long-term, real-world evidence and clinically-relevant data of INCO use in clinical practice in a global population.
Methods: Insight regarding protocol development was provided by an external study advisory committee. Participants 18 years of age or older, who have never received prior botulinum neurotoxin type A (BoNT-A) treatment, and are seeking treatment with INCO for UFL, will be enrolled at approximately 25 clinical sites in approximately 12 countries. Participants will be treated with INCO according to standard of care, and treatment, safety, and available effectiveness data will be collected. At each treatment visit, treatment variables will be recorded. Self-perception of age will be assessed using the validated FACE-Q Aging Appraisal Form and the Patient-Perceived Age Visual Analogue Scale. Severity of UFLs will be assessed by investigator's live assessment, and by participant self-assessment using the validated 5-point Merz aesthetic scales.
A global assessment of aesthetic improvement in appearance of UFL compared to the status prior to INCO treatment will be assessed by the participants using the Global Aesthetic Improvement Scale. Participant satisfaction with treatment will be assessed by direct questioning. Participants will undergo repeated treatments as needed and according to standard of care for up to 3 years, and will be contacted by phone, email, or site visit 4 to 6 weeks after each treatment to assess satisfaction. A blood sample will be collected from participants at the end-of study visit for the assessment of neutralizing antibodies.
Conclusions: The study design described here is, to our knowledge, the first of its kind for a real-world-evidence, observational study, which will follow the treatment journey of BoNT-naïve participants receiving facial cosmetic procedures for up to 3 years continuously. Data collected in this clinical study will enable a general assessment of the long-term safety, efficacy, and patient satisfaction associated with continuous treatments with INCO administered as needed according to physician and participant discretion for UFLs under real-world conditions. Data from this study will also provide insight into aesthetic uses of INCO in a global patient population, and exploration of the potential effects of neutralizing antibodies.
Funding: This study is sponsored by Merz Aesthetics.
Keywords: Aesthetics; Clinical study; Efficacy; IncobotulinumtoxinA; Patient satisfaction

AbobotulinumtoxinA Efficacy and Safety in Children With Upper Limb Spasticity Previously Treated With Botulinum Toxin

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Introduction: Primary endpoint analysis of this phase 3 study confirmed abobotulinumtoxinA (aboBoNT-A 8 U/kg and 16 U/kg) significantly

reduced hypertonia versus the 2 U/kg low-dose control and was generally well tolerated (Delgado, et al, 2021). However, the impact of previous botulinum neurotoxin (BoNT) injections on treatment outcomes remains largely unexplored.
Methods: We present subgroup analyses of a phase 3 study conducted in children (aged 2-17) with cerebral palsy (Gross Motor Function Classification System [GMFCS] Levels I-IV) and spasticity in ≥1 upper limb. In the first treatment cycle, 210 children were randomized to treatment with aboBoNT-A 2 U/kg, 8 U/kg or 16 U/kg into the primary target muscle group (PTMG; elbow or wrist flexors) and additional upper limb muscles. Children could be naïve to BoNT treatment or previously treated (with any BoNT formulation).
Results: In the modified intent-to-treat (mITT) population, 138 children had been previously treated with a BoNT formulation, and 70 children were new to treatment. At Week 6, previously treated children showed mean reductions (±SD in modified Ashworth scale (MAS_{PTMG}) scores of -1.4 ±1.0 in the 2 U/kg (n=45), -1.9 ±0.9 in the 8 U/kg (n=47), and -2.0 ±1.0 in the 16 U/kg (n=46) groups vs baseline. Children who were BoNT-naïve showed MAS_{PTMG} reductions of -1.5 ±1.2 (n=24), -2.0 ±1.3 (n=22), and -2.7 ±0.7 (n=24), respectively. Treatment differences were significant vs the control group for both subgroups treated with aboBoNT-A 16 U/kg and for the previously treated subgroup who received 8 U/kg. All children (all groups) showed improvement on the Physician Global Assessment. For this global assessment, the magnitude of improvement was slightly better for BoNT-naïve children in the 8 U/kg group than in other groups (2.3 vs 1.7-2.0 grade improvements in other subgroups). Adverse events during Cycle 1 (combined doses) were reported in a similar proportion of BoNT-naïve (52.8%) and previously treated (58.0%) children and most were considered unrelated to treatment. Eight children who had all been previously treated with BoNT reported experiencing muscular weakness.
Conclusions: These results demonstrate similar aboBoNT-A efficacy and safety profiles in children with upper limb spasticity who are new to BoNT treatment compared to those previously treated.
Funding: Ipsen
Keywords: AbobotulinumtoxinA; Clinical trial; Efficacy; Pediatric; Upper limb spasticity

Reference

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Comparative Efficacy of AbobotulinumtoxinA and OnabotulinumtoxinA for the Treatment of Refractory Neurogenic Detrusor Overactivity: An Indirect Treatment Comparison

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Introduction: Neurogenic detrusor overactivity (NDO) causes urinary incontinence (UI). AbobotulinumtoxinA (aboBoNT-A) and onabotulinumtoxinA (onaBoNT-A) reduce the frequency of weekly UI episodes. The objective was to compare the efficacy and safety of aboBoNT-A with onaBoNT-A in NDO.
Methods: The systematic literature review followed Cochrane database and National Institute for Health and Care Excellence (NICE) guidance. MEDLINE and other sources were searched for randomized controlled trials of botulinum toxin type A formulations (through August 2020). Results from two aboBoNT-A trials and four onaBoNT-A trials were included. Outcomes included mean change from baseline (CFB) in weekly UI episodes (2, 6, 12, and 24 weeks); proportion of patients with 100% reduction in UI episodes at 6 weeks; and treatment-emergent urinary tract infections (TE-UTI). Bucher indirect treatment comparisons (ITCs) were conducted.
Results: Six studies on three active interventions (aboBoNT-A 600 U, 800 U, and onaBoNT-A 200 U) were included in the ITC, connected via the comparator placebo.
Trends were consistent although not significant. aboBoNT-A 600 U had numerically greater reduction in weekly UI episodes versus onaBoNT-A, with mean (95% confidence interval [CI]) differences in CFB of -0.8 (-6.0,4.5) and -2.3 (-8.5, 3.9) at 6 and 12 weeks, respectively. At 6 weeks, aboBoNT-A 600 U had numerically greater odds of a 100% reduction in weekly UI episodes versus onaBoNT-A (odds ratio: 3.0 [95%CI: 0.60, 15.34]). Results were similar for aboBoNT-A 600 U and 800 U. Applying relative effect estimates to a common/average placebo anchor rate resulted in estimates of 54.5%, 45.9%, and 28.4% of patients achieving 100% reduction in weekly UI episodes at 6 weeks with aboBoNT-A 600 U, 800 U and onaBoNT-A 200 U, respectively. aboBoNT-A 600 U/800 U was associated with fewer TE-UTIs than onaBoNT-A 200 U (odds ratios: 0.64 [0.34,1.21] and 0.90 [0.48,1.66], respectively).
Conclusions: aboBoNT-A and onaBoNT-A offer significant efficacy in NDO, with improvement on aboBoNT-A numerically greatest, and difference in CFB between the two toxins increasing from week 6 to 12. Further studies are needed for better-powered comparisons that may find statistically significant differences.
Funding: This study was sponsored by Ipsen.
Keywords: Bladder; Botulinum neurotoxin type A; Comparative effectiveness; Neurogenic; Treatment decision; Urinary incontinence

Consecutive Headache-Free Days With OnabotulinumtoxinA Treatment in Patients With Chronic Migraine: A Pooled PREEMPT Analysis

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Introduction: The disability of chronic migraine prevents patients from performing daily activities, leading to substantial personal, societal, and familial disturbance. More consecutive days of headache freedom are associated with meaningful improvements in quality of life. The goal of this study was to evaluate the impact of onabotulinumtoxinA versus placebo on the number of consecutive headache-free days and days without moderate/severe headache in participants with chronic migraine.
Methods: This was a post hoc analysis of the phase 3, 24-week, randomized, double-blind PREEMPT clinical trials (NCT00156910, NCT00168428). A headache day was defined as a day with ≥4 continuous hours of headache, per participant diary; participants recorded headache severity as mild, moderate, or severe. Percentages of participants who experienced ≥7, ≥14, and ≥21 consecutive days without headache or without a moderate/severe headache that required acute medication were compared between onabotulinumtoxinA and placebo groups. Only diary data after the first dose were used to calculate consecutive days without headache. Data were pooled to improve precision. Two-tailed Fisher's exact tests were performed to compare onabotulinumtoxinA versus placebo during the double-blind phase.
Results: A total of 1384 participants were randomized to onabotulinumtoxinA (n=688) or placebo (n=696) in the PREEMPT trials. During the 28-day baseline screening phase, the mean number of headache days was 19.9 for onabotulinumtoxinA and 19.8 for placebo (P=0.498). During the double-blind treatment phase, significantly more participants treated with onabotulinumtoxinA than placebo experienced ≥7 (70% vs 64%; P=0.039), ≥14 (40% vs 31%; P<0.001), and ≥21 (26% vs 18%; P<0.001) consecutive headache-free days without acute medication use. Significant differences favoring onabotulinumtoxinA treatment remained when the analysis was restricted to participants who experienced ≥7 (74% vs 68%; P=0.018), ≥14 (42% vs 34%; P=0.003), and ≥21 (28% vs 21%; P=0.003) consecutive

moderate/severe headache-free days without acute medication use.
Conclusions: OnabotulinumtoxinA treatment resulted in significantly more consecutive headache-free days and moderate/severe headache-free days than placebo in chronic migraine patients.
Funding: Allergan (prior to its acquisition by AbbVie).
Keywords: Botox; Chronic migraine; Headache-free; Long-term; OnabotulinumtoxinA; PREEMPT

The Purification Process to Obtain a Complex-Free Highly Purified Botulinum Neurotoxin Type A1: RelabotulinumtoxinA

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Introduction: The use of *Clostridium botulinum* neurotoxins for aesthetic purposes is increasing. To date, most available products on the market are lyophilized and need to be reconstituted in saline before use. RelabotulinumtoxinA is a novel, stable, ready-to-use liquid formulation type A1 botulinum toxin (BoNT-A1) product. The ready-to-use formulation improves ease of use and minimizes the risk of dosing errors due to faulty reconstitution and is a pure BoNT-A1 free from complexing proteins and other protein additives. The drug substance (DS) manufacturing process is described, and some typical process data is presented.
Methods: Manufacture of the DS used in relabotulinumtoxinA starts with cultivation of a proprietary *C botulinum* type A1 strain. Following cultivation, three diafiltration and four chromatography steps are performed. The chromatography steps include ion-exchange and size-exclusion chromatography, with size-exclusion chromatography as the final polishing step. The entire manufacturing process uses single-use technology with no open handling of the potent toxin solutions. Each step is designed and has a specific purpose, eg, to remove cells and cell debris, to remove nucleic acids, or to separate BoNT-A1 from other process-related proteins. Purity and BoNT-A1 content were analyzed in fractions from all steps using sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) and a BoNT-A1–specific enzyme-linked immunosorbent assay (ELISA).
Results: During the ten-step manufacturing process, an approximately 140 times purer BoNT-A1 is obtained when comparing the DS to the harvested cultivation supernatant. This is illustrated by SDS-PAGE results, where the protein impurities are reduced throughout the purification process. Process parameters have been studied and the set ranges have been shown to secure a pure DS that is free from complexing proteins and stable when stored at <-70°C. The process yield is approximately 5 mg of BoNT-A1, which corresponds to about 20% over the purification process. The purity as determined by Ultra Performance Liquid Chromatography–Size-Exclusion Chromatography (UPLC-SEC) of the final DS is >98%.
Conclusion: An innovative production process has been developed where the BoNT-A1 is kept in liquid state throughout. The process yields approximately 5 mg of highly pure and complex-free BoNT-A1 that is used to manufacture relabotulinumtoxinA.
Funding: This project is funded by Galderma.
Keywords: BoNT-A1; Botulinum toxin; Chromatography; Liquid formulation; Single-use technology; Ready-to-use

Assessing the Effectiveness of AbobotulinumtoxinA Injections for Adult Lower Limb Spasticity in Routine Clinical Practice: Methodology and Baseline Data for the AboLiSh Study

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Introduction: While the efficacy and safety of abobotulinumtoxinA (aboBoNT-A) in reducing lower limb spasticity has been established in phase 3 studies, there is a paucity of information from real-life clinical practice. Goal attainment in lower limb spasticity management studies have been limited.
Methods: AboLiSh is an ongoing prospective, longitudinal, observational study (ClinicalTrials.gov Identifier: NCT04050527) exploring the real-world utilization and effectiveness of aboBoNT-A for lower limb spasticity. Ambulatory adult patients (≥18 years) with unilateral lower limb spasticity (able to take ≥5 steps with or without assistance) are treated in accordance with local prescribing guidelines to achieve individualized treatment goals. The primary endpoint is goal attainment as assessed using the cumulated (mean) Goal Attainment Scaling Leg T score, across all treatment cycles for each patient. We present an interim analysis of baseline data.
Results: The enrolled population includes 421 adults with lower limb spasticity, from 45 sites in 9 countries. The mean (±SD) age is 53.7 ±13.9 years, and 65.1% of patients are male. Most (96.4%) have spasticity due to acquired brain injury (stroke/trauma). At baseline, half of the patients have set active function primary goals (50.7%; 40.2% have set locomotion goals and 10.5% set transfer/standing goals). Other primary goals set include pain management (18.3%), range of movement (16.9%), involuntary movements (10.2%), passive function (1.9%), cosmesis/facilitation of therapy (0.7%), and other (1.2%). The median [Q1, Q3] total dose of aboBoNT-A is 600 [450, 900] units and the median number of muscles injected is 4 [3, 5]. Overall, 74.0% of Cycle 1 injections were given using ≥1 guidance technique (ultrasound 42.9%, electromyography 41.0%, electrical stimulation, 32.3%).
Conclusions: The ongoing AboLiSh study will inform on goal setting and goal achievement in multiple treatment cycles with aboBoNT-A for lower limb spasticity as well as help identify real-life drivers influencing clinical decision making for these patients.
Funding: Ipsen
Keywords: AbobotulinumtoxinA; Goals; Lower limb spasticity; Observational study

Extensor Digitorum Brevis and DIRECTION: Two Studies in Progress Comparing the Duration of Response of AbobotulinumtoxinA With Other Native Botulinum Toxins

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Introduction: Three commercially available botulinum neurotoxin type A toxins (BoNT-As) are used to treat adult upper limb spasticity (AULS), each varying in potency and duration of response. There is a paucity of clinical study data comparing the pharmacodynamics, safety, and efficacy of BoNT-As, and such data would support physicians in making informed treatment choices. The Extensor Digitorum Brevis (EDB) study (NCT04970407) will assess the duration of response of BoNT-As in healthy volunteers using an established pharmacodynamic model, and the DIRECTION study (NCT04936542) will explore the safety and efficacy of 2 BoNT-As in the treatment of AULS.
Methods: The EDB study is a phase I, randomized, double-blind, head-to-head study in 45 healthy volunteers. Using a dosing ratio of 2.5:1 (in line

with licensed indications), we will compare abobotulinumtoxinA (aboBoNT-A) 40 U, onabotulinumtoxinA (onaBoNT-A) 16 U, and incobotulinumtoxinA (incoBoNT-A) 16 U in 15 volunteers per arm. The aim of the EDB study is to show the superiority of aboBoNT-A versus onaBoNT-A or incoBoNT-A in terms of duration of response. The primary outcome will be the total amplitude of the compound muscle action as a relative change from baseline at week 28. DIRECTION is a post-marketing, multicenter, interventional, randomized, double-blind, 2x2 crossover study in 564 participants. Participants will be randomized to 1 of 2 sequences, with 282 participants per sequence: aboBoNT-A (900 U) followed by onaBoNT-A (360 U), and vice versa. The primary objective of DIRECTION is to demonstrate the noninferiority of aboBoNT-A versus onaBoNT-A by comparing the rate of treatment-emergent adverse events from injection to 12 weeks after injection. Secondary outcomes will focus on superiority of duration of response and various efficacy endpoints.

Conclusions: These studies will add to the understanding of the safety, efficacy, and duration of response of commercially available BoNT-As, thereby informing physician treatment choices. The EDB study will provide comparative data on the duration of response of native BoNT-As, and DIRECTION will further characterize safety and duration of response to allow informed decisions for care optimization.

Funding: These studies are funded by Ipsen.

Keywords: AbobotulinumtoxinA; Clinical study; Efficacy; Extensor Digitorum Brevis; Safety; Spasticity

Dosing, Treatment Intervals, and Treatment Satisfaction With OnabotulinumtoxinA Over Time in the Adult Spasticity International Registry (ASPIRE)

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Introduction: This study aims to examine the relationship between real-world dosing and treatment intervals of onabotulinumtoxinA (onabotA) for adult spasticity.

Methods: The prospective, observational, international Adult Spasticity International Registry (ASPIRE; NCT01930786) collected data for multiple etiologies over 2 years (N=730). OnabotA dosing and treatment intervals were at the physician's discretion. Patients were split into treatment interval groups (N=2373): <12, 12-14, 15-17, 18-20, and ≥21 weeks. Treatment adherence was defined as those receiving ≥3 treatment sessions with onabotA over the 2-year period. Patient satisfaction items were assessed, and adverse events (AEs) captured.

Results: OnabotA doses were generally constant over the treatment interval groups, with the lowest mean dose 335 U (for ≥21 weeks) and highest dose 387 U (for 12-14 weeks). Patients naïve to toxin at baseline received slightly lower doses (205-372 U) (in each treatment interval group than non-naïve [346-396 U]). Most patients received doses ≤400 U across all treatment interval groups: <12 (63%), 12-14 (66%), 15-17 (70%), 18-20 (75%), and ≥21 (76%) week groups. Dosing increased slightly over treatment sessions, from 355 U at session 2 to 394 U at session 7. Treatment-adherent patients reported high rates of satisfaction over treatment sessions: 81-92% stated their most recent onabotA treatment helped their spasticity, 73-82% were satisfied/extremely satisfied with how long they felt onabotA working, and 91-96% planned to continue using onabotA to treat their spasticity. Most commonly reported AEs were fall (5.5%), urinary tract infection (2.6%), and muscular weakness (2.6%).

Conclusions: Dosing of onabotA to treat adult spasticity remained consistent over treatment sessions, regardless of the length of the treatment interval, with most patients receiving onabotA within the approved dosing ranges. High patient satisfaction was observed, with no new safety signals.

Keywords: Dosing; OnabotulinumtoxinA; Spasticity; Treatment intervals

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A. Zuzek is a full-time employee of AbbVie and may hold AbbVie stock.

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A Novel Approach to New Onset Hemiplegic Shoulder Pain With Decreased Range of Motion Using Targeted Diagnostic Nerve Blocks: The ViVe Algorithm

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Introduction: Hemiplegic shoulder pain (HSP) is the most common pain disorder after stroke with incidence estimates of 30%-70%. It is associated with reductions in function, interference with rehabilitation, and a reduced quality of life. Onset may occur as soon as a week after stroke in 17% of patients. Management of HSP represents a complex treatment pathway with a lack of evidence to support any single treatment. The pain has heterogeneous causes. In the acute setting, decreased range of motion in the shoulder can be due to early onset spasticity, capsular pattern stiffness, glenohumeral pathology, or complex regional pain syndrome (CRPS). As contracture can form in up to 50% of patients after stroke, effective management of the painful shoulder and upper limb with decreased range of motion requires assessment of each possible contributor to the disorder. The anesthetic diagnostic nerve block (DNB) is known

to differentiate spasticity from contracture and other disorders of immobility and can be useful in determining an appropriate treatment pathway, including botulinum neurotoxin (BoNT). BoNT is not on label for the management of shoulder spasticity in many countries, and therapeutic usage of BoNT may result in treatment failure due to ongoing pain if the correct etiology of the pain and loss of range is not established.
Objective: To create a diagnostic algorithm to differentiate between the causes of HSP in the stiff, painful shoulder in the subacute setting, using diagnostic techniques including the Budapest Criteria for CRPS, and DNB for spasticity and pain generators.
Results: Examination of each joint in the upper extremity with HSP may differentiate each diagnosis with the use of an algorithm. Pain and stiffness isolated to the shoulder may be differentiated as primary shoulder pathology; sensory suprascapular DNB or intraarticular/subacromial injection can assist in differentiating adhesive capsulitis, arthritis, or rotator cuff injury. CRPS may affect the shoulder, elbow, wrist, and hand, and can be evaluated with the Budapest Criteria. Spasticity can be differentiated with the use of motor DNB. A combination of these disorders may cause HSP, and the proposed treatment algorithm may help in selecting a systematic treatment pathway.
Conclusion: HSP is a disabling disorder with poor treatment outcomes and inconclusive evidence to treat. This algorithm could lead to better outcomes for patients and more effective and directed use of botulinum toxins and other targeted treatments. It could improve the outcomes of BoNT therapy in this complex disorder through accurate diagnosis of the cause of the loss of range.
Keywords: Adhesive capsulitis; Complex regional pain syndrome; Hemiplegia; Muscle spasticity; Stroke

Reference

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Long-Term Safety and Tolerability of Repeated Treatments With OnabotulinumtoxinA in Children with Neurogenic Detrusor Overactivity

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Introduction: OnabotulinumtoxinA is approved for the treatment of neurogenic detrusor overactivity (NDO) in adults inadequately managed with anticholinergics and was shown to be effective and well tolerated in children in a randomized, double-blind study (Clinicaltrials.gov, NCT01852045). Given the paucity of long-term data in children we report on the continued safety in these patients after repeated onabotulinumtoxinA treatments.
Methods: This was a multicenter, double-blind, long-term, repeat-treatment extension study (Clinicaltrials.gov, NCT01852058) in patients who entered from the preceding study where they received one onabotulinumtoxinA treatment. Data were integrated across both studies. All patients (5-17 years) were on clean intermittent catheterization and could receive dose escalations based on their response to the preceding treatment (50 U, 100 U, or 200 U onabotulinumtoxinA [not to exceed 6 U/kg] studied).
Results: Among patients enrolled, 95, 90, 55, and 11 patients received at least 1, 2, 3, or 4 treatments with study medication, respectively. The safety profile was similar across doses and consistent after repeat treatments (Table). Most commonly reported treatment-emergent adverse events (TEAEs) within the first 12 weeks were urinary tract infection (UTI) and bacteriuria. There were no cases of autonomic dysreflexia, neutralizing antibodies, and no TEAEs related to distant spread of toxin. Two cases of pyelonephritis (100 U; cycle 2) occurred $\geq$ 231 days after treatment and were not considered treatment related. Six cases of nonrelated hydronephrosis occurred between 85 to 309 days after injection. No meaningful changes in renal function (based on estimated glomerular filtration rate) were noted in any patient.
Conclusions: OnabotulinumtoxinA continued to be well tolerated in up to four repeated treatments in pediatric patients with NDO with similar safety profiles across dose groups. TEAEs were primarily urological with no new safety concerns.

Table
Most Common TEAEs ($\geq$15% of patients in any treatment group)

Preferred term, n (%)	OnabotA 50 U	OnabotA 100 U	OnabotA 200 U	Total
Cycle 1	**N=31**	**N=39**	**N=25**	**N=95**
Total TEAEs	23 (74.2)	31 (79.5)	19 (76.0)	73 (76.8)
UTI	10 (32.3)	14 (35.9)	5 (20.0)	29 (30.5)
Bacteriuria	5 (16.1)	7 (17.9)	5 (20.0)	17 (17.9)
Headache	2 (6.5)	7 (17.9)	2 (8.0)	11 (11.6)
Cycle 2	**N=9**	**N=45**	**N=36**	**N=90**
Total TEAEs	7 (77.8)	34 (75.6)	31 (86.1)	72 (80.0)
UTI	1 (11.1)	22 (48.9)	8 (22.2)	31 (34.4)
Bacteriuria	1 (11.1)	9 (20.0)	2 (5.6)	12 (13.3)
Blood urine present	2 (22.2)	4 (8.9)	2 (5.6)	8 (8.89)
Leukocyturia	2 (22.2)	1 (2.2)	3 (8.3)	6 (6.7)
Cycle 3	**N=5**	**N=16**	**N=34**	**N=55**
Total TEAEs	4 (80.0)	10 (62.5)	21 (61.8)	35 (63.6)
UTI	0	4 (25.0)	8 (23.5)	12 (21.8)
Blood urine present	2 (40.0)	1 (6.3)	5 (14.7)	8 (14.5)
Bacteriuria	0	3 (18.8)	4 (11.8)	7 (12.7)
Cycle 4	**N=3**	**N=4**	**N=4**	**N=11**
Total TEAEs	3 (100.0)	2 (50.0)	4 (100.0)	9 (81.8)
Blood Urine present	2 (66.7)	2 (50.0)	2 (50.0)	6 (54.5)

Keywords: Neurogenic detrusor overactivity; OnabotulinumtoxinA; Pediatric
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Israel Franco has served as a consultant/advisor to Allergan, an AbbVie company, and is chief science officer of FIAS Inc (a software company) and chief science officer for Gogoband Inc (a software and bedwetting device maker).
Eric Dobremez has served as a consultant for Allergan, an AbbVie company.

Piet B. Hoebeke has served as a consultant for Allergan, an AbbVie company, and Astellas.
Wilson Titanji, Irina Yushmanova, and Brenda Jenkins are employees and shareholders of AbbVie.
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Long-Term Use of Botulinum Toxin: Is Primary Goal Achievement Sustainable for Poststroke Spasticity?

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Introduction: Botulinum toxin type A (BONT-A) is an effective therapeutic strategy in focal/regional poststroke spasticity.[1] However, maintenance of treatment success with BONT-A in patients undergoing long-term treatment is not clear. Thus, this study aims to analyze the success rate for achievement of primary goals in patients receiving long-term treatment with BONT-A.
Methods: A post hoc analysis of prospective, observational data of patients with poststroke spasticity treated with BONT-A from 2001 to 2021. Patients were separated into two groups according to duration of BONT-A treatment (<10 years vs ≥10 years). The success rate for achievement of the primary goal (mapped to the International Classification of Functioning, Disability and Health of the World Health Organization)[2] was compared for the two groups. Statistical analysis was performed using the Mann–Whitney U test and Chi-Square test, considering a P value of ≤0.05 as significant.
Results: A total of 288 patients with 2635 BONT-A treatment sessions were included in the analysis. Patients' mean age was 63.6±12.8 years, and the majority were male (56.3%; n=162). Mean time from stroke to first BONT-A treatment was 1.9±3.6 years, and the majority of patients had an ischemic stroke (64.9%; n=187). Median treatment sessions per patient were 6 (minimum: 1; maximum: 63), and the majority were treated in both limbs (62.0%; n=1632). AbobotulinumtoxinA was used in 65.8% (n=1734) of the treatment sessions (1113.1±376.1 U), onabotulinumtoxinA in 17.9% (n=472; (410.6±183.9 U), and incobotulinumtoxinA in 16.3% (n=429). Due to missing data, 1230 treatment sessions were included in a subanalysis investigating treatment success, with 994 injections included in the <10-year group and 236 in the ≥10-year group. Although the main primary goal in both groups was involuntary movement control, it assumed a significantly greater role in the ≥10-year group (43.2% vs 25.1%, P<0.001). The majority of patients in both groups presented an expected or greater than expected outcome on the Goal Attainment Scaling (GAS) assessment (<10 years: 70.2% vs ≥10 years: 75.9%; Table 1), although the success rate was significantly greater in the ≥10-year group (P=0.049). While the mean GAS outcome T-score was similar between the groups (<10 years: 48.6±4.9 vs ≥10 years: 49.0±4.5, P=0.243), the mean change in GAS score was significantly (P=0.001) higher in the ≥10-year group (12.2±4.5) compared to the <10-year group (11.5±5.0).
Conclusions: Primary treatment goals seem to change with long-term BONT-A administration. Although involuntary movement control was the most frequent treatment goal in the short-/mid-term treatment group (closely followed by mobility), it assumed a much greater role as a primary objective in the long-term (≥10-year) group. This may be due to a change in treatment paradigm from improvement in functioning to symptom/deficit control, as well as the higher persistence of upper limb limitation. Significantly higher success rate per goal and change in GAS scores were observed in the ≥10-year BONT-A treatment group, although no difference was found in mean GAS outcome T-score. This demonstrates that, even after 10 years of BONT-A treatment, primary goals can still be achieved, and treatment success may even be higher versus short-/mid-term treatment. Thus, patients should continue to receive BONT-A treatment in the long term, if there are realistic treatment goals, because success rates are high.
Keywords: Botulinum toxin; Long-term treatment; Spasticity; Stroke; Treatment success.

Table 1
Primary Treatment Objectives and GAS Results.

		<10 Years (N=994)	≥10 Years (N=236)	P Value
Primary Goals According to ICF	ROM Maintenance	104 (10.5%)	17 (7.2%)	<0.001*
	Pain	188 (18.9%)	40 (16.9%)	
	Involuntary Movement	249 (25.1%)	102 (43.2%)	
	Active Function	114 (11.5%)	16 (6.8%)	
	Passive Function	112 (11.2%)	26 (11.0%)	
	Mobility	223 (22.4%)	35 (14.8%)	
	Treatment Facilitation	4 (0.4%)	0 (0.0%)	
GAS	Initial T-Score	37.1 ± 1.3	36.8 ± 1.1	<0.001†
	T-Score Achieved	48.6 ± 4.9	49.0 ± 4.5	0.243†
	Goal ≥0	698 (70.2%)	179 (75.9%)	0.049*
	Goal <0	296 (29.8%)	57 (24.2%)	
	Change	11.5 ± 5.0	12.2 ± 4.5	0.001†
	Change ≥10	726 (73.0%)	185 (78.4%)	0.099*
	Change <10	268 (27.0%)	51 (21.6%)	

ROM=Range of Motion.
* Chi-Square Test
† Mann-Whitney U Test.

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Final Analysis of a Real-World, Retrospective Study in the United Kingdom Evaluating Treatment Outcomes of AbobotulinumtoxinA in Adult Focal Spasticity

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Introduction: AbobotulinumtoxinA (Dysport®; aboBoNT-A) is an effective treatment for adult focal spasticity. Despite being approved since 1990, real-world, long-term data from large, retrospective databases on the use of aboBoNT-A in this indication are scarce, especially in the United Kingdom.

Methods: DYSCOVER (NCT04604379) is a multicenter, non-interventional, retrospective study of treatment outcomes of aboBoNT-A for adult focal spasticity. The primary objective of the study was to describe the dose and injection interval of aboBoNT-A with single and repeated treatment cycles. Data were recorded for adult patients who received ≥1 injection of aboBoNT-A during the observation period (a minimum of 1 treatment cycle and a maximum of 52 weeks). Patients were naive to treatment with any botulinum toxin type A preparation 6 months prior to initiation of aboBoNT-A. All patients were treated according to local routine practice (aiming to adhere to national guidelines) and in line with the Summary of Product Characteristics for aboBoNT-A. Here, we present baseline characteristics and treatment practice from the final analysis.

Results: Of 108 patients included in the study population, 56% were male and the median weight at baseline was 64 kg. The median age of patients at first injection was 52.5 years. The most common underlying neurological condition was stroke, which affected 61% of patients. Most patients had unilateral spasticity, while bilateral spasticity occurred in 24% of patients with lower limb spasticity and 11% of patients with upper limb spasticity. Additionally, 25% of patients had spasticity in both their upper and lower limbs. There was a median interval of 43.7 months between the diagnosis of a neurological condition and first injection and 6.9 months between the diagnosis of spasticity and first injection. Patients presented with a variety of comorbidities, including anxiety or depression (14%), symptoms of constipation (8%), and urinary tract infection (6%). The median Modified Ashworth Scale score at baseline was 2.0 for all muscles assessed, with the exception of elbow pronators, which had a median score of 1.5. The median interval between injection cycles was 140 days. The number of injection cycles patients experienced throughout the observation period ranged from 1 to 4. Values for median dose per treatment cycle are provided in the table.

Table
Median Dose of AboBoNT-A Per Treatment Cycle

Treatment cycle	1 (n=108)	2 (n=82)	3 (n=44)	4 (n=7)
Total dose, units (U) Median dose, U (Q1, Q3)	500.0 (300.0, 700.0)	500.0 (500.0, 750.0)	500.0 (387.5, 700.0)	400.0 (300.0, 500.0)

Conclusions: This real-world dataset from the United Kingdom will support clinical practice by providing a much-needed insight into the characteristics and treatment of adult patients with focal spasticity treated with aboBoNT-A.

Funding: This study was funded by Ipsen.

Keywords: AbobotulinumtoxinA; Baseline characteristics; Focal spasticity; Methodology; Real-world data

Resolution of Two Steps in Botulinum Neurotoxin Serotype A1 Light Chain Localization to the Intracellular Plasma Membrane

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Introduction: The botulinum neurotoxins (BoNT) are highly potent neurotoxins produced by several *Clostridium* strains.[1,2] BoNT is an AB toxin composed of the catalytic light chain (LC) and the heavy chain (HC), which contains the translocation/receptor-binding domains.[3,4] Among the seven BoNT serotypes (A-G), BoNT/A is the most potent.[5,6] Within BoNT/A, there are nine subtypes, BoNT/A1-9, which have > 84% amino acid homology.[7,8] BoNT/A3 is functionally unique in that it has a short duration of action. Assessment of GFP-LC/A fusions showed that GFP-LC/A1 and GFP-LC/A3 localized to different intracellular compartments, membrane and cytosolic, respectively.[9] Previous research from the Barbieri laboratory found that the N-terminal 1-17 residues (N-terminus) of LC/A1 are necessary for membrane localization.[10] An amino acid sequence alignment of LC/A1 and LC/A3 showed a region of low homology spanning residues 268-357. This region of the low homology domain (LHD) possesses ~60% homology between LC/A1 and LC/A3 and is unique to LC/A3. Additionally, sequence analysis indicated that while most LC/A3 sequences had an identical N-terminus, one variant, LC/A3 NCBI #ABY56337, possessed two unique amino acid variations (Q^7P and $V^{14}G$). While re-sequencing the same strain revealed the two amino acid variations to most likely be a sequencing error, a recombinant LC/A3 containing them proved useful to our studies and was termed LC/A3V, relative to LC/A3 Loch Maree (LC/A3LM).

Methods: GFP-LC/A3-A1 chimeras tested the contribution of the N and/ or LHD for intracellular localization, where GFP-LC/A3-A1 chimeras contained either the N-terminus, the LHD, or both N-terminus and LHD of LC/A1. For steady-state imaging, GFP-LC/A3-A1 chimeras were transfected into neuro-2a cells as previously described,[11] fixed with 4% paraformaldehyde after an overnight incubation, and imaged for GFP fluorescence. Live-imaging was performed ~5 hours post transfection of GFP-LC/A3-A1 chimeras into neuro-2a cells at 10-second intervals for 10 minutes on a Nikon Eclipse Ti2 confocal microscope equipped with a W1 spinning Disc, Orca Flash CMOS camera at 60x (CFI Plan Apo λ, 1.4 NA objective).[12] To examine toxicity, full-length recombinant BoNT/A3V (rBoNT/A3V) was expressed in atoxic *C botulinum* strain Hall A-*hyper*/tox^{-}[13] and purified following the protocol used for BoNT/A1.[14] BoNT/A3V was administered intraperitoneally to groups of four female ICR mice and observed for 96 hours for signs of botulism and death.

Results: Assessing LC/A3V-A1 chimeras, steady state and live cell imaging determined contributions of the N-terminus and LHD domains for LC/A membrane localization. LC/A3V possessed a cytosolic or transient membrane association by steady state or live cell imaging, respectively. LC/A3V-A1-N-terminus and LC/A3LM had cytosolic and puncta phenotypes, indicating that the N-terminus of LC/A1 and LC/A3LM had affinity with intracellular vesicles. LC/A3V-A1-LHD had a cytosolic and plasma membrane phenotype at steady state and showed accumulation at the plasma membrane in live imaging experiments. LC/A3V-A1-N-terminus/-LHD was primarily plasma membrane–associated, possessing an intracellular phenotype indistinguishable from LC/A1.

In an ICR mouse model of botulism, BoNT/A3V was ~6-fold less potent than BoNT/A3LM and 10- to 20-fold less potent than BoNT/A1.

Conclusions: Two regions within LC/A1, the N-terminus and LHD, contribute sequentially to the plasma membrane localization observed. Additionally, intracellular localization correlates to potency, as BoNT/A3V, which is cytosolic, is ~6-fold less potent than BoNT/A3LM, which is predominantly vesicle-associated, and 10- to 20-fold less potent than BoNT/A1, which is predominantly plasma membrane–associated.

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Keywords: Botulinum neurotoxin; Botulinum neurotoxin serotype A1; Botulinum neurotoxin serotype A3; Cellular microbiology; SNAP-25; Toxins

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Morphological Evidence for the Presence of Nerve Sprouting and Stable Poly-Innervation in Human Orbicularis oculi Muscles Treated With Repeated Injections of Botulinum Neurotoxin Type A

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Botulinum neurotoxin type A (BoNT/A) has long been used for a number of pathological conditions, including movement disorders. BoNT/A, which blocks quantal acetylcholine (ACh) release at the neuromuscular junction (NMJ) resulting in transitory skeletal muscle paralysis, has shown its effectiveness in the management of benign blepharospasm, a form of focal dystonia. In patients with blepharospasm, repeated intramuscular injections of BoNT/A are performed, since slow functional recovery of neuromuscular transmission in the affected muscles occurs. Despite this limitation, BoNT/A not only safely relieves patients of their dystonia symptoms, but in addition substantially improves their quality of life. However, in a small percentage (3.6%) of patients, the BoNT/A-treatment is no longer effective in relieving blepharospasm symptoms, and it is necessary to perform upper myectomy of the orbicularis oculi muscle. In the present work, we used surgical waste muscle specimens from 14 patients, treated with repeated BoNT/A injections (abobotulinumtoxinA, Dysport®), and incobotulinumtoxinA (Xeomin®). These patients had no detectable neutralizing antibodies, and their last BoNT/A injection ranged from a few days to 120 days before the surgery. These muscle specimens were compared to specimens removed in normal, BoNT/A-naïve subjects during blepharoplasty. Morphological analyses, using confocal laser microscopy and staining with fluorescent alpha-bungarotoxin, and immunostaining with both β-III tubulin and neurofilaments, revealed that the innervation pattern of motor nerve terminals, the muscle nicotinic ACh receptors (nAChR), and the NMJs were quite different and more complex in BoNT/A-treated muscles than in control muscles. A conspicuous finding in BoNT/A-treated muscles was the stable polyneuronal innervation of muscle fibers. Indeed, multiple endplates innervated by different axons on individual muscle fibers was a prominent feature, while control muscles were consistently mono-innervated and NMJs were much simpler. It is clear that the functioning of a muscle fiber controlled by several axons that may asynchronously fire action potentials and trigger asynchronous contractions cannot be physiologically favorable. The increase in the proportion of poly-innervated muscle fibers may be related to both the stimulation of nerve sprouting (due to muscle inactivity caused by BoNT/A) and the absence of nerve terminal elimination. In addition, the presence of convergent innervation from several (3-4 motor axons) to a unique endplate was another new finding. During development, the dynamic structures composing the NMJ undergo rapid formation and elimination, and in rodents, nonessential synapses are eliminated. Interestingly, synapse elimination has also been observed in mature rodent NMJs during synaptic remodeling produced by a single BoNT/A injection. The new findings that we report raise several questions on the origin and factors contributing to the plasticity changes observed.

Keywords: Blepharospasm; Botulinum neurotoxin type A; Motor nerve sprouting; Orbicularis oculi muscle; Persistent poly-innervation; Skeletal neuromuscular junctions

IncobotulinumtoxinA Demonstrates Safety and Prolonged Duration of Effect in a Dose-Ranging Study for Glabellar Lines: Final Study Results

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Introduction: There is an increasing demand for a longer duration of effect from botulinum toxin A products. A 2-stage, phase 2, randomized, double-blind study was conducted to assess the duration of effect and safety of incobotulinumtoxinA (INCO) doses higher than the US Food and Drug Administration–approved 20 units (U) for glabellar frown lines (GFL). The stage 1 primary efficacy and safety results were reported previously. Here, we report the results of the final analysis (stage 1 and 2), including primary and secondary efficacy and safety endpoints (Kerscher et al, 2021).
Methods: A total of 241 subjects with moderate-to-severe GFL were randomized to receive a single treatment with either 20 U (N=61), 50 U (N=60), 75 U (N=61), or 100 U (N=59) INCO. The primary efficacy endpoint was duration of ≥1-point improvement from baseline assessed by investigator at maximum frown on the Facial Wrinkle Scale.
Results: The median duration of effect was 175 days for the 20 U group (95% confidence interval [CI] 142, 185), 185 days for the 50 U group (95% CI 182, 205), 210 days for the 75 U group (95% CI 182, 217), and 215 days for the 100 U group (95% CI 183, 237). The incidence of treatment-related adverse events was low across all doses, and no treatment-related serious adverse events were reported.
Conclusions: INCO doses up to 100 U were well tolerated, consistent with the known safety profile of 20 U, and increasing dose resulted in prolonged duration of effect for GFL.

Funding: This study was sponsored by Merz Aesthetics.
Keywords: Aesthetics; Clinical trial; Efficacy; Glabellar frown lines; Higher doses; IncobotulinumtoxinA

Reference

Kerscher M, Fabi S, Fischer T, et al. IncobotulinumtoxinA demonstrates safety and prolonged duration of effect in a dose-ranging study for glabellar lines. *J Drugs Dermatol.* 2021;20(10):1052-1060.

The Role of Botulinum Toxin Type A in a New Skin Quality Consensus

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Introduction: Although skin quality is a key factor in human attractiveness[1,2] and aesthetic treatment choice, there is no standardized criteria used to define it.[3] A global panel of dermatologists and aesthetic physicians reviewed relevant studies to determine parameters that define skin quality and help determine associated treatment options[4], including botulinum toxin type A (BoNT-A).
Methods: A panel of 10 dermatologists and aesthetic physicians convened to establish a consensus on skin quality criteria and treatment options. The advisory board met virtually due to the COVID-19 global pandemic and the health risks associated with face-to face meetings. The virtual nature of the consensus discussions, which spanned 6 weeks, offered a unique and independent way to capture member contributions, and conferred several advantages over a conventional, in-person meeting that typically lasts for only a few hours. For example, the advisory board members could have more in-depth discussions in the virtual format over the 6 weeks and respond to other member's inquiries at their convenience. Members also had time to more deeply consider their responses and review presentations or relevant research that they otherwise would not have been able to in a live, face-to-face meeting.
Consensus was guided by the two co-chairs of the advisory board. A modified version of the Delphi method[5] was used to arrive at consensus. Members accessed an independent online platform to review and vote on statements on skin quality criteria and treatment options. Consensus was defined as: strong consensus = greater than 95% agreement; consensus = greater than 75% to 95% agreement; majority consensus = greater than 50% to 75% agreement; no consensus = less than 50% agreement. All consensus panel members acted independently, made a significant contribution to the work reported, gave final approval, and agreed to be accountable for all aspects of the work.
Results: There was strong consensus that skin quality can be described across all ethnicities by four emergent perceptual categories (EPCs): skin tone evenness, skin surface evenness, skin firmness, and skin glow. Improving the EPCs can require a multilayered treatment strategy, and BoNT-A is a treatment option for several of the parameters, including skin surface evenness (ie, pore size, wrinkles, and scars) and glow. IncobotulinumtoxinA has a low potential for induction of immunogenicity, which makes it ideal for evolving aesthetic procedures that require larger amounts of BoNT-A[6] and intradermal injections, which may be more immunogenic than intramuscular.
Conclusions: BoNT-A is a treatment option for improving skin quality parameters, as established by consensus of a global panel of dermatologists and aesthetic physicians.
Funding: This activity was sponsored by Merz Aesthetics, Raleigh, NC, USA.
Keywords: Aesthetic treatment; Botulinum toxin type A; Consensus; Skin quality

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A Phase IV, Prospective, Observational, Multicenter Study Evaluating the Effectiveness and Safety of AbobotulinumtoxinA in Pediatric Lower Limb Spasticity (PLLS)

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Introduction: In view of the wide range of presentations and patient needs, it is becoming increasingly recognized that the treatment of spasticity, including the assessment of treatment goals, needs to be individualized. This prospective study assessed the longitudinal attainment of patient-centered, function-related goal attainment scale (GAS) T-scores after repeated abobotulinumtoxinA (abo-A) injections for ≤30 months (≤10 cycles) in the US.
Methods: Eligible patients aged 2-17 with PLLS were recruited from investigators' clinical practices. Prescription decisions were made independent of study enrollment. GAS T-scores were assessed for each injection cycle and goals could be re-defined at each injection visit; scores of ≥50 reflect goal achievement. Adverse events (AEs) were reported.
Results: Of 210 patients in the effectiveness population, 77.6% (n=163) were previously treated with a botulinum neurotoxin. Available Gross Motor Function Classification System (GMFCS) levels showed that 31.3% (n=61/195) of patients were non-ambulatory (GMFCS Level IV/V). Mean cumulated GAS T-score was 51.1 (SD±9.3). Overall, 75.2% of patients achieved their primary goals. Across all cycles, the mean number of muscles injected ranged from 5.5 (±2.9) to 7.0 (±3.7); the mean number of injection sites ranged from 8.1 (±2.7) to 9.9 (±5.7), with gastrocnemius muscle injections being most common (85.7%). Injection guidance techniques were used in

>70% of patients in Cycles 1–6; electrical stimulation was most frequently used (>50%). In the safety population (n=102/242 [42.1%]), 392 treatment-emergent AEs, which were generally mild to moderate, were reported. A total of 35 AEs (n=15/242 patients [6.2%]) were deemed treatment related.
Conclusions: Overall, goals were achieved as or better than expected in the majority of patients. Abo-A was well tolerated, with a low incidence of treatment-related AEs. These results confirm that abo-A is an effective treatment option, with a positive risk-benefit profile for PLLS.
Funding: Ipsen.
Keywords: AbobotulinumtoxinA; GAS T-score; Pediatric lower limb spasticity; Treatment goals

Real-World Botulinum Toxin Type A Treatment Patterns in Patients With Cervical Dystonia

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Introduction: Limited information is available on real-world botulinum toxin treatment patterns among patients with cervical dystonia (CD) in the United States. We describe annual injection frequency and toxin-switching rates for botulinum toxin type A in the treatment of CD.
Methods: Medical claims data, pharmacy claims data, and enrollment information from the Truven MarketScan database (TMD) and the Optum Clinformatics™ Data Mart (CDM) among adults with continuous health plan enrollment and ≥1 medical claim for CD were assessed between 2016 and 2019. Index toxin was defined as the first toxin observed on a medical claim during the study period; index date was date of first toxin with a CD diagnosis. Included patients were analyzed three ways. The "annual" and "pooled" cohorts focused on each year (2016-2019) and across years, respectively.
The "pooled" cohort allowed each patient to contribute multiple years of full enrollment with a CD-related toxin. The "treatment initiators" cohort included patients who did not have any CD-related toxin claims in the 6-month baseline period.
Results: Across the TMD and CDM datasets, the mean annual numbers of administrations across each year for all toxins ranged from 2.40 to 2.63 in 2016, 2.43 to 2.75 in 2017, 2.34 to 2.77 in 2018, and 2.39 to 3.02 in 2019. Mean annual numbers of administrations across all toxins in the pooled cohort ranged from 2.44 to 2.75 and 2.35 to 2.59 in the treatment initiators cohort. For each year (2016-2019), the percentage of patients switching from index toxin ranged from 0.8% to 1.6% for onabotulinumtoxinA, 3.2% to 9.7% for abobotulinumtoxinA, and 5.6% to 10.6% for incobotulinumtoxinA. Switch rates for onabotulinumtoxinA were lower versus the other toxins in the pooled (1.2%-2.7% vs 6.8%-13.9%) and treatment initiators (1.1%-1.6% vs 6.5%-9.2%) cohorts.
Conclusions: In the real world, injection frequency is similar across toxins in the treatment of CD with patients switching less from onabotulinumtoxinA compared to other botulinum toxin type A therapies.
Funding: This study was sponsored by AbbVie.
Keywords: Botulinum toxin type A; Drug administration schedule; Treatment frequency; Treatment switching

Real-World Botulinum Toxin Type A Treatment Patterns in Patients With Spasticity

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Introduction: Botulinum toxin is indicated for treatment of spasticity, but limited information is available on real-world botulinum toxin treatment patterns in the United States. We describe annual injection frequency and toxin-switching rates for botulinum toxin type A in the treatment of spasticity.
Methods: This study assessed medical claims data, pharmacy claims data, and enrollment information from the Truven MarketScan database (TMD) and the Optum Clinformatics™ Data Mart (CDM) among adults with continuous health plan enrollment and ≥1 medical claim for spasticity between 2016 and 2019. Index date was the date of index toxin (first toxin observed on a medical claim during the study period) with a spasticity diagnosis. Included patients were analyzed 3 ways. The "annual" and "pooled" cohorts focused on each year (2016-2019) and across years, respectively. The "pooled" cohort allowed each patient to contribute multiple years of full enrollment with a spasticity-related toxin. The "treatment initiators" cohort comprised patients who did not have any spasticity-related toxin claims in the 6-month baseline period.
Results: Across the TMD and CDM datasets, mean annual numbers of administrations across each year for all toxins ranged from 1.62-2.26 in 2016, 1.98-2.28 in 2017, 2.06-2.52 in 2018, and 2.11-2.29 in 2019. Mean annual numbers of administrations for all toxins in the pooled cohort ranged from 1.97-2.33 and 2.08-2.81 in the treatment initiators cohort. The percentage of patients switching from index toxin for each year (2016-2019) ranged from 0.8%-1.9% for onabotulinumtoxinA, 2.4%-9.0% for abobotulinumtoxinA, and 4.7%-16.1% for incobotulinumtoxinA. Switch rates for onabotulinumtoxinA compared to other botulinum toxins were 1.1%-3.2% vs 3.0%-16.4% in the pooled cohort and 1.3%-1.9% vs 2.2%-12.5% in the treatment initiators cohort.
Conclusions: In the real world, injection frequency is similar across toxins in the treatment of spasticity. Patients with spasticity switch less from onabotulinumtoxinA compared to other botulinum toxin type A therapies.
Funding: This study was sponsored by AbbVie.
Keywords: Botulinum toxin type A; Drug administration schedule; Muscle spasticity; Treatment frequency; Treatment switching

Prevalence of Spasticity-Related Pain in Children/Adolescents With Cerebral Palsy

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Introduction: Chronic pain is one of the most common complaints accompanying cerebral palsy (CP), but it is underrecognized and undertreated. This analysis determined the prevalence and intensity of spasticity-related pain (SRP) in children/adolescents (C/As) with CP using baseline data from three prospective trials in the incobotulinumtoxinA international pediatric phase 3 study program.
Methods: Baseline data from the TIM, TIMO and XARA trials were pooled. In all three studies, SRP was assessed in C/As aged 2-17 years with lower limb (LL) and/or upper limb (UL) spasticity ([Gross Motor Function Classification System Expanded and Revised [GMFCS-E&R] levels I-V]; Ashworth Scale score ≥2) using the Questionnaire on Pain caused by Spasticity

(QPS); both self-reports (direct or via interviewer) and parent/caregiver (P/C) observer reports were included. A C/A was considered to have SRP if any QPS key item score was rated >0 at baseline. Individual QPS modules were descriptively analyzed.
Results: At baseline, 331 and 155 C/As and 841 and 444 P/Cs completed at least one item of the relevant LL and UL QPS module, respectively. The presence of LL or UL SRP with at least one activity at baseline was respectively reported by 81.9% and 69.7% of C/As and observed by 85.9% and 77.7% of P/Cs. For both LL and UL SRP, intensity and frequency were higher with more demanding activities, irrespective of who completed the QPS. P/Cs indicated that SRP altered many of their children's behaviors, such as activity level, posture, mood, facial expression, eating, sleeping, and interactions with others.
Conclusion: This pooled analysis of self-reported and P/C-observed QPS data indicates that a substantial proportion of C/As with CP and LL and/or UL spasticity experience SRP, and that pain is associated with more demanding activities. The high SRP prevalence, along with its negative consequences, emphasizes the need for effective, early and long-term pain management in C/As with CP.
Funding: This research was supported by Merz Pharmaceuticals GmbH.
Keywords: Cerebral palsy; Movement disorders; Muscle spasticity; Paediatric; Pain; Prevalence

Improvements in Upper Limb Spasticity-Related Pain in Children/Adolescents With Cerebral Palsy After IncobotulinumtoxinA Injections

Florian Heinen [a,*], Michaela Bonfert [a], Petr Kanovský [b], A. Sebastian Schroeder [a], Henry G. Chambers [c], Edward Dabrowski [d], Thorin L. Geister [e], Angelika Hanschmann [e], Michael Althaus [e], Marta Banach [f], Deborah Gaebler-Spira [g]

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Introduction: This is an analysis of the effects of incobotulinumtoxinA (incoA) on upper limb (UL) spasticity-related pain (SRP) over multiple treatment cycles (ICs) in children/adolescents (C/As) with cerebral palsy (CP) using pooled data from two prospective trials in the incoA international pediatric phase 3 study program.
Methods: In the TIMO and XARA studies, C/As aged 2-17 years with CP-associated unilateral or bilateral UL spasticity received incoA for up to 4 ICs that could be adjusted for individual needs. Data from all incoA doses were combined. SRP was assessed with the Questionnaire on Pain caused by Spasticity (QPS); C/A- (direct or via interviewer) and parent/caregiver (P/C)-completed modules were used. The pain population included all C/As for whom a key QPS item score was >0 at baseline (using the 10-point graphic Wong-Baker FACES® Pain Rating Scale); post-baseline scores of 0 indicated complete pain relief.
Results: Data from 155 C/As and 388 P/Cs with data for at least one item of the respective UL QPS module were included. UL general pain was reported by 69 C/As at baseline; 39.7% and 41.8% of patients treated with incoA were pain-free by week 4 of IC1 and IC4, respectively (P<0.001 vs baseline for all ICs). C/A-reported mean UL QPS general item intensity scores improved by 1.7 and 2.2 points for patients treated with incoA at week 4 of IC1 and IC4, respectively (P<0.001 vs baseline for all ICs). P/Cs observed UL general pain in 277 C/As at baseline; 28.3% and 38.2% of patients treated with incoA were pain-free by week 4 of IC1 and IC4, respectively (P<0.001 vs baseline for all ICs). C/A-reported and P/C-observed improvements were generally greater with demanding tasks than at rest and were more pronounced with increasing incoA ICs.
Conclusion: In addition to muscle tone regulation, incoA provided sustained pain relief across multiple ICs for children with CP and UL SRP, even when they were engaged in demanding tasks.
Funding: This research was supported by Merz Pharmaceuticals GmbH.
Keywords: Botulinum toxin type A; Muscle spasticity; Movement disorders; Paediatric; Cerebral palsy; Pain

Improvements in Lower Limb Spasticity-Related Pain in Children/Adolescents With Cerebral Palsy After IncobotulinumtoxinA Injections

Florian Heinen [a,*], Michaela Bonfert [a], Petr Kanovský [b], A. Sebastian Schroeder [a], Henry G. Chambers [c], Edward Dabrowski [d], Thorin L. Geister [e], Angelika Hanschmann [e], Michael Althaus [e], Marta Banach [f], Deborah Gaebler-Spira [g]

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Introduction: This is an analysis of the effects of incobotulinumtoxinA (incoA) on lower limb (LL) spasticity-related pain (SRP) over multiple treatment cycles (ICs) in children/adolescents (C/As) with cerebral palsy (CP) using pooled data from three prospective trials in the incoA international pediatric phase 3 study program.
Methods: In the TIM, TIMO, and XARA studies, C/As aged 2-17 years with CP-associated unilateral or bilateral LL spasticity received incoA for up to 4 ICs that could be adjusted for individual needs. Data from all incoA doses were combined. SRP was assessed with the Questionnaire on Pain caused by Spasticity (QPS); C/A- (direct or via interviewer) and parent/caregiver (P/C)-completed modules were used. The pain population included all C/As for whom a key QPS item score was >0 at baseline (using the 10-point graphic Wong-Baker FACES® Pain Rating Scale); post-baseline scores of 0 indicated complete pain relief.
Results: Data from 330 C/As and 839 P/Cs with data for at least one item of the respective LL QPS module were included. LL general pain was reported by 178 C/As at baseline; 35.3% and 49.4% of patients treated with incoA were pain-free by week 4 of IC1 and IC4, respectively (P<0.001 vs baseline for all ICs). C/A-reported mean LL QPS general item intensity scores improved by 2.1 and 2.8 points for patients treated with incoA at week 4 of IC1 and IC4, respectively (P<0.001 vs baseline for all ICs). P/Cs observed LL general pain in 568 C/As at baseline; 25.2% and 34.1% of patients treated with incoA were pain-free by week 4 of IC1 and IC4, respectively (P<0.001 vs baseline for all ICs). C/A-reported and P/C-observed improvements were generally greater with demanding tasks than at rest and were more

pronounced with increasing incoA ICs.
Conclusion: In addition to muscle tone regulation, incoA provides sustained pain relief across multiple ICs for children with CP and LL SRP, even when they are engaged in demanding tasks.
Funding: This research was supported by Merz Pharmaceuticals GmbH.
Keywords: Botulinum toxin A; Cerebral palsy; Movement disorders; Muscle spasticity; Pain; Paediatric

Novel, Genetically Engineered SNAP-25 Reporter Constructs for BoNT-A and BoNT-E Toxin Activity Measurement

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Introduction: Potency testing of botulinum neurotoxin is a critical component in both research and commercial production of botulinum toxins. However, current methods used in potency testing are limited because of readout constraints. Therefore, we have designed five genetically modified versions of synaptosome-associated protein of 25 kDa (SNAP-25) for toxin activity measurement of botulinum neurotoxin type A (BoNT-A) and botulinum neurotoxin type E (BoNT-E). All constructs are suitable for expression in cell lines, cells derived from embryonic stem cells, and cells derived from inducible stem cells with potency readout by Western blotting (WB), enzyme-linked immunosorbent assay (ELISA), or apoptosis. The constructs can be used as standalone reagents or incorporated in cell systems.
Methods: Five genetically modified SNAP-25 constructs were designed in PCDNA-Sport 6 eukaryotic expression vectors and transfected into human embryonic kidney (HEK)-293 cells using Lipofectamine™ 3000 Transfection Reagent. These constructs had short DIABLO sequences of various lengths, which are exposed upon BoNT-A cleavage and thereby induce apoptosis in cells. To enable antibody detection, constructs also had N-terminal Myc and C-terminal FLAG tags. Genetically modified SNAP-25 reporter constructs were affinity-purified from the cultures 48 hours (h) post transfection. The five different constructs were evaluated for in vitro cleavage using BoNT-A and BoNT-E and analyzed with WB and ELISA. Apoptosis was evaluated (using ApoTox-Glo™ Triplex Assay) by transfecting peptides representing the BoNT-cleaved constructs into HEK-239 cells using Pierce Protein Transfection Reagent. Expression of the constructs in neurons derived from human-inducible pluripotent stem and HEK-293 cells was also investigated.
Results: For this study five different SNAP-25 constructs, designated reporter 1-5, were generated for potency determination of BoNT-A and BoNT-E. Using these we have shown that:
1) SNAP-25 reporters 1, 2, and 4 can be expressed in stem cells and continuous HEK-293 cells.
2) Cleavage of SNAP-25 reporter 1 can be detected using ELISA with antibodies directed toward the Myc and FLAG affinity tags.
3) SNAP-25 reporters 1, 2, and 4 are most effectively cleaved by BoNT-A and that SNAP-25 reporters 3 and 5 show intermediate cleavage by BoNT-A.
4) SNAP-25 reporters 1, 2, and 4 can be cleaved by several BoNT-A formulations (relabotulinumtoxinA, abobotulinumtoxinA, incobotulinumtoxinA, and onabotulinumtoxinA), as well as BoNT-E.
5) The peptide resulting from cleavage of SNAP-25 reporter 2 and 4 by BoNT-A and BoNT-E effectively induces apoptosis in cells.
6) Cleavage of SNAP-25 reporter 1 could be detected at 80 pg relabotulinumtoxinA (10% cleavage) after 22 h incubation; this limit of detection was not determined for the other BoNT/A formulations or for BoNT/E.
Conclusions: We have designed genetically modified SNAP-25 constructs that can be used as general tools for BoNT-A and BoNT-E potency determination in standalone assays or incorporated into cell systems.
Funding: This project is fully funded by Galderma.
Keywords: Apoptosis; BoNT-A; BoNT-E; ELISA; Reporter constructs; SNAP-25

Clinical Immunogenicity of DaxibotulinumtoxinA for Injection in Glabellar Lines Including Subjects With Multiple Exposures: Pooled Data From the SAKURA Phase 3 Trials

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Introduction: DaxibotulinumtoxinA for Injection (DAXI) is a novel botulinum toxin type A (BoNTA) product containing purified 150-kD core neurotoxin (daxibotulinumtoxinA) in addition to a proprietary stabilizing peptide (RTP004) and other excipients. As with any new biologic product, DAXI has the potential to be immunogenic and elicit antibody formation. Therefore, investigation of DAXI's immunogenic potential is an important component in the overall clinical safety assessment, including the responses after repeated drug administration.
Methods: The presence of neutralizing antibodies to daxibotulinumtoxinA and binding antibodies to daxibotulinumtoxinA or RTP004 was assessed in adults enrolled in the phase 3 glabellar line clinical program, comprising two double-blind, placebo-controlled, single-dose studies (SAKURA 1 and 2) and an open-label safety study (SAKURA 3) of up to three repeat treatments of 40 U DAXI. Binding antibodies were detected by a validated direct-binding enzyme-linked immunosorbent assay (ELISA). The testing paradigm consisted of the industry standard multi-tiered approach and included screening, confirmatory assay, and titration assay. Samples from subjects testing positive for daxibotulinumtoxinA-binding antibodies were further evaluated for the presence of neutralizing antibodies in the mouse protection assay. The effect of anti–drug-binding antibodies on treatment response and duration of clinical benefit was assessed. Safety was evaluated with respect to the occurrence of immune-related adverse events.
Results: Overall, 2786 subjects received DAXI (n=882, $\geq$ 2 treatments; n=568, 3 treatments) and 2823 subjects were exposed to RTP004 as DAXI (n=2786) or placebo (n=203) (n=914, $\geq$ 2 exposures; n=702, 3 exposures). Of these, 2737 and 2772 had both evaluable pre- and post-treatment samples for binding antibodies to daxibotulinumtoxinA and RTP004, respectively. At baseline, 12 of 2737 (0.4%) subjects were found to have binding antibodies to BoNTA and 66 of 2772 subjects (2.4%) had detectable antibody titers to RTP004. Treatment-emergent anti–daxibotulinumtoxinA-binding antibodies were detected in at least one study sample in 20 subjects (0.7%), and one subject (<0.1%) with binding antibodies at baseline demonstrated an increased titer. No subject developed neutralizing antibodies to daxibotulinumtoxinA. Treatment-induced anti–RTP004-binding antibodies

were detected in 35 (1.3%) subjects. No subjects had treatment-boosted anti–RTP004-binding antibodies. In the majority of subjects, the binding antibodies were transient and not present in the final sample. No subject had binding antibodies to both daxibotulinumtoxinA and RTP004. All subjects with treatment-induced binding antibodies to daxibotulinumtoxinA or RTP004 achieved a response of none or mild glabellar line severity at Week 4 following each DAXI treatment cycle, and duration of clinical response was not different in treatment cycles when antibodies were detected compared with those in which no antibodies were recorded. No subjects with binding antibodies to daxibotulinumtoxinA or RTP004 reported any immune-related adverse events.

Conclusions: This is the first large-scale analysis of the risk of antibody formation to DAXI. No subjects developed neutralizing antibodies to daxibotulinumtoxinA. Results from this study suggest that DAXI administration results in a low incidence of antibody formation. For the small percentage of subjects who developed transient binding antibodies to daxibotulinumtoxinA or RTP004, these were found to not impact clinical efficacy, safety, or duration of action.

Funding: The study was funded by Revance Therapeutics, Inc.

Keywords: Anti-drug antibodies; Botulinum toxin type A; DaxibotulinumtoxinA; Glabellar lines; Immunogenicity

Treatment of Cervical Dystonia Using Shorter IncobotulinumtoxinA Injection Intervals Improves Patient-Reported Outcomes in Those With Inadequate Benefits From Standard Intervals

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Introduction: There is individual variation in the reported waning of botulinum toxin (BoNT) treatment benefit in patients with cervical dystonia (CD), even among patients who experience a favorable peak response. Thus, some patients prefer injection intervals shorter than the standard 12 weeks. This study assesses whether individualized treatment intervals can lead to improved patient experience without compromising safety. The objective was to assess the impact of 2 different injection schedules of incobotulinumtoxinA on patient-reported assessments in CD.

Methods: An open-label, randomized, phase IV study (CD Flex; NCT01486264) was designed to compare 2 incobotulinumtoxinA injection intervals (short-flex: 8±2 weeks [N=142]; long-flex: 14±2 weeks [N=140]) in BoNT-responsive subjects with CD who report typical waning of clinical benefit at <10 weeks. Subjects received 8 injections over a period of up to 2 years. Patient-reported outcomes (4 weeks post-injection 8) included satisfaction (10-point scale), patient-reported global response (9-point Likert scale), and the CD impact profile (CDIP-58). Additional endpoints included a physician-assessed global response and a clinical global impression of severity.

Results: Subject satisfaction was significantly improved vs study baseline over 8 cycles in the short-flex group (mean change=1.2 points, P=0.0007), but not in the long-flex group. A significant improvement was also observed in the short-flex group in the physician-assessed global impression of severity 4 weeks after injection 8. Most domains of the CDIP-58 analysis (pain/discomfort, sleep, annoyance) demonstrated numerical trends favoring the short-flex group. At 4 weeks post-injection 8, a similar distribution of scores was observed for both groups on the subject- and physician-rated global response assessments with no relevant difference between groups. No differences in safety profile were noted.

Conclusions: Subjects with shorter incobotulinumtoxinA injection intervals reported improved satisfaction after 8 injections. Trends favoring short-flex were observed in both the CDIP-58 analysis and physician-rated clinical global impression of severity. Evidence suggests that individualizing injection intervals to treat CD may improve patient-reported outcomes without compromising safety.

Funding: This study was sponsored by Merz Pharmaceuticals, LLC.

Keywords: Botulinum toxin; Cervical dystonia; IncobotulinumtoxinA; Movement disorders

Factors Associated With Favorable Response in Real-World Use of Botulinum Toxin Type A Products for Adult Patients With Upper Limb Spasticity

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Introduction: Clinical trials have shown that botulinum toxin type A (BoNT-A) can enable achievement of treatment goals related to pain management, involuntary movements, range of motion, passive function, and active function or mobility in people with upper limb spasticity (ULS). However, real-world data are limited. This analysis aimed to examine factors associated with a favorable response (including the agent used), as well as outcomes associated with treatment response (including quality of life), in a real-world setting.

Methods: ULIS III (NCT02454803) was an international, multicenter, non-interventional, prospective, longitudinal (2-year) study of adult patients with ULS treated with abobotulinumtoxinA (aboBoNT-A), onabotulinumtoxinA (onaBoNT-A), or incobotulinumtoxinA (incoBoNT-A). Full study description and primary findings are presented elsewhere (Turner-Stokes et al, 2021). In the current analysis of ULIS III data, patients were excluded from the analysis if they changed BoNT-A formulation during follow up. Response was defined as a ≥10-point increase in the cumulated Goal Attainment Scaling (GAS) T-score vs baseline. Patient quality of life was assessed with the EQ-5D scale (US and Australia only). Multivariate logistic regression analysis of response was used to evaluate the impact of baseline characteristics, administration method, dosing, and use of rehabilitation therapy.

Results: Overall, 828 patients provided cumulated GAS-T-score data during the study (555 in the aboBoNT-A, 196 in the onaBoNT-A, and 77 in the incoBoNT-A groups). In the multivariate analysis, prognostic factors for response were: use of injection guidance techniques, patient's sex, and

type of BoNT-A preparation used. AboBoNT-A response rates across treatment cycles were significantly higher than onaBoNT-A ones (P<.001), but no statistically significant difference was found between aboBoNT-A and incoBoNT-A (P=0.864). Patients for whom injection guidance techniques were used for ≥75% of injections and female patients were more likely to achieve a response. At the last visit, mean (standard deviation) change in EQ-5D index from baseline was 0.05 (0.22) for responders and 0.00 (0.21) for non-responders. All responders had a trend to be less frequent users of antispasticity medications, including baclofen (30.8% vs 39.9%), pain medication (eg, gabapentin, pregabalin, amitriptyline; 9.1% vs 11.6%), opioids (2.2% vs 3.4%), neurolytic agents (0.3% vs 1.3%) during the study period, compared to treatment non-responders.

Conclusions: These real-life data suggest that aboBoNT-A is an effective treatment choice for ULS patients. AboBoNT-A was associated with a higher responder rate in this study, which could be linked to improved quality of life and less need for concomitant medications.

Funding: This study was sponsored by Ipsen.

Keywords: Botulinum neurotoxin type A; Cost-effectiveness analysis; Quality of life; Spasticity costs

Reference

Turner-Stokes L, Jacinto J, Fheodoroff K, et al and the Upper Limb International Spasticity (ULIS-III) study group. Longitudinal goal attainment with integrated upper limb spasticity management including repeat injections of botulinum toxin A: Findings from the prospective, observational Upper Limb International Spasticity (ULIS-III) cohort study. *J Rehabil Med.* 2021;53(2):jrm00157.

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: The objective of this study was to evaluate the efficacy and safety of DaxibotulinumtoxinA for Injection (DAXI) vs placebo for cervical dystonia (CD). DAXI is a novel botulinum toxin type A formulation with a proprietary peptide excipient. We report results of a multicenter, Phase 3 double-blind, placebo-controlled trial.

Methods: Adults with moderate-to-severe CD, randomized 1:3:3 (placebo, DAXI 125 U, DAXI 250 U), were followed for ≤36 weeks post-treatment. The primary endpoint was change from baseline (CFB) in Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) score averaged at Weeks 4 and 6. Safety was also evaluated.

Results: Three hundred one subjects were randomized; placebo (n=46), DAXI 125 U (n=125), DAXI 250 U (n=130). Demographics were similar across cohorts. Mean±SE TWSTRS CFB at the primary timepoint was -4.3±1.8 placebo, -12.7±1.3 DAXI 125 U (P<0.0001 vs placebo), and -10.9±1.2 DAXI 250 U (P=0.0006 vs placebo). DAXI dose groups did not statistically differ. TWSTRS subscales showed similar improvement: 30-33%, 25-26%, and 11-12%, respectively, for DAXI 125 U, 250 U, and placebo. Clinician Global Impression of Change (CGIC) and Patient Global Impression of Change (PGIC) demonstrated improvement (a little to very much better) with DAXI consistent with the primary endpoint (CGIC, 77-78% DAXI vs 46% placebo; PGIC, 71-73% DAXI vs 41% placebo). Most DAXI-treated subjects were somewhat satisfied to very satisfied at Week 4 (DAXI 125 U 69.6%; DAXI 250 U 62.3%) and Week 6 (DAXI 125 U 68.8%; DAXI 250 U 66.2%), consistent with the primary endpoint. Median duration of effect was 24.0 and 20.3 weeks for DAXI 125 U and DAXI 250 U, respectively, defined as time to loss of 80% peak treatment benefit.

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 with DAXI 125 U and DAXI 250 U, respectively.

Conclusions: Treatment with DAXI was safe and efficacious with a meaningful reduction in CD symptoms, high subject satisfaction, and median duration of effect of 20.3-24.0 weeks.

Funding: The study was funded by Revance Therapeutics, Inc.

Keywords: Botulinum toxin type A; Cervical dystonia; DaxibotulinumtoxinA; Subject satisfaction; Treatment

IncobotulinumtoxinA for Refractory Neuropathic Pain Following Breast Cancer Surgery

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Introduction: Recently published French guidelines recommend botulinum toxin type A (BoNT/A) as a second-line treatment for peripheral neuropathic pain (PNP).[1] Persistent PNP after breast surgery is a real challenge.[2-4] We report the efficacy of BoNT/A injected around the pectoral nerves to treat refractory PNP following breast cancer surgery.

Method: This retrospective cohort study included women presenting with severe chronic neuropathic pain (rated on the numerical rating scale [NRS] at rest >6/10) following breast cancer surgery. All patients were treated with second-line therapies, including capsaicin patches and BoNT/A injected around the pectoral nerves using ultrasound guidance.

Results: Four patients were included and received 50 UI BoNT/A. After 3 months pain was reduced by >70 % for 3 patients. Two patients needed a second injection that induced >80% reduction of pain. No adverse event was noted at 9 months.

Conclusion: This retrospective, open-label case series suggests the need for further clinical research in the management of PNP refractory to current treatment following breast surgery.

Keywords: Efficacy; IncobotulinumtoxinA; Postsurgical neuropathic pain; Retrospective study

Table
Patient characteristics and previous treatments

Patient	Age (years [yrs])	Oncologic Treatment	First-Line Pain Treatment	Second-Line Pain Treatment	Duration and Intensity of Pain
1	52	Quadrantectomy Axillary lymphadenectomy Radiotherapy Letrozole	Opioids Bromazepam Acetaminophen	Amitriptyline Pregabalin Capsaicin patches (2)	4 yrs DN4: 6/10 NRS: 6/10 Allodynia +++
2	69	Mastectomy and node picking	Amitriptyline Bromazepam Acetaminophen NSAIDs	Amitriptyline Capsaicin patches (2)	2 yrs DN4: 6/10 NRS: 6/10 Allodynia +
3	78	Mastectomy Axillary lymphadenectomy Radiotherapy Letrozole	Opioids Pregabalin Acetaminophen	Capsaicin patches (3)	7 yrs DN4: 7/10 NRS: 6/10 Allodynia +
4	39	Mastectomy and node picking	Pregabalin Nefopam NSAIDs	Amitriptyline Capsaicin patches (4)	3 yrs DN4: 5/10 NRS:5.5/10 Allodynia +

Capsaicin patches (number); DN4 questionnaire for neuropathic pain.

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Current Perspectives on the Management of Cervical Dystonia Among Global Clinicians

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Introduction: Botulinum toxin is considered first-line treatment for cervical dystonia (CD); however, there are still unmet needs in CD management. This study aims to gain the perspectives of clinicians who treat patients with CD regarding assessment and treatment goals, disease awareness, intervention, and follow up.
Methods: Three online surveys (Survey Monkey®) each consisting of about 25 clinician- and patient-related questions were circulated to neurology, movement disorders, and physiatry clinicians (Survey 1 [n=29]; Survey 2 [n=51]; Survey 3 [n=21]). Individual survey results were collated, and overall percentage scores calculated.
Results: Seventy-six percent (22/29) of surveyed clinicians considered the Toronto Western Spasmodic Torticollis Rating Scale most appropriate for assessing CD. Most clinicians (27/29;93%) set treatment goals and reported that their patients expected them to be met within 3 months. Patients were most likely to have been symptomatic for 1-2 years prior to referral; delayed referrals were largely due to poor recognition of early symptoms. Respondents felt general practitioners and neurologists would benefit most from additional information on CD. Most respondents agreed that patient awareness could be raised via social media (38/47;81%), and diagnosis expedited via patient advocacy groups (41/47;87%). Most respondents (20/21;95%) reported using botulinum toxin (predominantly onabotulinumtoxinA) as first-line therapy, often with other therapies/procedures. The majority of respondents regarded 3 treatment cycles as necessary to optimize treatment outcomes (13/17;76%) and reported low discontinuation rates of 0-20% (12/17;71%).
Conclusions: Survey results suggest the need to address educational gaps to increase patient awareness and clinicians' understanding of diagnostic criteria. The results also suggest that an optimal long-term treatment strategy requires patient and clinician agreement on at least 3 treatment cycles to assess safety and efficacy, which may lessen early discontinuation.

Keywords: Botulinum toxin; Cervical dystonia; Disease awareness; Treatment strategy
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HAJ has no conflicts of interest to disclose.
SS has no conflicts of interest to disclose.
PF has served as a speaker for Allergan, an AbbVie company.

The First FDA-Approved Batches of Botox®

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This paper is dedicated to Edward J. Schantz (1908-2005).
Botulinum toxin (BTX) has become an important pharmaceutical that is used worldwide for the clinical treatment of a wide variety of neurological disorders. In contrast to the extensive knowledge of clinical development of BTX by Alan B. Scott and other physicians, much less is known about the manufacture and validation of BTX for human use. The manufacture and quality validation of the first FDA-approved batches of BTX (79-11 and 88-4) were performed at the Food Research Institute/University of Wisconsin-Madison in the 1970's and early 80's by Ed Schantz and subsequently (1985 to mid-1990's) on a daily basis with Eric Johnson. The first clinical batches were prepared primarily by methods developed by Ed Schantz and others at Camp Detrick during World War II, using a special *Clostridium botulinum* fermentation strain from Harvard. The crude fermentation batches of BTX were purified by non-chromatographic methods and validated for essential properties including purity, protein subunit composition, and specific toxicity. These criteria for high-quality BTX were accepted by the FDA, which allowed advance to the clinic. Initial batches (79-11 and 88-4) together with Alan Scott's clinical studies resulted in the approval of BTX on December 31, 1989, for clinical applications. These initial batches were used for 6-8 years until newer batches were prepared and approved by the FDA. These initial approved batches (79-11 and 88-4) were later acquired by Allergan and named Botox® in 1992.

Physicians' Use of Perioperative Botulinum Toxin Injections in Treatment of Spastic Limbs: A Cross-Sectional National Survey

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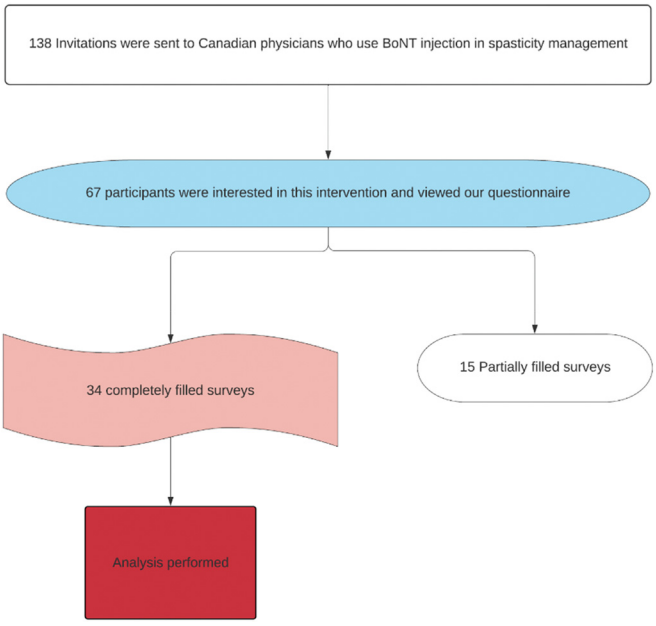
Objective: To investigate the practice patterns of Canadian physicians who use perioperative botulinum toxin (BoNT) injections to improve outcomes of surgeries performed on spastic limbs.
Design: A cross-sectional national survey composed of an invitation email and an 18-item questionnaire was disseminated by the national Physical Medicine and Rehabilitation (PM&R) society to 138 physician members involved in spasticity management.
Participants: Thirty-four physicians (25%) fully completed the survey.
Main Outcome Measures: Participants completed an online questionnaire that examined the practice patterns and surgical outcomes associated with perioperative BoNT injections.
Results: The majority (n= 21, 84%) of Canadian physicians who inject BoNT perioperatively to improve outcomes of surgeries performed on spastic limbs are specialists in PM&R practicing in academic settings. Most respondents (74%) used BoNT injections for perioperative treatment for patients with limb spasticity undergoing surgery. Sixty-five percent of physicians used BoNT preoperatively, 21% intraoperatively, and 24% postoperatively. Of the physicians who performed BoNT injections preoperatively, 6% performed BoNT injections 7-12 weeks preoperatively, 32% performed BoNT injections 4-6 weeks preoperatively, 47% performed BoNT injections 2-3 weeks preoperatively, and 15% performed BoNT injections 0-1 week preoperatively. A majority (85%) of physicians responded that injecting BoNT perioperatively may improve a patient's surgical outcome and all (100%) of the participants stated that BoNT did not contribute to any perioperative complications or adverse effects. Qualitative responses emphasized that successful outcomes from the perioperative BoNT were linked to enhanced collaboration with surgeons and that more research is needed to determine the optimal timing of perioperative BoNT.
Conclusion: Canadian physicians—mostly PM&R specialists—administer perioperative BoNT to improve outcomes of surgeries performed on spastic limbs. The optimal timing for perioperative BoNT was suggested to be 2-3 weeks before the surgery by 47% of survey respondents. All participating physicians responded that perioperative BoNT did not contribute to any known perioperative complications or adverse events. This study highlights the importance of conducting more robust research to better understand the optimal timing for perioperative BoNT injection, enhance collaboration between physicians and surgeons, and increase awareness of perioperative BoNT when planning for surgeries on spastic limbs.
Funding: This study did not receive any grants or funding from public, commercial, or not-for-profit sources.
Keywords: BoNT; Botulinum toxin; Perioperative; Spasticity; Surgical outcomes



Flowchart.

Table 1
Demographics of Participants and Intervention Outcomes

Physician Demographics	n (%)	Intervention Demographics	n (%)
Specialty		**BoNT use (# of years)**	
PM&R	31 (91%)	0-9 years	11 (32%)
Orthopedic Surgery	2 (6%)	10-19 years	16 (47%)
Plastic Surgery	1 (3%)	20-29 years	6 (18%)
		>30 years	1 (3%)
Province		**BoNT conditions treated**	
British Columbia	15 (44%)	Stroke	30 (88%)
Ontario	10 (29%)	Traumatic brain injury	30 (88%)
Quebec	5 (15%)	Cerebral palsy	30 (88%)
Alberta	1 (3%)	Multiple sclerosis	30 (88%)
Saskatchewan	1 (3%)	Spinal cord injury	31 (91%)
Manitoba	1 (3%)		
New Brunswick	1 (3%)		
		Perioperative BoNT use	
		0-4 years	7 (20.5%)
		5-9 years	5 (15%)
		10-14 years	4 (12%)
		15-19 years	8 (23.5%)
		>20 years	1 (3%)
		Do not use	9 (26%)

Table 2
Perioperative Use of BoNT

	n (%)		n (%)
Perioperative use	25 (74%)	**BoNT Formulations**	
Preoperative	22 (65%)	OnabotulinumtoxinA	33 (97%)
Intraoperative	7 (21%)	IncobotulinumtoxinA	24 (71%)
Postoperative	8 (24%)	AbobotulinumtoxinA	18 (53%)
Preoperative use		**Setting of Perioperative use**	
0-1 weeks pre-op	5 (15%)	Academic Hospital	24 (71%)
2-3 weeks pre-op	16 (47%)	Non-Academic Hospital	4 (12%)
4-6 weeks pre-op	11 (32%)	Community/Private Practice	6 (18%)
7-12 weeks pre-op	2 (6%)		
Not notified of date	14 (41%)		
Perioperative surgical outcomes		**Perioperative adverse effects**	
Improves	29 (85%)	No adverse effects	34 (100%)
Does not improve	3 (9%)		
Unanswered	1 (3%)		
Perioperative barriers to treatment		**BoNT Guidance Techniques**	
Time constraints	13 (38%)	Electromyography	24 (71%)
Lack of evidence	6 (18%)	Electrical stimulation	30 (88%)
		Ultrasound	21 (62%)
		CT fluoroscopic guidance	3 (9%)
Colleague perioperative treatment			
Similar	0 (0%)		
Differs	19 (56%)		
Unsure	14 (41%)		
Unanswered	1 (3%)		

Table 3
Themes and Responses

Tips and pearls of wisdom for using perioperative BoNT injections
"Relationships with surgeon. Teach your surgical colleagues why it is helpful"
"Discuss with surgeon what is being done and what treatment options are available. Work with surgeons who have expertise in neurologic patients"
"Optimal timing can be difficult to coordinate with patient/surgeon/clinical time"
"Appears to reduce postoperative pain particularly in the leg with standing"
How/why did you start injecting BoNT into spastic limbs perioperatively?
"Excellent relationship with surgeons"
"Asked by surgeon colleague to do it"
"Clinical encounter—surgeon initiated the question"
"To help with pain and spasticity management"
What improved surgical outcomes have you noticed with BoNT injections?
"Improved pain control. Decreased tendon rupture"
"Improved pain control, better positioning for casting and bracing"
"Ease of post-op positioning, immobilization and healing of fractures"

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Real-World Cost and Utilization Analysis of Botulinum Toxin Agents in Blepharospasm: A National Retrospective Cohort Study

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Introduction: We conducted a retrospective cohort study using a US claims database to assess real-world costs, dosing, and wastage of incobotulinumtoxinA (INCO) and onabotulinumtoxinA (ONA) for the treatment of blepharospasm.
Methods: Three full calendar years (2018-2020) of data from the IQVIA Health Plan Claims Database were used for this real-world cost and utilization analysis. Outpatient claims were identified for inclusion based on a Current Procedural Terminology (CPT) code of 64642, an ICD-10 diagnosis code of G24.5 (blepharospasm), and a J-code of J0585 (ONA) or J0588 (INCO). The primary outcome of the study was cost per claim for INCO and ONA, respectively. Dose per claim, wastage per claim, and cost per patient per year were assessed as secondary endpoints. For the cost per year analysis, data were included from adult patients who were diagnosed with blepharospasm and who received ≥2 injections of ONA or INCO spanning a ≥12-month period. Limitations of this retrospective analysis include but are not limited to non-interchangeability of toxins, limited data, as well as no analysis of available discounts or other price reductions.
Results: A total of 46,540 claims (40,661 ONA and 5,879 INCO) met criteria for inclusion in the claims cost analysis. Most of the claims were for female patients (70.5%) and submitted by ophthalmologists (70.0%). The mean cost per claim was $601 for ONA versus $404 for INCO ($P<0.001$). The mean dose per claim was 48.0 units for ONA and 46.5 units for INCO ($P<0.001$), which translated to a mean dose ratio of 0.97 to 1.00 for INCO to ONA. The mean wastage per claim was 71% higher for ONA than for INCO (55.0 units versus 32.1 units; $P<0.001$). A total of 5,285 adult patients (4,501 ONA and 784 INCO) met criteria for inclusion in the cost per year analysis. The mean cost per patient per year was significantly higher for ONA than for INCO ($1,765 versus $976; $P<0.001$).
Conclusions: These real-world claims data demonstrate that INCO and ONA were utilized with a dosing ratio of 0.97 to 1.0, consistent with available literature from active comparator studies that demonstrate similar safety and efficacy at comparable doses for treatment of adults with blepharospasm or cervical dystonia. However, significant cost savings for payers can be realized through the use of INCO for blepharospasm patients, which demonstrates a lower cost per claim and unique opportunities to minimize wastage.
Funding: Dr. Kazerooni was an employee of Merz Pharmaceuticals, LLC, at the time of the analysis; Dr. Rootman, Dr. Goldberg, and Dr. Cohen received no compensation or funding for this research project.
Keywords: Blepharospasm; Cost; Dosing; IncobotulinumtoxinA; OnabotulinumtoxinA; Wastage

Disparities in Access to Spasticity Chemodenervation Specialists in the United States: A National Analysis of Medicare Data

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Introduction: The objective was to explore the variations in access to spasticity chemodenervation specialists across several geographical, ethnic, racial, and population density factors.
Methods: This was a retrospective analysis on 2017 Medicare Provider Utilization and Payment Data: Physician and Other Supplier dataset from the Center for Medicare & Medicaid Services, which includes data on 34 million fee-for-service beneficiaries. The primary outcome was Medicare beneficiaries per provider. Providers with substantial adult spasticity chemodenervation practices (SASCPs) were included (defined as having ≥11 unique Medicare patients with claims for Current Procedural Terminology codes related to chemodenervation for spasticity). Ratios were assessed across census-defined geographical regions as well as hospital referral regions (HRRs). Urban was defined as being part of a metropolitan statistical area with population ≥ 500,000.
A multivariate linear regression model for the top 100 HRRs by beneficiary population was created, using backward stepwise selection to eliminate variables with P values > 0.10 from final model.
Results: There was a total of 566 providers with SASCPs, the majority of which were neurologists or physiatrists (546/566; 96.5%). Unadjusted results showed lower access in non-urban versus urban areas in the form of higher patient:provider ratios (83,106 vs 51,897). Access ratios were also lower in areas with ≥25% Hispanic populations (141,800 vs 58,600). Multivariate linear regression results showed similar findings with urban HRRs having significantly lower ratios (-45,764 [P=0.004] versus non-urban) and areas with ≥25% Hispanic populations having significantly higher ratios (+96,249 [P=0.003] vs <25% Hispanic areas). Factors such as proportion of population that was white, black, or on Medicaid was not found to be predictive.
Conclusions: This study found that patients in non-urban and highly Hispanic communities face inequities in access to chemodenervation specialists. Future studies should venture to confirm whether findings are limited to this specialization or are part of a larger issue in access to healthcare specialty services. Additionally, strategies to improve access for these underserved communities should be explored.
Funding/Disclosures: This research received no external funding. Dr. Kazerooni was an employee of Merz Pharmaceuticals, LLC, at the time of the analysis. Mr. Healy and Dr. Verduzco-Gutierrez received no compensation or funding for this research project. Dr. Verduzco-Gutierrez has been a consultant for Merz Pharmaceuticals, LLC, Allergan Inc, Ipsen Biopharmaceuticals Inc, Medtronic USA Inc, and Piramal.
Keywords: Access; IncobotulinumtoxinA; Medicare; Spasticity

Effectiveness of Early AbobotulinumtoxinA Injections in Patients With Upper Limb Spastic Paresis After Traumatic Brain Injury in Real Clinical Practice: Results of a Multicenter Observational, Non-Interventional Prospective Study (Adults with post Traumatic Brain Injury (TBI) upper limb (UL) spasticity – APTULS)

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Introduction: About 80% of traumatic brain injury (TBI) patients develop movement disorders. One of the most frequent manifestations of motor disorders is spastic paresis of the upper extremity (UE). In spastic paresis, spasticity is the most disabling factor, causing loss of limb function, development of contractures, pain, and disability. Treatment of spasticity is effective in the early period following TBI, but there are few studies on the use of botulinum neurotoxin therapy during this period.
Objective: To evaluate the effectiveness of abobotulinumtoxinA

(Dysport®; Abo-BTA) injections in reducing UE spasticity in the early post-TBI period in real clinical practice.
Methods: The study included 44 patients (79.55% men and 20.45% women). The mean age was 37.7±12.79 years with UE spasticity that developed no later than 12 weeks after TBI. The treatment protocol included guided injections of Abo-BTA into the UE muscles in accordance with the drug label. The average total dose of Abo-BTA injected into the UE muscles was 990.34± 95.12 units. Follow up was done 3-6 months post-treatment. Treatment efficacy was assessed by changes in muscle tone according to the Modified Ashworth Scale (MAS); measurement of spasticity according to the Modified Tardieu Scale (MTS); and the volume of UE active movements using goniometry.
Results: In the 3-6 months' posttreatment period, most patients showed a significant decrease in muscle tone on the MAS; a reduction in the spasticity angle according to the MTS; and an increase in the range of active movements in the shoulder, elbow, and wrist joints of the paretic UE ($P<0.05$). A moderately strong correlation was found between the degree of sensory impairment and the severity of spasticity in the UE elbow joint according to the MAS (the degree of spasticity varied by 15.79%). A total of 29.5% of patients developed non-serious adverse events, which is a rate similar to the data indicated in the drug label.
Conclusions: Abo-BTA is effective and safe for the treatment of UE spasticity in the early period after TBI.
Keywords: AbobotulinumtoxinA; Dysport®; Muscle tone; Spasticity; Upper extremity

Reference

Khatkova SE, Diagileva VP, Krylova LV, Pokhabov DV, Arutiunian AG. Effectiveness of early abobotulinum toxin (Dysport®) injections in patients with upper limb spastic paresis after traumatic brain injury in real clinical practice: Results of a multicenter observational non-interventional prospective study (Adults with post Traumatic Brain Injury (TBI) upper limb (UL) spasticity – APTULS). *Neurol Neuropsychiatr Psychosom.* 2021;13(5):48-55. In Russian.

Speech-Induced Cervical Dystonia

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Introduction: Action-specific focal dystonias[1] affect an isolated body part and are triggered by a specific action. Here we report a young female patient with a rare form of action (task)-specific cervical dystonia induced by speaking.
Methods: Case report and literature review.
Results: A female patient without past medical problems developed complaints at the age of 19 when she had progressive retrocollic posture spasms when speaking. She was first referred for psychiatric evaluation, and escitalopram was initiated without effect. She was referred to our department after one year of complaints. MRI of the head and full metabolic test panel were negative. She was treated with injections of incobotulinumtoxinA (total dose: 200 U) into the bilateral splenius capitis (using ultrasound guidance) and sternocleidomastoid muscles, resulting in almost complete recovery for 2 months. She had 5 treatment sessions with increasing re-injection intervals to 5 months when her treatment was stopped with tolerable symptoms due to her plan to get pregnant.
Conclusions: Speech-induced cervical dystonia is a rare condition, with only two published idiopathic patients[2,3] and one tardive dyskinesia patient[4] in the literature. Our patient had a unique course with regression of symptoms following repeat incobotulinumtoxinA injections.
Keywords: Action (task)-specific dystonia; IncobotulinumtoxinA; Speech-induced cervical dystonia

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Botulinum Toxin Treatment of Refractory Joint Pain Syndrome in Osteoarthritis

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Introduction: Osteoarthritis (OA) affects more than 302 million people worldwide.[1] In the United States, more than 22.7 million people report mobility limitations associated with arthritis.[2] Older and/or overweight people and postmenopausal women are more likely to suffer from the disease. As life expectancy increases, the number of people with severe OA is expected to increase. The most common complaint is pain and stiffness in the affected joint. There is no unified approach to joint pain management presented in the clinical guidelines, and treatment techniques have different efficacy ratings.[3-6]
Systemic NSAIDs and intra-articular glucocorticosteroid injections have a high risk of adverse reactions or are contraindicated due to severe comorbidities in elderly patients. Comorbidity in elderly patients with OA may be a contraindication to joint replacement surgery, which raises the need for palliative care. Botulinum toxin type A (BoNT-A) formulations have been used for a long time in the treatment of many diseases. The safety and efficacy of BoNT-A has been confirmed by many randomized studies.[7,8] BoNT-A formulations do not have a pronounced systemic effect and can significantly reduce pain when injected into the joint affected by OA.[8-13]
Methods: We studied a group of 20 patients (14 women, 6 men) aged 45 to 81 years with knee joint lesions of varying severities (stages I-III). At baseline visit all patients were interviewed, assessed using a visual analog scale (VAS) for pain severity, and had clinical examination with goniometry and radiography of the joint performed. Most patients were contraindicated for arthroplasty because of somatic diseases (11 patients), and 9 patients refused surgery for personal reasons. Pain syndrome was assessed by patients at 4 to 8 on the VAS. All patients received intra-articular injections of 200 U per joint of BoNT-A (incobotulinumtoxinA) under ultrasound guidance, and 100 U were distributed among the muscles actively involved in contracture formation. On day 21, after intra-articular injection, patients were scheduled for a follow-up visit, at which the primary examination algorithm was repeated.
Results: At the end of treatment (day 21), 15 patients had a pronounced and stable reduction in pain (pain score 0-2 on the VAS). Four patients had non-significant pain reduction (pain score of 3-4 on the VAS). One patient had no positive dynamics. Nineteen patients experienced an increase of walking endurance, more movement in the affected joints, and improved quality of life. During the treatment period, two patients noted local reactions to the injection (local hyperemia), which resolved on its own; no other adverse events were recorded.
Conclusions: Intra-articular injections of incobotulinumtoxinA are effective and safe for relieving pain syndrome in OA. BoNT-A can be used in

patients with a severe comorbid background since it does not negatively affect the accompanying somatic pathology.
Keywords: IncobotulinumtoxinA; Intra-articular injection; Joint pain; Osteoarthritis

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Treatment of Muscle-Tonic and Myofascial Syndromes in Patients With Lower Limb Length Mismatch With Botulinum Toxin Type A

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Introduction: Secondary changes in the musculoskeletal system are often associated with a number of musculoskeletal defects: pathology in various parts of the spine and large joints of the lower extremities; degenerative-dystrophic diseases of the spine; upper-lower jaw joint deficiency; and lower limb length mismatch (LLLM). LLLM often results in asymmetry of the paired skeletal muscles, which is the trigger for muscle-tonic syndrome (MTS), myofascial syndrome (MFS), and, in the future, dystonia.
We aimed to confirm the presence of MTS and MFS in patients with LLLM and create an algorithm for the examination of these patients.
Methods: We examined a group of patients with MTS (70 men and 80 women aged 18 to 60 years) and MFS. All patients underwent orthopedic examination with anthropometric measurements and a 3D scan (three-plane pose examination) if LLLM was diagnosed. Superficial electromyographic (EMG) examination was performed to detect tone asymmetry in the paired muscles. All patients underwent LLLM correction with custom insoles (soft frame orthoses) with compensation under the control of a 3D scanner. Pain syndrome was assessed using a visual analogue scale (VAS). Botulinum toxin (BoNT) therapy was administered for tone asymmetry correction and pain relief. In MTS patients, the drug dosage was calculated as 5 IU per injection site, whereas in MFS patients, the dosage was 10-15 IU per injection site.
Results: The orthopedic examination using anthropometric measurements revealed an LLLM of 0.4 cm to 1.5 cm. A 3D scan confirmed the following deficits: oblique pelvis position (1.5 to 6 degrees) with asymmetry of the shoulder girdle and elevation of the shoulder on the short leg side in 86% of the patients (57 men and 72 women). This group of patients showed asymmetry of tone and pain (5-7 points) in the temporalis, masseter, trapezius, rhomboid, longissimus dorsi, and quadratus lumborum muscles averaging from 20% to 97%. One month after wearing compensatory insoles, EMG monitoring in 96 patients (64% of the study subjects: 61 men and 35 women) showed at least 30% reduction in asymmetry of tone in the paired muscles and relief of pain syndrome (1-2 points). Subsequently, the patients were prescribed an individual course of physical therapy (asymmetric exercises with post isometric relaxation and stretch therapy) and wearing of a compensatory orthosis. The asymmetry of tone in the paired muscles decreased on average by 20%-25% in 54 patients (36% of the subjects, 9 men and 45 women), but complete symmetry was not achieved (asymmetry was 35%-45%), and pain syndrome persisted. After 3 weeks of BoNT treatment, the pain syndrome disappeared (1-2 points), and EMG examination showed insignificant asymmetry of tone in the paired muscles.
Conclusions: LLLM causes pain syndrome and asymmetry of muscle tone in the paired muscles. Continuous wearing of limb-length compensatory orthoses produced improvement in postural balance and harmonization of tone in a number of patients. However, when the degree of asymmetry is high, compensatory orthoses are insufficient to relieve pain and create symmetry, leading to MTS and MFS. This condition requires BoNT therapy.
Keywords: Botulinum toxin; Muscle hypertonia; Muscle-tonic syndrome; Myofascial syndrome; Pain

Does the Guidance Method Affect Dosing of Botulinum Toxin in Writer's Cramp?

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Introduction: Writer's cramp (WC) is a rare form of focal, idiopathic dystonia. Although botulinum neurotoxin (BoNT) lacks an approval in this condition, this drug is often considered the first-line treatment. Electrical stimulation (ES)-based guidance is most frequently used to safely direct BoNT injections to target muscles. However, more recently, ultrasound (US) guidance is also being used for BoNT injections in limb dystonia. The main objective of this work was to determine how the guidance method (electrical stimulation or ultrasound) may affect the dosing of BoNT in WC.
Methods: The study was registered with the French National Data Protection Commission (reference: DEC20-058). We retrospectively examined the medical records of consecutive patients treated with botulinum neurotoxin type A (BoNT-A) injections for WC (presenting as flexion of the fingers or of the hand; because they are rare, other forms were not included). A patient was included in the study if he/she had received at least three ES-guided BoNT injections followed by at least three US-guided injections. We studied the doses in the following muscles: flexor carpi ulnaris (FCU), flexor carpi radialis (FCR), flexor digitorum profundus (FDP), and flexor hallucis longus (FHL).
A mixed linear model (fixed effect: guidance method) was used to compare the doses injected according to each guidance method. A mixed linear

model with random effect was used to assess changes in dose over time according to each guidance method. Quantitative data were expressed as median (minimum-maximum). Doses were expressed in units of onabotulinumtoxinA.
Results: Thirty-four patients were included (22 women and 12 men; age at first injection: 44.8 years (40.0-50.7); number of injection cycles using ES guidance: 12.0 (9.0-19.0); number of injections using US guidance: 8.0 (7.0-9.0). The following doses were higher with ES than with US guidance: mean dose of BoNT by muscle (2.9 versus 2.7; $P<.001$) and dose in the FDP, 6 (0-12) versus 3 ([0-10]; $P=.046$). The following results were lower with ES than with US guidance: dose in the FCU, 0 ([0-10] versus 3 ([0-18]; $P=.004$) and number of injected muscles, 2 (2-3) versus 3 ([2-4]; $P=.014$). Using electrical stimulation, the dose increased over time in the FCU and in the FCR; the total dose decreased, as well as the dose in the FDP and the FHL. Using ultrasound, the doses seemed more stable over time (except for a decrease of the total dose), but the follow up was short.
Conclusions: Our results show that the total dose of BoNT injected in WC was lower with ultrasound guidance than with electrical stimulation guidance, especially in the FDP. Using electrical stimulation guidance, we had to reduce the dose in the smaller muscles (probably because of side effects) and to increase the dose in the larger muscles (probably to improve the outcome).
Funding: None
Keywords: Botulinum neurotoxin dose; Electrical stimulation; Ultrasound; Writer's cramp

Is There a Role for BoNT in the Management of Depression?

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Over the past several years, we and other researchers have provided evidence from several randomized clinical trials (RCTs) for the fast, robust, and enduring alleviation of unipolar depression symptoms by a single injection of botulinum neurotoxin (BoNT) in the glabellar region (ie, the corrugator supercilii and procerus muscles). There are several meta-analyses, and the most recent by Schulze et al[1] (Figure) has been adequately examined[2] with respect to relatively large effect sizes (d = 0.98), methodological issues, and mode of action.
The most favored therapeutic rationale for BoNT is the facial feedback hypothesis. The theory suggests that besides manifesting our emotions, facial expressions also modulate affective states via proprioceptive afferent nerve pathways. With the injection of BoNT into specific parts of the glabellar region, the facial muscles that are thought to be involved in the expression of negative emotions relax, and thus their perception is mitigated. Animal and initial functional imaging studies in humans corroborate these assumptions; however, they also raise other possible modes of action.
In light of these findings and our more than 10 years of clinical experience with the use of BoNT in psychiatric settings, the current and a putative future role of BoNT in the management of depression will be critically discussed.

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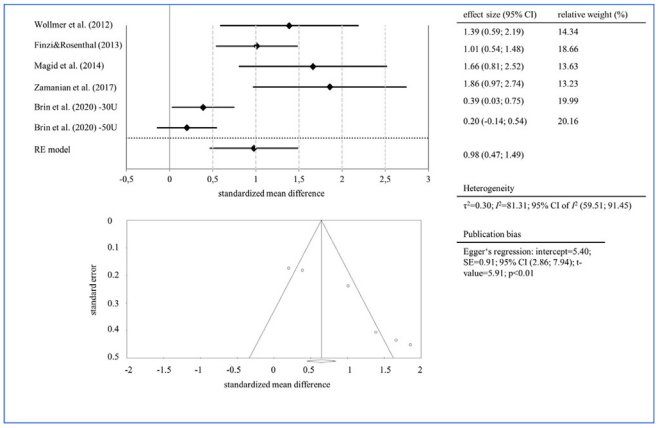

Fig. Meta-analysis of RCTs comparing BoNT vs placebo treatment of depression. Forest plot and funnel plot for the interaction model of BoNT vs placebo (T0 vs T1).[1]

A Phase III, Single-Center, Randomized Controlled Trial Comparing Clinical and Cost-Effectiveness Analyses of Botulinum Toxin Uterine Injections Versus Placebo for Severe Dysmenorrhea and Chronic Pelvic Pain of Uterine Origin After Standard Therapeutic Failure

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Introduction: Severe dysmenorrhea and chronic pelvic pain (CPP) of uterine origin may be followed by psychosocial consequences and professional absenteeism.[1,2] Despite negative magnetic resonance imaging and laparoscopy, currently available treatments (analgesics, anti-inflammatory drugs, and hormones) are ineffective with low quality-of-life gains.[3,4] As published previously, injections of botulinum toxin (BT) type A under hysteroscopy into uterine myometrium revealed significant decrease of patient-reported symptoms and improvement of global quality of life scores at 8 and 12 weeks postinjection.[5] The aims of this study are to evaluate the clinical efficacy and cost-effectiveness of BT use for severe dysmenorrhea/CPP after treatment failure.
Methods: A phase III, single-center, randomized controlled trial comparing BT versus placebo in 100 participants. Randomization will be centralized. Study investigators, analysis team, and subjects will be blinded. Participants will be informed and will sign informed consent forms. Data will be collected and recorded in a secure electronic platform before enrollment, at inclusion, at 8 weeks, and 4 months follow up.
Main clinical outcome measure: Patient Global Impression of Improvement. Secondary clinical outcomes: Health-Related Quality of Life (HRQoL) measured by the Endometriosis Health Profile; generic health-related quality of life measured using the EQ-5D-3L questionnaire, Female Sexual

Function Index, dysmenorrhea and dyspareunia numerical rating scale, visual analogue pain scale, side effects, drug tolerance, surgical complications, and global patient satisfaction. Effectiveness and cost outcomes: Quality-adjusted life years (QALYs), direct and indirect costs, net social benefit, incremental cost-effectiveness ratios.
Results: This study will provide reliable evidence on the effectiveness of a novel therapy for patients suffering from severe dysmenorrhea and CPP of uterine origin, in the event of standard therapeutic failure. The health economic evaluation will provide evidence to guide collective decision-making on marketing authorization for this clinical indication.
Conclusions: Evidence-based clinical and cost-effectiveness data for BT will guide the proposal and implementation of a new treatment for severe dysmenorrhea and CPP of uterine origin.
Funding: Merz Pharmaceuticals GmbH
Keywords: Clinical trial; Cost-effectiveness; Efficacy; IncobotulinumtoxinA; Pelvic perineal pain; Women

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Intraoperatively Administered AbobotulinumtoxinA Alleviates Pain After Surgery and Improves General Wellness in a Pig Model

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Introduction: Pain after surgery remains a significant healthcare challenge. Here we tested the activity of abobotulinumtoxinA (Dysport®; aboBoNT-A) in a postsurgical pain model in young domestic pigs.
Methods: A full-skin and muscle incision and retraction (SMIR) on the lower flank was followed with an intradermal injection of either aboBoNT-A (100, 200, 400 U/pig), its vehicle (saline), or wound infiltration of liposomal extended-release bupivacaine (n=6/group). Animals were assessed for mechanical sensitivity (von Frey test), distress behaviors, latency to approach the investigator, locomotor activity, and wound inflammation/healing for up to 5 days following surgery. At the end of the study immunohistochemical analyses of the total and cleaved synaptosome-associated protein of 25 kDa (SNAP-25) as well as pain-related biomarkers were performed in animals treated at 400 U aboBoNT-A or saline.
Results: AboBoNT-A treatment reversed allodynia partially, starting day 1 and fully, starting day 3, reduced distress, and normalized approaching responses starting 6h postsurgery. Bupivacaine reversed mechanical allodynia for 24 hours after surgery but failed to reduce distress or to normalize approaching responses. Locomotor activity, wound inflammation, and healing were similar across the groups. Cleaved SNAP-25 was absent in the skin and dorsal root ganglia, but present in the ipsilateral dorsal horn of aboBoNT-A–treated animals. A marked decrease in GFAP staining and moderate reduction in IbA1 staining was seen in aboBoNT-A-treated animals in comparison to vehicle-treated controls. The substance P and calcitonin gene-related peptide (CGRP) labelling in the lumbar spinal cord or in the dorsal root ganglia were similar in treated and control groups.
Conclusions: Intraoperative aboBoNT-A can be considered for clinical evaluation as an option for safe, non-opioid analgesia with enduring pain relief and improved emotional wellbeing after surgery.
Funding: This study was sponsored by Ipsen.
Keywords: Behaviour; Botulinum neurotoxin; Cleaved SNAP-25; Spinal cord; Surgical pain

Analgesic Activity of AbobotulinumtoxinA in Postoperative Pain Models in the Pig is Driven by the Route of Administration: New Highlights on the Mechanism of Action

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Introduction: The management of pain after surgery continues to rely heavily on opioid drugs, despite side effects. The mode of action of the allodynic activity of abobotulinumtoxinA (Dysport®; AboBoNT-A) was investigated in postsurgical pain models in the pig.
Methods: Intradermal (id), subcutaneous (sc), intramuscular (im), and nerve block (nb) injections were performed with AboBoNT-A (200 U/pig) or a saline solution 15 days before a full-skin incision in pigs. Animals were assessed for mechanical evoked pain (von Frey test) and distress behaviors for up to 8 days following surgery. At the end of the study, transcriptomic and immunohistochemical analyses were performed.
Results: AboBoNT-A treatment reduced allodynia and distress in the id-injected animals only. Cleaved-synaptosome-associated protein of 25 kDa (c-SNAP25) was observed in the skin (arrector muscles, periarterial nerve endings) and focally in the ipsilateral dorsal horn of the lumbar spinal cord in id-injected animals. In other groups, significantly lower amounts of c-SNAP25 were detected in the skin (sc group only) and spinal cord (sc, im, and nb).
In the brain, traces of c-SNAP25 were detected in the cuneate, trigeminal, and red nuclei in the brain with an identical pattern in id and im groups. Interestingly, there was no staining in nuclei implicated in pain regulation, indicating that toxin effects most likely occurred at the skin level, dorsal root ganglia (DRG), and/or spinal cord.
Transcriptomic analyses performed on the ipsilateral dorsal horns of the lumbar spinal cord comparing the saline to AboBoNT-A in id-injected animals revealed an increased expression of 36 genes (up to x14, $P<0.01$) and a decreased expression of 44 genes (up to -93%, $P<0.01$). Pathway analyses suggested an involvement of axonemal dynein assembly, glutaminergic pathway regulation, interleukin-1 (IL-1) and inflammatory cell migration/chemotaxis, and ERK1/2 cascade.
Conclusions: This study confirms a long-term analgesic activity of AboBoNT-A in this model, highlights the importance of the route of administration, and provides new data on the mode of action.
Funding: This study was sponsored by Ipsen.
Keywords: Behavior; Botulinum neurotoxin; Immunohistochemistry; Pain; Spinal cord; Transcriptomic

Screening and Development of 9-Hydroxy-4*H*-Pyrido[1,2-*a*] Pyrimidin-4-One–Based Bifunctional Inhibitors of BoNT-A/LC

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Introduction: Botulinum neurotoxin type A (BoNT/A) is the most toxic substance known with LD_{50} estimated to be 1-2 ng/kg in humans.[1,2] The half-life of this toxin spans several months to a year, and current pharmacotherapeutic approaches are on a timescale of hours to days. To overcome the limitations of reversible inhibitors, the Janda lab has been working on the development of a small molecule, covalent BoNT inhibitor strategy. Recently, our research has pivoted towards developing bifunctional inhibitors containing a warhead that covalently tethers to the active site scaffold in addition to the metal-chelating, zinc-binding group (ZBG). [3-5] In order to overcome the pitfalls of hydroxamate-based bifunctional inhibitors, through screening and structure-activity relationship analyses (SAR), we have developed 9-hydroxy-4H-pyrido[1,2-a]pyrimidin-4-one (PPO)–based bifunctional inhibitors.

Methods: *In silico* screen of eMolecules diverse set (1 million molecules) by Atomnet resulted in 33 compounds of interest, which were further evaluated against 10 nM BoNT/A LC in a Fluorescence Resonance Energy Transfer (FRET) assay to find the hit-PPO. A library of 43 additional PPO-based derivatives were synthesized and screened with continuous SNAPtide FRET assay and a motor neuron cell assay. As shown in Figure 1, SAR were analyzed and supported by docking of hit compounds into the X-ray crystal structure of BoNT/A LC. Based on the binding pose, bifunctional compounds with a methanethiosulfonate (MTS) reactive group were designed, synthesized, and evaluated to obtain k_{inact}/K_i (a more accurate measure of reactivity and inhibitory potency than IC_{50}). Endpoint FRET assay, exhaustive enzyme dialysis, and mass spectrometry were used to validate the covalent modification of the inhibitors.

Results: PPO library screen resulted in a hit-2 (PPO-benzyl) with IC_{50} of 0.9 μM against BoNT/A LC. A sub library created by SAR based on hit-2 was evaluated with motor neuron cell assay and SNAPtide assay. It resulted in a lead (PPO-4-phenylbenzyl) with IC_{50} of 0.33 μM (SNAPtide assay), and cell IC_{50} of 13 μM. Based on docking results (Figure 1B), the ideal linker length was determined to be ~6-7 Å from C8, as this would be an ideal position for the covalent tether. Optimally designed bifunctional inhibitor–containing MTS warhead (PPO-81) showed k_{inact}/K_i of 2810±150 $M^{-1}s^{-1}$, which is four-fold higher than the analogous dichlorocinnamic hydroxamate (DCHA) scaffold ($k_{inact}/K_i = 514±17$). The endpoint assay indicated long-term, time-dependent inhibition. Covalent modification of Cys165 of BoNT/A LC by PPO-81 was confirmed by high-resolution mass spectrometry (HRMS).

Conclusions: Using *in silico* screening and utilizing medicinal chemistry tools, a new class of BoNT/A LC inhibitors was discovered based on a PPO scaffold. Compounds showed promising inhibition in a motor neuronal cell assay for BoNT/A activity. PPO-81 showed four-fold improvement over hydroxamate-based MTS predecessor. Bifunctional mechanism of this inhibitor was confirmed via HRMS and dialysis studies.

Keywords: Bifunctional inhibitors; BoNT light chain; Botulinum neurotoxin type A; FRET assay; Zinc-chelating inhibitors

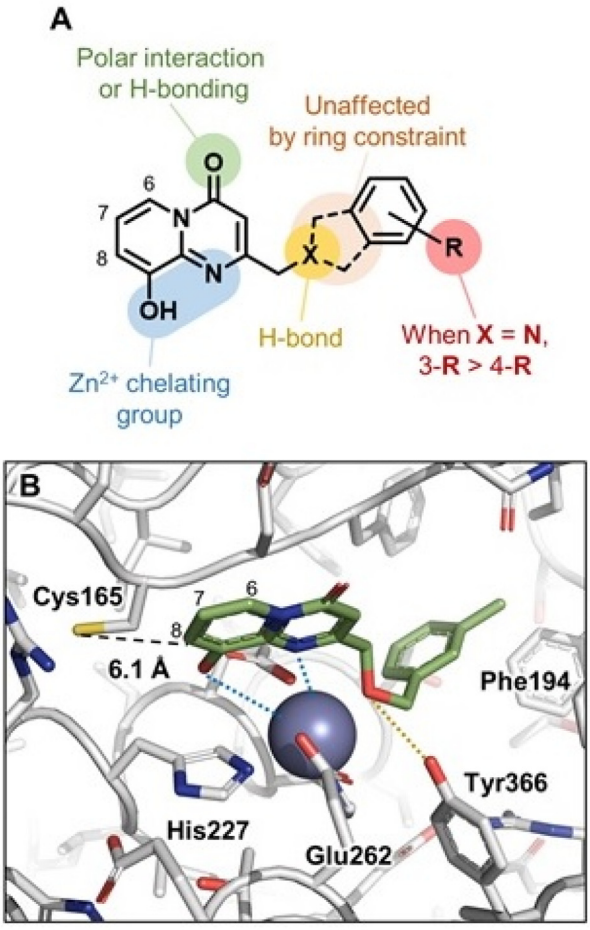

Fig. 1. A. Summary of SARs for PPO compounds, with ring numbering. **B.** Docking of 3-methylbenzyl PPO derivative (green) in the active site of BoNT/A LC (PDB 4HEV). Dark grey sphere represents Zn^{2+}. Numbering corresponds to ring numbering in (A).

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Comparison of Primary Rat Adult DRG and Human Nociceptors for BoNT Characterization

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Introduction: One of the therapeutic areas for which botulinum neurotoxins (BoNTs) have been investigated is the treatment of pain, with research suggesting that BoNTs can provide benefits related to effects on cholinergic control of nociceptive and antinociceptive systems. The establishment of pain in vitro models is essential for further investigating the biological relevance of BoNTs in pain.

Methods:

- Establishment of primary adult dorsal root ganglion (aDRG) cultures from rat and human-induced pluripotent stem cell (hiPSC)–derived sensory neurons.
- Characterization of the main pain markers of both models by gene expression analysis and immunocytochemistry.
- Evaluation of BoNT/A and LHn/A effect using synaptosome-associated protein of 25 kDa (SNAP-25) cleavage assays.
- Comparison of the expression levels of pain markers and the effect of BoNT molecules on aDRG and hiPSC-derived sensory neurons.

Results: aDRG neurons were cultured from adult rats in different culturing plate formats for up to 14 days. hiPSC-derived sensory neurons were provided by Censo Biotechnologies and cultured in 96-well plates for up to 5 weeks.

For both models, the expression of key pain markers such as Neurofilament Heavy Chain (NEFH), calcitonin gene-related peptide (CGRP), neurotrophic receptor tyrosine kinase 1 (NTRK1), transient receptor potential vanilloid subtype 1 (TRPV1), and P2X purinoceptor 3 (P2X3) was shown by RNAseq analysis and confirmed by immunocytochemistry studies. While RNAseq analysis suggested that hiPSC-derived neurons expressed lower levels of CGRP and NTRK1 compared to primary aDRG cultures, immunocytochemistry studies showed comparable expression levels of these markers in both cell models (Figure).

Treatments of both neuronal cell populations with recombinant botulinum neurotoxin type A (rBoNT/A) showed that the level of intoxication differed between the two models with aDRG neurons showing significantly lower potencies and maximum SNAP-25 cleavage compared to hiPSC-derived neurons.

Conclusions: Expression of several of the pain markers was observed in aDRG and hiPSC-derived sensory neurons. However, the expression levels of the pain markers CGRP and NTRK1 was different between these two neuronal types, when analyzed by RNAseq. Both aDRG and hiPSC-derived sensory neurons are sensitive to soluble (*N*-Ethylmaleimide–Sensitive Factor) Attachment Protein Receptor (SNARE) cleavage by BoNT molecules, showing clear potential as an in vitro model for the study of botulinum neurotoxins in pain.

Funding: This study was sponsored by Ipsen.

Keywords: Adult dorsal root ganglion neurons; Human-induced pluripotent stem cells; SNAP-25 cleavage; RNAseq

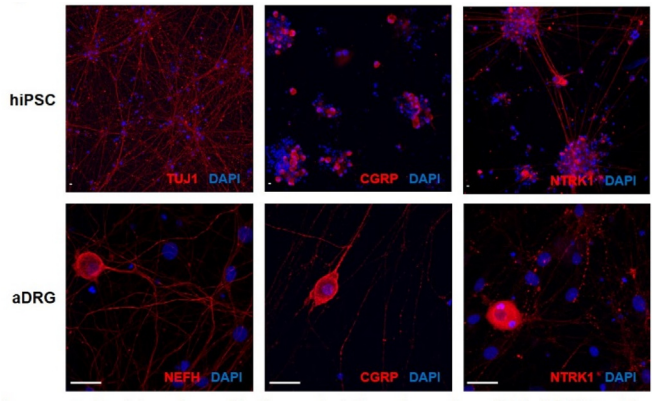

Fig. Expression of pain markers in the hiPSC and aDRG neurons. Immunocytochemistry using antibodies against the pain markers TUJ1 (hiPSC only), CGRP, NTRK1, and NEFH (aDRG only) in cultured hiPSC and aDRG neurons. Images were obtained using confocal microscopy at magnifications of 20x (hiPSC) and 40x (aDRG). Scale bar=10 μm.

Neurotoxin Use in a Presumed Case of Stiff Person Syndrome

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Case Presentation: A 50-year-old female with a history of osteoporosis, alcohol abuse, and Barrett's esophagus presented with progressive lower extremity weakness, difficulty walking, and frequent falls, resulting in multiple fractures. Her other symptoms included Raynaud's disease, worsening cachexia with 50-pound weight loss in six years requiring percutaneous endoscopic gastrostomy (PEG) placement, and hypertonia with contractures in all extremities.

She underwent extensive work up for various primary and secondary neuromuscular disorders, which was significant for positive anti–glutamic acid decarboxylase (GAD)-65 antibody and electromyography/nerve conduction study (NCS) evidence of severe length-dependent polyneuropathy and features possibly consistent with stiff person syndrome. Other differentials included an undetermined inflammatory myopathy, given consistently elevated muscle-specific enzymes (creatine phosphokinase [CPK], aspartate aminotransferase [AST], aldolase) and muscle biopsy results showing "features of an inflammatory myopathy without overt inflammatory cell infiltration, muscle atrophy, or inclusion bodies." Testing for common infiltrative diseases, plasma cell dyscrasias, endocrine abnormalities, infectious diseases, autoimmune/connective tissue diseases, and paraneoplastic antibodies were negative. The patient underwent treatment with plasma exchange, rituximab, steroids, and intravenous immunoglobulin without improvement in her symptoms. She tried valium, baclofen, and tizanidine for her hypertonia with only marginal benefits.

On examination in November of 2020, the patient presented with dystonia in bilateral hands, more significant on the left. The patient was not able to use her fingers to hold items or feed herself. She received a total of 400 units of onabotulinumtoxinA in her bilateral flexor pollicis longus (FPL), bilateral flexor pollicis brevis (FPB), bilateral adductor pollicis (AP), right flexor digitorum superficialis (FDS), right flexor digitorum profundus (FDP), and left lumbricals 1-4, without significant improvement. In August of 2021, after discussing reasonable, modest goals of treatment, the patient received a total of 1000 units of abobotulinumtoxinA in her bilateral FDS, right FDP, bilateral FPB, and bilateral lumbricals 1-4 with increased active and passive range of motion and improvement in her Functional Independence Measure (FIM) scores, including increased independence in toileting, bathing, and dressing within two weeks, while participating in daily rehabilitation. Further assessments are forthcoming.

Discussion: Spasticity and hypertonia can be managed with a variety of interventions, including oral medications, chemodenervation, phenol or ethanol neurolysis, intrathecal baclofen, and orthopedic interventions for contractures. Given the patient's cachexia and poor response to oral medications, she was an appropriate candidate for injections. AbobotulinumtoxinA has been approved by the FDA for blepharospasm, cervical dystonia, and moderate-to-severe glabellar lines.[1] However, in this case, abobotulinumtoxinA was used for functional improvement in activities of daily living. AbobotulinumtoxinA is believed to have a clinically longer duration of action given the greater amounts of active neurotoxin in the injection[2] and may spread more diffusely.[1] In this case, botulinum toxin injections with associated goal-directed daily therapy led to improved function.

Keywords: AbobotulinumtoxinA; Botulinum toxin; Hypertonia; OnabotulinumtoxinA; Stiff person syndrome

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Botulinum Toxin Type A Duration Enhancement by Mu-Conotoxin CnIIIC

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Introduction: Duration of action is a key feature of botulinum toxin type A (BoNT/A) with clinical relevance. Yet, after more than three decades of research, it remains challenging to substantially enhance duration[1] other than by increasing the dose. We report the unexpected finding that co-injecting BoNT/A and mu-conotoxin CnIIIc—a specific blocker of muscular Nav1.4 voltage-gated sodium channels—significantly enhances BoNT/A duration of action in three preclinical models.
Methods: CnIIIc was obtained by peptide synthesis,[2] solubilized in saline, and mixed with commercial BoNT/A before injection. For digital abduction score (DAS) experiments,[3] rats were injected in the tibialis anterior and scored until muscle relaxation was no longer detectable. Area under the DAS curve (DAS AUC) was used for group comparison. For free running wheel experiments,[4] rats were injected in the gastrocnemius and the daily run distance was monitored until full recovery. For electrophysiological experiments, mice were injected in the soleus and evoked end-plate potentials (ePP) were recorded in the injected muscle.[5]
Results: Dose-dependent myorelaxation induced by CnIIIc was detectable in DAS experiments from 2 hours to 2 days with peak at 24 hours. BoNT/A doses were calibrated to obtain similar reference pharmacodynamics across different commercial sources. Myorelaxation was detectable between 6 hours and 12 days, with a peak at 2 days. Myorelaxation was also measured after injection of a fixed dose of BoNT/A mixed with increasing doses of CnIIIC. As expected, the combination with CnIIIC induced fast myorelaxation, which started within two hours, as with the CnIIIC alone. Surprisingly, the duration of action of BoNT/A was enhanced by the presence of CnIIIC since the effect of the combination lasted several days longer than BoNT/A alone. BoNT/A enhancement was dependent on the CnIIIC dose and was observed with all three BoNT/A formulations tested (abobotulinumtoxinA, incobotulinumtoxinA, and onabotulinumtoxinA). BoNT/A enhancement in duration was further confirmed by free running wheel experiments and by electrophysiology experiments, where the ePP signal at the neuromuscular junction was significantly lower in mice injected with the CnIIIc-BoNT/A combination than those injected with BoNT/A alone.
To rule out a direct molecular interaction between CnIIIC and BoNT/A, CnIIIC was mutated at two residues to lower its channel-blocking activity in vitro. Mutations completely abrogated BoNT/A enhancement. Staggered injections of CnIIIC and BoNT/A were also tested: BoNT/A activity was enhanced within a certain time frame between injections.
Conclusions: Our results show that mu-conotoxin CnIIIC enhances the activity of BoNT/A and suggest an underlying synergistic biological mechanism. To our knowledge, this is the first report describing an enhancement of BoNT/A activity by postsynaptic inhibitors of muscle contraction. Most interestingly, the degree of enhancement seen in our models suggests clinical meaningfulness, and investigation of use in humans seems warranted.
Keywords: Botulinum toxin type A; Botulinum toxin type A combination; Conotoxin; Duration; Neuromuscular junction; Preclinical

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Efficacy Study of AbobotulinumtoxinA in the Rhino Mouse Model of Acne

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Introduction: Besides neuronal cells, BoNT/A has been suggested to target other cell types such as fibroblasts or keratinocytes.[1, 2] These cells play a key role in skin conditions. Maintaining a high-quality sebum secretion is also essential to avoid premature aging.[3]
Methods: The rhino mouse was used for this study, as the utriculi it develops in the skin are very similar to comedones, accompanied by oily skin, which are representative hallmarks of acne.[4] Briefly, anaesthetized animals were injected via the intradermal route (ID; four sites of injection) by either vehicle or 0.1, 0.3, and 1 unit (U) abobotulinumtoxinA (Dysport®) per mouse. A reference group was administered with adapalene gel 0.1% (daily local application) for 15 days. The body weight and thickness of the dorsal skin were measured on days 1, 5, 10, and 15; erythema and scaling were recorded at the same time. On day 15, animals were ethically euthanized, and skin samples were collected for histology, enzyme-linked immunosorbent assay (ELISA), and lipidomic assays.
Results: AbobotulinumtoxinA administered ID at 0.1 U and 0.3 U per mouse was well tolerated. AbobotulinumtoxinA 1 U per mouse induced a transient loss of muscle tone associated with a slight body weight loss after which mice recovered a good health status. AbobotulinumtoxinA did not show any significant effect on utricle surface area but induced a significant anti-inflammatory effect on the dermis at the two highest doses. Moreover, abobotulinumtoxinA showed neither side effects commonly observed with local retinoids nor hyperplasia nor dermis inflammation. No change in skin interleukin 1 (IL-1) alpha cytokine levels was evidenced with abobotulinumtoxinA, whereas a dose-dependent increase of substance P concentration in the skin was recorded, suggesting that abobotulinumtoxinA induces neuropeptide accumulation in tissue by inhibiting exocytosis mechanisms. Lipidomic analysis showed that abobotulinumtoxinA significantly increased the sebum concentration of several lipid species presenting skin-protecting properties.
Conclusions: These data suggest that abobotulinumtoxinA administered ID has skin rejuvenating, anti-inflammatory, and moisture-boosting properties.

Funding: This study was sponsored by Ipsen (manufacturer of abobotulinumtoxinA).
Keywords: AbobotulinumtoxinA; Aging; Lipids; Mouse; Sebum; Skin

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Table
Macroscopic, histological, and biochemical skin properties of rhino mice at D15 after either daily treatment with adapalene or a single dose of abobotulinumtoxinA at Day 1.↗: increase; ↘: decrease; —: no change versus vehicle group (saline).

Parameters (D15)	Daily Adapalene	AbobotulinumtoxinA (intradermal injection at Day 1)
Skin thickness	—	—
Erythema	↗	—
Scaling	↗	—
Sebaceous gland surface	↗	—
Utricle surface	↘	—
Epidermis thickness	↗	—
Dermis inflammation	↗	↘
Keratinocyte proliferation	↗	—
Fibroblast proliferation	↗	—
Sebocyte proliferation	—	—
SP (skin)	—	↗
IL-1α (skin)	↘	—
Fatty acids (sebum)	↘	↗
Cholesterol (sebum)	—	↗
Waxes (sebum)	—	↗

A Virtual Reality Platform to Facilitate Training on Treatment of Lower Limb Spasticity With OnabotulinumtoxinA

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Introduction: OnabotulinumtoxinA is safe and effective for treating lower limb spasticity (LLS). Treatment outcomes and adverse events may be affected by injector training and experience. Treatment of LLS with onabotulinumtoxinA requires an understanding of muscle anatomy, dosing, and injection techniques. A pilot study was conducted to assess the benefits of a virtual reality (VR)–based training platform.
Methods: A VR-based platform employing an immersive experience with haptic technology was designed to provide supplemental training on the use of onabotulinumtoxinA for LLS to enhance knowledge of muscle anatomy and selection, and improve injection competency. In a pilot program conducted from December 2020 through August 2021, United States–based medical students, residents, and fellows underwent one-on-one VR-based training on lower limb anatomy and injection guidance, then assessed the platform by completing surveys before and after training.
Results: One hundred forty medical trainees (6 medical students, 124 residents, and 10 fellows) from 21 academic centers completed pre–VR-training surveys; 111 completed post–VR-training surveys. Average learning time per VR session was ~43 minutes. The percentage of trainees who were very comfortable with localization of lower limb muscles increased from 8% pre–VR-training to 14% post–VR-training. Percentages of trainees identifying all correct responses almost doubled from pre–VR-training to post–VR-training when asked about possible functions of the flexor digitorum longus (12% vs 22%) and which muscles to consider injecting in a hypothetical poststroke patient (21% vs 40%). Pre–VR-training, 34% of respondents indicated that a VR-based training tool would be extremely useful versus 52% post–VR-training. The primary features that trainees found beneficial were the realistic feel of needle insertion/removal (82%) and the ability to use injection guidance (79%).
Conclusions: Preliminary results indicate a potential need to improve baseline knowledge and onabotulinumtoxinA injection technique in the treatment of LLS among medical trainees. A brief (<60 minutes) supplemental training session using a VR-based platform resulted in improvements in muscle localization and injection considerations. Optimal VR-based training duration and potential additional interventions to augment learning and improve trainees' knowledge base need to be further defined.
Funding: This study was sponsored by AbbVie.
Keywords: Botulinum toxin type A; Muscle spasticity; Neurotoxins; Spastic paraplegia; Training technique; Virtual reality, instructional

Real-World Evidence for the Safety and Efficacy of CGRP Monoclonal Antibody Therapy Added to OnabotulinumtoxinA Treatment for Migraine Prevention in Adult Patients With Chronic Migraine

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Introduction: The objective of this study was to collect real-world data to improve the understanding of the safety, tolerability, and potential benefit of adding a calcitonin gene-related peptide (CGRP) monoclonal antibody (mAb) to onabotulinumtoxinA (onabotA) for chronic migraine (CM).
Methods: This was a retrospective, longitudinal study conducted using data extracted from a single clinical site's electronic medical records (EMR) of adult patients (≥ 18 years) with CM treated with ≥ 2 consecutive cycles of onabotulinumtoxinA before ≥ 1 month of continuous onabotulinumtoxinA and CGRP mAb (erenumab, fremanezumab, or galcanezumab) combination treatment according to the prescribing physician's discretion. Safety and efficacy (monthly headache days [MHD]) were recorded at first mAb prescription (index) and up to 4 onabotA visits ~3, 6, 9, and 12 months post-index.

Results: EMR were collected for 192 patients; 148 met eligibility criteria. Fifty-seven percent of patients were prescribed erenumab, 42.3% fremanezumab, and 0.7% galcanezumab. Patients in this study received onabotA treatment for an average of 2.6 years before the initiation of combination treatment. With onabotA treatment alone, 88.1% of patients experienced a reduction in MHD and 35% had a $\geq$ 50% reduction in MHD. Mean (standard deviation [SD]) MHD were 20.4 (6.6) before onabotA and 14.0 (6.9) before the addition of a mAb. There were additional significant reductions in MHD at the first visit (~3 months) and at all subsequent visits (Figure) following the initiation of combination therapy. OnabotA was discontinued by 42 (28.2%) patients and a mAb by 50 (33.6%) patients. The most common reasons for discontinuing either treatment were lack of insurance coverage (40%) and lack of effect (34%); 14% discontinued a mAb and none onabotA due to safety/tolerability. Adverse events (AEs) were reported by 18 patients (12.1%); no serious AEs were reported.

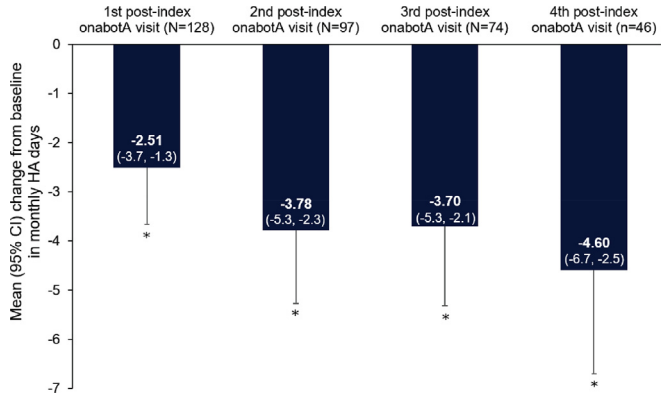

Fig. Change from baseline in monthly headache day frequency during combination treatment with onabotulinumtoxinA and CGRP mAbs. Baseline (N=149) mean (95% confidence interval [CI]) MHD frequency = 14.0 (12.9, 15.1). *Indicates 95% CI does not include zero. HA=Headache.

Conclusions: In this real-world study, onabotA was effective at reducing MHD and the addition of a CGRP mAb was safe, well tolerated, and associated with incremental reductions in MHD for those who remained on the combination treatment. No new safety signals were identified. The majority of patients who discontinued either or both treatments reported lack of insurance coverage as the reason. There are limitations inherent to observational studies, notably the lack of a placebo arm for comparison. Prospective, real-world and controlled trials are needed to further evaluate the safety and quantify the benefits of this combination treatment paradigm for people with CM.

Funding: Allergan (prior to its acquisition by AbbVie)

Keywords: Calcitonin gene-related peptide; Concomitant treatment; Migraine prevention; OnabotulinumtoxinA; Real-world; Safety

Disclosures:

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Controlling Post-Stroke Spasticity With Botulinum Toxin in the Long Term: Which Limbs and How Many Muscles Are We Treating?

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Introduction: Botulinum toxin type A (BoNTA) is an effective therapeutic strategy for post-stroke spasticity. This study aimed to analyze the evolution of treatment approach regarding the limbs treated with BoNTA and the number of muscles injected over time in patients treated for $\geq$10 years in a reference center.

Methods: We performed a retrospective analysis of data prospectively and non-interventionally collected from the clinical files of patients diagnosed with stroke and treated with BoNTA for $\geq$10 years. We analyzed the number of injections in the upper limb (UL), lower limb (LL), and combined upper and lower limbs (UL+LL), as well as the number of muscles per limb treated at the 1st (T^1), 5th (T^5), 10th (T^{10}), and 20th (T^{20}) injection cycles.

Results: A total of 24 patients was included, and the total number of injections was 646 (minimum [min]:11; maximum [max]: 63; average: 26.91 injections/patient). The percentage of patients who received at least 20 injections was 70.83%. The average treatment time was 13.48 years (min: 10; max: 18 years). The proportion of patients who had only the UL treated increased over time (T^1=16.66%; T^5=25%; T^{10}=25%; T^{20}=20.83%). For the LL, there was a tendency for the proportion to remain the same in the early stages followed by an increase (T^1=8.33%; T^5=8.33%; T^{10}=8.33%; T^{20}=33.33%). For the combined UL+LL, the proportion decreased over time (T^1=75%; T^5=66.66%; T^{10}=66.66%; T^{20}=45.83%).

The average number of muscles treated in the UL showed an increasing trend: T^1=5.25 (min: 3; max: 7); T^5=6.5 (min: 5; max: 9); T^{10}=6.6 (min: 5; max: 9); T^{20}=7.6 (min: 4; max: 9). In the LL, we observed the opposite: T^1=5.5 (min: 5; max: 6); T^5=5 (min: 5; max: 5); T^{10}=4 (min: 3, max: 5); T^{20}=2 (min: 2; max.2). In patients treated simultaneously in the UL+LL, there was a trend toward an increase followed by a decrease in the average number of muscles injected: at T^1=9.89 (min: 6; max: 13), T^5=11.50 (min: 7; max:16), T^{10}=10.59 (min: 6; max:15), and T^{20}=8.45 (min: 4; max: 12).

Conclusions: The frequency of injections of UL only or LL only increased, while there was a progressive reduction of injections of both limbs over time. This suggests a progression toward more focused goals. The increasing average number of muscles injected in the UL corroborates this theory since functional deficits of this segment tend to be more permanent and limiting of activity/participation. We can also speculate that once patients need less muscles injected in the LL, injectors have the opportunity to optimize their approach by treating more UL muscles. Other factors that may have played a role but which were not addressed for this abstract are dosing and frequency of injections (reported by our group: Martins, et al, 2021; Rosa, et al, 2021) and adjuvant therapies (Abreu, et al, *Toxicon*. 2022).

The finding that there is a very large percentage of patients who received 20 or more injections reinforces the proposition that the benefits of this treatment continue to exist in the long term.

Keywords: Goal of treatment; Long-term use of BoNTA; Post-stroke spasticity

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How Do Goal Categories Influence the Most Frequently Targeted Muscles for Post-Stroke Spasticity? Twenty Years of Experience of a Reference Center

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Introduction: Botulinum toxin type A (BoNT-A) is effective in controlling post-stroke spasticity. This study aimed to analyze the limbs and muscles injected according to therapeutic goals over time.
Methods: This is a post hoc analysis of prospective, observational data from a designated population of post-stroke spasticity (PSS) patients treated from 2001 to 2020. We obtained demographic data and number of injections and determined the most frequently targeted limbs and most frequently injected muscles for each primary goal category.
Results: The sample consisted of 288 PSS patients (56.3% female) and a total of 2635 injections. We recorded 1848 primary goals divided into two groups: symptoms/deficits (SD): 56.1% and activity/function (AF): 3.9%, subdivided into upper limb only (UL), lower limb only (LL), and upper and lower limbs (UL+LL). For SD, **involuntary movement control** was reported in 28.5% (n=527; UL=189; LL=18; UL+LL= 320). The most frequently injected muscles in this category were flexor digitorum superficialis (FDS), 70.39%; biceps brachii (BB), 64.89%; brachialis (BA), 49.33%; gastrocnemius medialis (GM), 45.54%; and gastrocnemius lateralis (GL), 45.35%.
Pain/discomfort corresponded to 16.9% (n=313; UL=88; LL=27; UL+LL=198). The most frequently injected muscles were FDS, 57.18%; GM, 54.31%; subscapularis (SS), 52.39%; GL, 51.43%; and BB and pectoralis major (PM), 50.47% each.
Maintaining ROM corresponded to 10.7% (n=198; UL=54; LL=30; UL+LL=114). The most frequently injected muscles were FDS, 71.21%; GM, 52.52%; GL, 5.52%; flexor digitorum longus (FDL), 45.45%; and BB, 47.47%.
For AF, **facilitation of mobility** was found in 22% (n=406; UL=13; LL=114; UL+LL= 279). The most frequently injected muscles were GM, 80.04%; GL, 77.58%; soleus (S), 66.25%; FDL, 59.35%; and FDS, 47.78%.
Active function corresponded to 10.7% (n=197; UL=76; LL=8; UL+LL=113). The most frequently injected muscles were FDS, 73.6%; GL, 47.71%; GM, 46.70%; FCR, 42.63%; and BB, 41.11%.
Passive function corresponded to 10.6% (n=196; UL=97; LL=7; UL+LL= 92). The most frequently injected muscles were FDS, 83.16%; flexor carpi radialis (FCR), 52.55%; BB, 51.02%; and flexor carpi ulnaris (FCU), 49.48%.
Therapy facilitation corresponded to 0.6% (n=11; UL=1; LL=1; UL+LL=9). The most frequently injected muscles were GM, GL, and FDS, 81.81% each; flexor pollicis longus (FPL), flexor digitorum profundus (FDP), FCR, and FCU 54.54% each.
Conclusions: Different goals as well as different limbs targeted seemed to determine muscle selection and frequency of injections in this real-world cohort analysis.
Keywords: Muscles injected; Post-stroke spasticity; Primary goals

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Quality of Life in Patients With Spasticity Treated With Botulinum Toxin

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Introduction: Functionality is affected in those suffering from spasticity.[1] Botulinum toxin type A (BoNTA) is a treatment with type A evidence for spasticity management in adults and children.[2] One measure of treatment efficacy is improvement in functionality. In this sense, health-related quality of life (HRQOL) is useful to assess the effectiveness of health interventions and to measure functionality.[3] Therefore, in this study, we retrospectively evaluated EuroQol-5D (quality of life assessment interview) responses in a group of patients with spasticity to examine the effect of BoNTA in routine clinical practice.
Methods: We evaluated 211 clinical records of patients with spasticity between 2016 to 2018 who received a single-blind BoNTA injection (incobotulinumtoxinA or abobotulinumtoxinA), in at least two sessions. Patients were administered the EuroQol-5D quality of life assessment interview (EQ-5D, adults and EQD-5D-Y, children) and the Patient Global Impression of Change (PGIC) scale by telephone. Ashworth Scale scores were recorded prior to BoNTA application and subsequently at weeks 4 and 8 to assess efficacy.
Results: The sample consisted of 161 patients. The mean age was 21.4 years (standard deviation: 18.7), and the most frequent diagnosis was cerebral palsy (n=100, 62.1%). A total of 96.9% of patients reported perceived clinical improvement with respect to baseline, and improvement in their health status and quality of life, according to the EuroQol-5D. BoNTA significantly reduced spasticity per year of treatment, according to the Ashworth Scale. There were no serious adverse events. Adults presented a higher frequency of anxiety/depression and children a better health status at the beginning of treatment. At the final evaluation, children reported better health status, less pain, and less anxiety/depression. Both groups reported an improvement in the rest of the quality-of-life dimensions, and more than 90% of patients in both groups reported clinical improvement compared to the beginning of treatment.
Conclusions: From the patient's perspective, there was clinical and HRQOL improvement when treated with BoNTA. Self-assessment is an alternative means of measuring the effect of BoNTA on clinical and quality of life variables that is adapted to a real-world clinical setting
Funding: This study received a grant from Merz Pharma S.A. de C.V.

Keywords: Botulinum toxin type A; IncobotulinumtoxinA; Quality of Life; Spasticity

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Subject-Relevant Outcomes of On-Label 50 U AbobotulinumtoxinA Treatment for Moderate-to-Severe Glabellar Lines Across Three Individual Trials

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Introduction: The 50 U dose of abobotulinumtoxinA (ABO) is approved for glabellar line (GL) treatment. Here we present efficacy, subject satisfaction, and safety results from three recent clinical trials using this dose, with a focus on ≥1-grade glabellar line improvement and subject satisfaction, reflecting clinical outcomes of significance for the subjects.
Methods: Subjects with moderate-to-severe GL were treated with 50 U ABO and followed for 6 to 9 months in three studies (NCT03736928, double-blind, Phase 2; NCT03960957, double-blind, Phase 3; NCT03687736, open-label, Phase 4). Evaluations included investigator- and subject-assessed GL severity scale (GLSS), a subject satisfaction questionnaire, subject-reported onset of effect (diary), and adverse events.
Results: In each study, 80, 224, and 120 subjects were evaluated, the majority achieving improvement in GLSS. Median time to onset of effect was 2 days in all three studies. At Month 6, 53%, 46%, and 37% of subjects, respectively, in each trial maintained ≥1-grade improvement in investigator-assessed GLSS, and at Month 9, 18% of subjects in the Phase 2 trial still had ≥1-grade improvement. The majority of subjects were satisfied with their treatment and found the result to be natural looking up to Month 6 in all three trials. Treatment-related adverse events were mostly mild, and none were serious.
Conclusions: ABO 50 U for glabellar line treatment was efficacious and well tolerated across all three trials, with rapid onset, and ≥1-grade improvement and subject satisfaction lasting for up to 6-9 months after injection.
Funding: Research funded by Galderma Research & Development, LLC
Keywords: ABO; AbobotulinumtoxinA; Glabellar lines; Subject satisfaction

Subject Satisfaction With AbobotulinumtoxinA for Moderate-to-Severe Glabellar Lines: A Randomized, Dose-Escalation, Double-Blind Study

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Introduction: AbobotulinumtoxinA (ABO) is approved for glabellar line (GL) treatment using a dose of 50 U. Subject satisfaction with GL treatment using higher ABO doses up to 125 U vs placebo was evaluated as part of a Phase 2 study. The primary endpoint was Month 1 composite response, defined as a ≥2-grade Glabellar Line Severity Scale (GLSS) improvement and a GLSS score of 0 or 1 assessed by both investigator and subject.
Methods: In this 9-month, double-blind study (NCT03736928), subjects received an ABO dose of 50 U, 75 U, 100 U, or 125 U, or placebo. Assessments included a subject satisfaction questionnaire, 3 FACE-Q scales (Psychological Function, Appraisal of Lines, and Perceived Age), GLSS at maximum frown, and safety. Time to return to baseline GLSS score was assessed concurrently on both investigator and subject scales.
Results: Around 80 subjects were evaluated per group. Subject satisfaction was high after treatment; >90% were satisfied with their appearance and agreed that they looked natural and appeared refreshed at Month 1. Subjects' well-being improved, and they were less bothered about their glabellar lines and perceived themselves as younger up to Month 9. At Month 9, 18% (50 U), 26% (75 U), 35% (100 U), and 31% (125 U) retained a ≥1-grade improvement (investigator GLSS). The median time to return to baseline GLSS scores was 226, 240, 252, and 256 days, respectively. Treatment was generally safe across all doses.
Conclusions: ABO was efficacious and well-tolerated across all doses from 50 U to 125 U, with ≥1-grade severity improvement maintained for up to 9 months. Subjects reported natural results and high rates of satisfaction, sustained to Month 9, as well as a wider positive impact on well-being and age assessment. Subject satisfaction is an aspect of clinical importance, which captures treatment effect over time.
Funding: Research funded by Galderma R&D, LLC.
Keywords: AbobotulinumtoxinA; ABO; Dose-escalation; FACE-Q; Glabellar lines; Subject satisfaction

OnabotulinumtoxinA, Nerve Stimulation Devices, Mirabegron, and Anticholinergics Versus Best Supportive Care for Overactive Bladder: An Updated US Cost-Effectiveness Analysis

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Introduction: Refractory overactive bladder (OAB) can be managed by various treatments: onabotulinumtoxinA (onabotA), implantable sacral nerve stimulation (SNS) devices, percutaneous tibial nerve stimulation (PTNS), mirabegron, and anticholinergic medications. New SNS device models (eg, rechargeable adaptations) entered the market subsequent to findings of past cost-effectiveness (CE) analyses.

Methods: A Markov model was updated to compare the CE of treatment options to best supportive care (BSC, ie, behavioral therapy, incontinence pads, occasional catheterization) over 10-year and 15-year time horizons from a Medicare payer perspective. Treatment options included onabotA, rechargeable Axonics r-SNM System™, non-rechargeable Medtronic InterStim I (7-year longevity) and II (4.4-year longevity), PTNS, mirabegron 25 mg and 50 mg, and anticholinergics (solifenacin [5 and 10 mg] and tolterodine ER [4 mg]). Resource utilization, adverse event rates, treatment discontinuation, medical/ pharmacy costs, efficacy estimates, and battery life duration (for SNS devices) were derived from published sources. Costs and quality-adjusted life-years (QALYs) were discounted at 3% per year. Outcomes were reported as total and incremental QALYs and costs, and incremental CE ratios (ICERs) relative to BSC.
Results: Over 10 years, total QALYs ranged from 7.070 (tolterodine) to 7.179 (onabotA) versus 7.069 for BSC. Total costs ranged from $13,193 (tolterodine) to $32,561 (Medtronic InterStim II) versus $11,914 for BSC. ICERs relative to BSC ranged from $35,307 per QALY gained (onabotA) to $1,238,440 per QALY gained (mirabegron 25 mg) (Table).

Table

Time horizon:10 years	Total costs, $	QALYs	Incremental costs vs BSC, $	Incremental QALYs vs BSC	ICER (per QALY gained), $
BSC	11,914	7.069	–	–	–
OnabotulinumtoxinA	15,802	7.179	3,888	0.110	35,307
Axonics r-SNS Device	21,694	7.136	9,780	0.068	144,676
Medtronic InterStim I	27,123	7.125	15,209	0.057	267,982
Medtronic InterStim II	32,561	7.125	20,647	0.057	363,788
PTNS	14,681	7.106	2,767	0.037	74,496
Mirabegron (25 mg)	17,035	7.073	5, 121	0.004	1,238,440
Mirabegron (50 mg)	17,028	7.073	5,115	0.005	1,088,616
Solifenacin (10 mg)	14,830	7.072	2,916	0.004	816,372
Solifenacin (5 mg)	14,822	7.073	2,908	0.005	634,835
Tolterodine ER (4 mg)	13,193	7.070	1,279	0.002	668,541

BSC, best supportive care; ER, extended release; ICER, incremental cost-effectiveness ratio; PTNS, percutaneous tibial nerve stimulation; QALY, quality-adjusted life year; SNS, sacral neuromodulation system.

Conclusion: OnabotA is the most cost-effective treatment (at $35,307/QALY gained) for patients with OAB in the US. Other treatments considered in this model (except PTNS; $74,496/QALY) were not cost-effective, yielding ICERs above the $100,000/QALY threshold.
Keywords: Anticholinergics; Best supportive care; Cost-effectiveness; Mirabegron; OnabotulinumtoxinA; Nerve stimulation devices
Disclosures:
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BM has served as a consultant and investigator for Allergan, an AbbVie company, and as a consultant for Astellas, Urovant, and Medtronic.
JMT has served as a consultant for Allergan, an AbbVie company, and Urovant, and as a speaker for Allergan, an AbbVie company, Astellas, and Urovant.
DS has served as a consultant for Urovant and Antares Pharma, and as a speaker for Teleflex and Allergan, an AbbVie company.
AP has served as a speaker for Allergan, an AbbVie company, and received royalties from UptoDate.
CC is an investigator for Allergan, an AbbVie company, and Cook MyoSite.
VBN is a consultant for Allergan, an AbbVie company.
AT, PG, and **AL** are employees of Allergan, an AbbVie company.
VN has served as an investigator for Allergan, an AbbVie company, Astellas, and Cook MyoSite.

Botulinum Toxin and Goal Setting: What Matters to the Spasticity Patient and the Rehabilitation Team?

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Introduction and Objectives: The GAS-eous Tool (Goal Attainment Scaling— Evaluation of Outcome for Upper-Limb Spasticity) has introduced a unified approach to goal setting and outcome assessment in the management of spasticity. Goal setting that involves the patient and rehabilitation team is essential for treatment success. Furthermore, rehabilitation outcomes improve if patients are engaged in goal setting.[1] There is a wide range of individual treatment goals, and patients have different expectations and needs for spasticity treatment.[2] The objective of this study was to evaluate the main goals of spasticity treatment with botulinum toxin type A (BoNT-A), goal achievement, and injection procedures at two Physical Medicine and Rehabilitation (PM&R) Departments.
Methods: This is an international, multicenter, retrospective study conducted at two PM&R Departments in Lisbon, Portugal, and Bilbao, Spain. The medical records of spasticity patients treated with BoNT-A during the first 9 months of 2021 were analyzed. Goal Attainment Scaling (GAS) was performed for each previous BoNT-A treatment goal at between 12 and 16 weeks post injection, usually at the reinjection consultation. BoNT-naïve patients were excluded. For patients with multiple injection records, only the first reinjection session was considered for data analysis.
Results: A total of 83 patients, 56 from Lisbon and 27 from Bilbao, with a mean age of 56.23± 11.85 years, and predominantly male (56.63%; n=47) were included. The majority of patients had a stroke diagnosis (72.23%; n=60). Mean duration of spasticity was 13.87 ± 10.04 years. The majority of patients presented with hemiparesis (73.49%; n=61) and hemispasticity (65%; n=54).
Forty-two patients received abobotulinumtoxinA (807.19 ± 333.15), 37 onabotulinumtoxinA (258.11 ± 131.70), and 5 incobotulinumtoxinA (170±76). A mean of 6.12± 2.68 muscles was injected, and the lower limb was the most frequently injected (83.13%; n=69) limb. All muscles were injected using ultrasonographic guidance. Seventy (84.33%) patients utilized adjunctive therapies.
Globally, treatment objectives assessed using the GAS-eous Tool included range of motion maintenance (62.65%; n=52), mobility facilitation (59.03%; n=49), passive function (25.30%; n= 21), active function (25.30%; n=21), pain control (25.30%; n=21), and involuntary movement control (24.10%; n=20). In 63 (75.9%) patients, two or more goals (maximum [max]: 4) were selected. Global mean GAS T-score was 48.65± 2.26 (Lisbon: 49.36±1.30; Bilbao: 47.08 ±3.54), and global mean change in GAS T-score was 9.86± 3.08 (max:18.6; minimum: 0; Lisbon: 9.7±3.24; Bilbao:10.9 ±2.68).
Conclusions: Individualized SMART (Specific, Measurable, Achievable, Realistic, and Timely) goal setting is critical for spasticity patients. The mean GAS T-score at follow up was consistent with that reported in the literature, reflecting the team's improving ability to negotiate and establish achievable goals (in our case slightly overestimating the expected outcome). The fact that the GAS score was not recorded at BoNT-A peak effect may conceal a more robust effect of BoNT-A treatment. The change in GAS T-score was close to clinically meaningful (>10). Most of the patients presented with chronic spasticity, suggesting that patients continue to receive benefit from repeated treatments. The GAS score change interval was not extended, which may indicate that chronic patients were already well managed and relatively stable.

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OnabotulinumtoxinA Is Efficacious and Well Tolerated in Male Patients With Overactive Bladder and Urinary Incontinence: Placebo-Controlled Treatment Cycle 1 Results From a Pooled Analysis of Four Randomized Trials

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Introduction: In clinical trials of onabotulinumtoxinA (onabotA) treatment of overactive bladder (OAB) with urinary incontinence (UI), most patients were females. This pooled analysis evaluates the efficacy and safety of onabotA in a large number (n=194) of male patients with OAB and UI.
Methods: Data were pooled from 4 randomized, double-blind, placebo-controlled trials (NCT00910845, NCT00910520, NCT01767519, and NCT01945489). Outcome measures included number of UI episodes/day and Incontinence Quality of Life (I-QOL) total summary score. All except NCT01945489 included Treatment Benefit Scale (TBS). Efficacy measures are reported as change from baseline at 12 weeks post−first treatment. Treatment-emergent adverse events (TEAEs) are reported for the first 12 weeks after treatment.
Results: Pooled ITT population (N=1564) included 194 males (12.4%) and 1370 females (87.6%). Baseline demographic and disease characteristics, including mean age and UI episodes/day, were similar between sexes and treatment groups. At week 12, mean changes from baseline in UI episodes/day were -2.2 and -1.3 in males treated with onabotA or placebo, respectively; decreases in females were -3.0 and -1.1, respectively. Males and females treated with onabotA achieved mean I-QOL increases above minimal important difference of 10 points. A positive response on the TBS at week 12 was 44.3% (onabotA) and 27.5% (placebo) in males, and by 66.1% (onabotA) and 29.7% (placebo) in females. Overall incidence of TEAEs in the 12 weeks after treatment was higher in females (39.4%) than males (34.7%). Most common TEAEs in males were dysuria, increased residual urine volume, and haematuria, while in females, urinary tract infection, dysuria, and bacteriuria were most common. Urinary retention was lower in females (3.6%) than males (5.2%).
Conclusions: Results show onabotA was efficacious and well tolerated in males and females. TEAEs were limited to local effects on bladder in both; no new safety concerns were found.
Funding: Allergan, an AbbVie Company
Keywords: Incontinence; Male; Overactive bladder
Disclosures:
V Nitti has served as an investigator for Allergan (an AbbVie company), Astellas, and Cook MyoSite.
DA Ginsberg has served as an investigator for Allergan (an AbbVie company).
A Kohan has received consultancy fees and/or has been an investigator for Allergmelian, Ipsen, and Medtronics.
K McCammon has served as an investigator for Allergan (an AbbVie company).
B Jenkins, I Yushmanova, and **A Boroujerdi** are employees of AbbVie.
C Chapple has served as a consultant for Astellas Pharma, Bayer Schering Pharma AG, Contura, Ferring, Symimetic, Takeda, and Urovant Sciences; served as a speaker for Allergan (an AbbVie company) and Astellas Pharma; served as an investigator for Allergan (an AbbVie company), Astellas Pharma, Bayer Schering Pharma AG, and Poiesis Medical; served as an author for Astellas Pharma; received a patent with Symimetic.

Diagnosis and Management of a Cervical Dystonia Population With a Large Proportion of Whiplash Injuries: Use of EMG Along with Standard Assessment

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Introduction: Cervical dystonia (CD) is the most common form of focal dystonia, but the diagnosis is often delayed for years.[1-3] This is not surprising since there are no specific diagnostic tests or laboratory studies required. The purpose of the study was to add an objective electromyography (EMG) exam to the standard assessment of CD to ensure diagnosis when the condition is less obvious. Another goal was to assess for associated medical conditions.
Methods: A retrospective chart review of patients diagnosed with cervical dystonia at an outpatient clinic of a tertiary academic medical center was performed**.** All patients underwent an EMG assessment of a minimum of three of the cervical muscles most frequently implicated in abnormal posture or pain. The presence of moderate-to-severe dystonia was used to confirm the diagnosis of CD.
Results: A total of 120 patients were diagnosed with CD, with a 70% predominance of females. The most common precipitating events were trauma, 67%, and overuse, 18%. Headache was present in 62% of patients (P <0.001, while 29% (P <0.05) of patients had thoracic outlet syndrome, both at significantly higher rates than the general population, based on published literature and national US epidemiologic data.[4-6] A greater proportion of individuals had hypermobility (20%) than the general population (P <0.052).[7]
Conclusions: With an objective EMG exam included in the assessment of CD, we found a significant correlation with cervicogenic headache and thoracic outlet syndrome. When these medical conditions are diagnosed alone, it may be appropriate to assess for CD and include an EMG examination**.**
Funding: None
Keywords: Cervical dystonia; Cervicogenic headaches; Electrodiagnosis; Hypermobility; Thoracic outlet syndrome

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Non-Destructive Catalytic Inactivation of Novel Recombinant Botulinum Neurotoxin Type A Variants Facilitates Safe Mass Spectrometry Analysis, Outside of Containment

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Introduction: Botulinum neurotoxins (BoNTs) are the most potent substances found in nature. Nevertheless, botulinum neurotoxin types A and B (BoNT-A/-B) have been widely developed for therapeutic use.[1] Advances in molecular biology techniques and rapid DNA synthesis have allowed a wide variety of novel BoNTs with alternative functions to be assessed as potential new classes of therapeutic drugs. Mass spectrometry assessment of in-process samples and final products, a significant prerequisite in drug development, is a process involving extremely laborious and time-consuming steps when conducted in containment laboratories. We developed processes that enable safe manipulation and accurate analysis of these hazardous samples out of containment, thus lessening the need for specialist resources and reducing drug development timelines.
Method: Briefly, a commercially available detergent (RapiGest™ SF) commonly used in mass spectrometry[2] was incubated with the active toxin under different experimental conditions. The treated samples were assayed for proteolytic activity following detergent treatment in an in vitro substrate cleavage assay, as well as a cell-based assay. A mass spectrometry protocol was developed using an inactive version of the toxin containing two mutations in the active site that completely block Zn^{2+} binding. The complete loss of toxin activity was confirmed prior to release from containment. Importantly, this mutant displays the expected mass for the light and heavy chains.
Conclusion: We have developed a protocol that facilitates the safe analysis by mass spectrometry of full-length botulinum neurotoxins outside of a safety cabinet or containment suite, thus removing the need to house such an instrument in a safety cabinet for dedicated use and the requirement for containment-trained staff to perform the analysis.
Keywords: BoNTs; Drug development; Inactivation; Mass spectrometry; RapiGest™
Funding: Ipsen

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History of Injection Guidance Practice in a Reference Botulinum Toxin Type A Clinic Over 20 Years

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Introduction: Botulinum toxin type A (BoNT-A) is an effective strategy in poststroke spasticity (PSS) management and requires accurate administration into target muscles.[1,2] This study aims to characterize the choice of injection guidance modalities for BoNT-A administration over time in a European Spasticity Clinic with 20 years of experience.
Methods: A post hoc analysis of prospective, observational data of PSS patients treated with BoNT-A from 2001 to 2021. Demographic data was analyzed, and injection guidance practice over the years was sorted into 3 groups: use of anatomic reference (AR), guidance using electrical stimulation (ES), or ultrasonographic guidance (US). All groups were analyzed for the following periods 2001-2005, 2006-2010, 2011-2015, 2016-2018, and 2019-2021. We examined the use of these methods in clinical practice over time. ES has been available since 2005 and US since 2016. Means of maximum BoNT-A dose percentages were compared among the following groups: guided methods (GM) and non-guided method (NGM) in the periods before and after 2016 when US was introduced. Statistical analysis was done with SPSS v.25.
Results: A total of 288 PSS patients were included; 126 (43.7%) were females. The median age was 54.3±12.5 years, and 187 (64.9%) had an ischemic stroke. A total of 2635 injections were analyzed, consisting of 1730 (65.66%) abobotulinumtoxinA, 429 (16.28%) incobotulinumtoxinA, and 470 (17.84%) onabotulinumtoxinA injections. Between 2001 and 2005, 96.2% (n=51) of injections used AR and 3.8% (n=2) ES; 2006-2010, 94.2% (n=226) used AR and 5.8% (n=14) ES; 2011-2015, 86.5% (n=993) used AR and 13.5% (n=155) ES; 2016-2018, 48.2% (n=344) used AR, 47.2% (n=337) ES, and 4.6% (n=33) US; 2019-2021, 17.9% (n=75) used AR, 44.5% (n=186) ES, and 37.6% (n=157) US. There were significant differences between GM and NGM in maximum BoNT-A dose percentages after the introduction of US guidance (GM vs NGM: 85.04±39.86% vs 79.67±41.28%, P=0.031); this was not seen before 2016 (GM vs NGM: 80.71±31.75% vs 80.32±33.40%, P=0.885).
Conclusions: Our data show that over time, US and ES have quickly gained ground relative to AR once these methods became available in our spasticity clinic. Furthermore, GM seemed to increase injector confidence, as shown by the use of much higher doses of BoNT-A. This was especially noticeable after the introduction of US in 2016, and after the publication of high-dose safety studies for the 3 toxin formulations.[3-5]
Keywords: Anatomic reference; Botulinum toxin; Guidance methods; Spasticity; Stroke

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How Do Injection Guidance Methods Relate to Doses Used, Targeted Limbs, And Goal Achievement?

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Introduction: Botulinum toxin type A (BoNTA) is an effective treatment for post-stroke spasticity (PSS) and requires accurate placement into target muscles.[1,2] This study aims to compare different BoNTA injection targeting methods with respect to doses used, targeted limbs, and therapeutic success.
Methods: A post hoc analysis of prospective, observational data collected from 2001 to 2021 was performed. Demographic data was analyzed, and the type of injection guidance divided into three groups: use of anatomic references (AR), electrical stimulation guidance (ES), or ultrasonographic guidance (US). The parameters of success utilized were: Goal Attainment Scale (GAS) ≥ 0 per primary goal and maximum dose percentage of BoNTA per limb—upper limb (UL), lower limb (LL), or upper and lower limbs (UL+LL)—were compared between groups. Statistical analysis was performed using SPSS Statistics v.25 software.
Results: A sample of 288 patients (43.7% females), encompassing 2635 injections of which 65.64% used AR, 26.97% ES, and 7.38% US, was analyzed. After excluding 1426 injections for lack of data, we analyzed 1209 injections, of which 55.91% used AR, 32.84% ES, and 11.25% US. The success rate was higher in the ES group (84.6%) when compared to the AR (84.5%) or US groups (79.4%), but no association was found between success rate and guidance method (AR vs ES: $X^2(1)=0.005$, $P=0.942$; ES vs US: $X^2(1)=1.987$, $P=0.159$; AR vs US: $X^2(1)=2.113$, $P=0.146$). BoNTA doses were different between groups ($F(2,741)=12.99$, $P=0.000$). The US group had higher doses for UL+LL than the AR and ES groups (AR vs ES: 96.79±30.80% vs 90.62±32.13%, $P=0.049$; AR vs US: 96.79±30.80% vs 112.07±40.18%, $P=0.000$; ES vs US: 90.62±32.13% vs 112.07±40.18%, $P=0.000$). There was no significant difference in mean doses for UL between groups ($F(2,344)=0.747$, $P=0.474$) (AR vs ES: 62.70±24.83 vs 65.72±22.51, $P=0.526$; AR vs US: 62.70±24.83 vs 61.70±24.03, $P=0.970$; ES vs US: 65.72±22.51 vs 61.70±24.03, $P=0.617$). Regarding the LL, there was a statistically significant difference in mean doses of BoNTA used ($F(2,115)=9.142$, $P=0.000$). The US group had higher mean doses of BoNTA injected than the AR group, but not the ES group (AR vs ES: 64.71 ±45.44% vs 93.06±57.21%, $P=0.039$; AR vs US: 64.71 ± 45.44% vs 123.89±74.33%, $P=0.000$; ES vs US: 93.06±57.21% vs 123.89±74.33%, $P=0.160$).
Conclusions: Our data showed similar success rates (% of goals scored GAS$\geq$0) between groups. The US group received higher maximum dose percentages of BoNTA when compared to the AR and ES groups, except for UL injections. We hypothesize that this may be related to the fact that recently available US guidance gave the injectors more confidence in using higher doses. On the other hand, data for US-guided injections are more recent, after high-dose studies had been published for the three BoNTA formulations.[3-5] It remains to be explored whether duration of the changes produced was similar between groups.
Keywords: Anatomical references; Botulinum toxin; Guidance methods; Spasticity; Stroke; Success rate

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A Cell-Penetrating Peptide (CPP) Binds Directly to and Enhances Membrane Binding of the Core Toxin of Botulinum Neurotoxin Serotype A (BoNT/A)

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Introduction: CPPs are small oligomeric peptides with sequences that facilitate transport of cargo across lipid bilayers through the activation of macropinocytosis. This has not been successful to date as there are currently no CPPs approved to deliver therapeutic cargoes. DaxibotulinumtoxinA for injection (DAXI) is a new BoNT/A product formulated with a proprietary 35-amino acid–long peptide (RTP004), which contains a CPP motif at each terminus. In clinical trials, DAXI has been shown to have a 24-week duration in glabellar lines[1] and cervical dystonia.[2] Previously, we reported RTP004 can increase BoNT/A thermostability.[3] We hypothesized that a direct binding interaction between the two molecules could increase stability. Here, we characterize, in vitro,the direct interaction between RTP004 and the 150-kDa BoNT/A molecule and show enhancement of toxin binding to artificial membranes and intact cells.
Methods: Circular dichroism and *in silico* electrostatic potential map generation, using the Advanced Poisson-Boltzmann Solver[4] and mapped to the protein in ChimeraX,[5] were used to better understand the secondary structure of RTP004 and the electrostatic potential of the core toxin, respectively. Binding and competition experiments using both enzyme-linked immunosorbent assay (ELISA) and surface plasmon resonance (SPR)-based assays determined if there was a direct interaction between RTP004 and the core toxin of BoNT/A. The effect of RTP004 on enhancing core toxin binding to artificial membranes (PIPosomes) and intact cells was assessed via SPR-based binding assays and infrared (IR) fluorescence-based, cell-binding assays, utilizing Neuro-2A (N2a) cells, respectively.
Results: First, the sequence and chiroptical analysis of RTP004 indicated that it is highly positively charged and adopts a polyproline type II helix. Next, the electrostatic potential of the BoNT/A 150-kDa core neurotoxin (PDB ID: 3BTA) was mapped and shown to be highly negatively charged allowing for a potential interaction between RTP004 and the core toxin of BoNT/A. Direct interaction was evaluated with ELISA and SPR-based binding data. Taken together, these data demonstrate a direct interaction between RTP004 and the 150-kDa core toxin in different environments with dissociation

constants in the nanomolar range (1-150 nM). Furthermore, we demonstrate in vitro that RTP004 can enhance binding of the 150-kDa core toxin to artificial membranes (PIPosomes) 2- to 3-fold relative to non-coated membranes. Finally, coating cells with RTP004 enhanced the binding of the toxin 2- to 3-fold vs non-coated cells. Control experiments with human serum albumin failed to show any interaction or enhanced core toxin binding.

Conclusions: Overall, we have demonstrated that RTP004 can directly interact with the 150-kDa BoNT/A core toxin in both static and dynamic environments. Additionally, this interaction can enhance binding of BoNT/A core toxin to both artificial membranes as well as intact cells.

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Keywords: DaxibotulinumtoxinA; Enzyme-linked immunosorbent assay; Human serum albumin; RTP004 peptide; Surface plasmon resonance

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OnabotulinumtoxinA for Treatment of Masseter Muscle Prominence: Secondary Results From a Phase 2, Dose-Escalation Study

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Introduction: Benign enlargement of the masseter muscles (masseter prominence) may appear as a wide, square, or trapezoidal lower face shape, which may be considered undesirable.

Methods: Adults aged 18-50 years with bilateral marked/very marked (grade 4/5) masseter prominence were randomized 1:4 to placebo or onabotulinumtoxinA ([onabotA] 24 U, 48 U, 72 U, 96 U) in a double-blind, dose-escalation study. On day 1, six injections were administered (3 injections/masseter) with optional re-treatment at day 180; patients were followed monthly for 1 year (day 90, primary time point; day 360 exit visit). Changes in lower facial width and mandibular facial angle (internal angle between horizontal and vertical lines that intersect at the apex of the mandibular angle) were measured, based on 2D image projections.

Results: In total, 187 patients (mean age 35.4 years; 81.8% female) were randomized (placebo, n=37; 24 U, n=37; 48 U, n=37; 72 U, n=37; 96 U, n=38). At day 90, changes in facial width were 0.4 mm for placebo and -4.7 mm, -4.3 mm, -5.0 mm, -5.6 mm for 24 U, 48 U, 72 U, and 96 U, respectively (all $P<0.001$ vs placebo). All onabotA doses provided significant improvements in reducing facial width up to 6 months after each treatment vs placebo (all $P\leq0.005$ vs placebo). At day 90, changes in mandibular facial angle were -1.1° for placebo and 4.3°, 4.2°, 4.3°, and 4.2° for 24 U, 48 U, 72 U, and 96 U, respectively (all $P<0.001$ vs placebo). Significant improvements from baseline in mandibular facial angle that increased toward a more oblique angle were maintained for up to 6 months after each treatment.

Conclusions: As reported previously, onabotA significantly reduced muscle volume vs placebo (all $P<0.001$; primary endpoint). Here, onabotA significantly reduced lower facial width and increased mandibular angle for all dose groups vs placebo in subjects with bilateral masseter muscle prominence. Significant reductions were observed for up to 6 months after each of 2 treatments with onabotA.

Keywords: Aesthetic; Clinical trial; OnabotulinumtoxinA; Masseter muscle

Funding/Disclosures:

J Rivers and S Humphrey are consultants, speakers, and investigators for Allergan, plc.

J Carruthers serves as consultant and investigator for Allergan plc.

S Liew serves as an investigator, speaker, and consultant for Allergan plc.

E Lee, B Bowen, and MF Brin are employees of Allergan plc and may own stock/stock options in the company.

This study was sponsored by Allergan Aesthetics, an AbbVie Company.

Immunostaining Procedure for Ganglioside Expression Evaluation in Human Neurons

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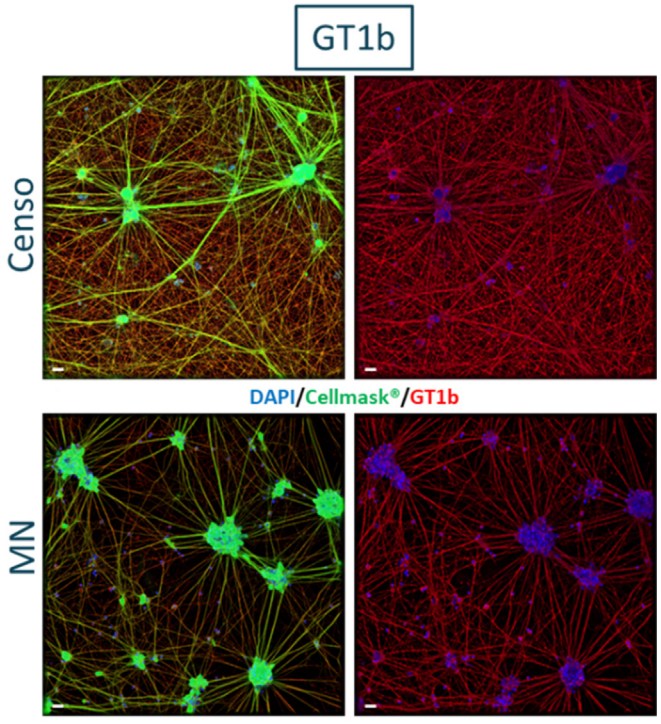
Introduction: To enter neurons, botulinum neurotoxins (BoNTs) bind the cell membrane using a 2-step mechanism: binding to polysialogangliosides (PSGs) that accumulate the toxins at the neuronal surface followed by binding to a protein receptor. All neurons do not express the same ganglioside population and all BoNTs do not bind the same ganglioside to enter the cell. Protein receptor expression is well known within cell types, whereas ganglioside expression is not. This may be due to a lack of a protocol to stain and quantify gangliosides in the neurons. Here we describe a method that allowed us to study ganglioside expression in vitro using immunocytochemistry (ICC), high-content microscopy, and image analysis.

Methods:

- Establishment of human-induced pluripotent stem cell (hiPSC)–derived sensory neuron and hiPSC-derived motoneuron cultures.
- Development and validation of a robust immunocytochemistry protocol for the gangliosides GT1b, GM1, and GD2.
- Image analysis, measurement, and comparison of the expression level of ganglioside between hiPSC-derived motoneurons and hiPSC-derived sensory neurons

Results: hiPSC-derived motoneurons from Fujifilm Cellular Dynamics (FCDI; MN in the figure below) and sensory neurons from Censo Biotechnologies (Censo in the figure below) were cultured in 96-well plates for 2 weeks and 5 weeks, respectively, as suggested by suppliers. Cells were fixed and ganglioside staining was performed without permeabilization. GT1b, GM1, and GD2 were observed in both hiPSC-derived neuron cultures. Since GT1b is one of the preferred forms of ganglioside recognized by BoNTs, its level of expression was evaluated by Imactiv-3D company by quantifying the fluorescent signal after ICC. The comparison of expression between both hiPSC-d models revealed a higher level of expression of GT1b in human motoneuron cultures compared to human sensory neurons.

Conclusions: This new method of expression quantification could be useful in identifying gangliosides associated with a specific cell type, thereby enabling the design of a new toxin able to target a cell population using specific gangliosides.



Immunocytochemistry using antibodies against GT1b in cultured hIPSC-derived Motoneurons and Sensory neurons. Images were obtained using confocal microscopy at magnifications of 20X. Scale bar = 25 µm

Funding: This study was sponsored by Ipsen.
Keywords: Ganglioside; Human-induced pluripotent stem cells; Immunocytochemistry

AbobotulinumtoxinA Doses in Upper and Lower Limb Spasticity: A Systematic Literature Review

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Introduction: Limb spasticity is a disabling potential consequence of stroke, traumatic brain injury, or progressive neurological disease.[1,2] Clinical studies have shown that abobotulinumtoxinA (AboBoNT-A) reduces both upper- and lower-limb spasticity[3,4]; however, there are concerns that physicians tend to delay such treatment or administer inadequate doses, to avoid the upper end of dose ranges specified in the summary of product characteristics or cited in the literature. Seeking insights into this issue, a systematic literature review was conducted to explore published evidence on the AboBoNT-A doses given intramuscularly in the upper and lower limb regardless of the etiology of spasticity.
Methods: Searches were conducted in November 2020 for clinical trials and real-world-evidence studies indexed in MEDLINE and Embase (via Ovid SP) or presented at three relevant conferences (2018-2020). Only studies reporting the mean/median AboBoNT-A dose or a clear dose range for a specific muscle were considered. Average muscle volume in cm^3 was determined for each muscle as reported by Holzbaur et al for upper limb[5] and Handsfield et al for lower limb[6]. Average AboBoNT-A doses (mean or median values depending on data availability) were then plotted against the average muscle volume to explore interrelationships between these two variables.
Results: Of the 1,781 unique records identified from the electronic databases, 349 were accepted for full-text review following initial screening. Of these, 53 articles, plus 3 conference abstracts ultimately met the eligibility criteria, resulting in the inclusion of 49 unique studies across 56 publications. Sample sizes across studies ranged from 9 to 456, with most studies (36/49; 73%) enrolling fewer than 100 patients each. Most studies reported only on upper-limb spasticity (31/49; 63%), with fewer reporting only on lower-limb spasticity (14/49; 29%) or both (4/49; 8%). Regarding the upper limb, mean, median or fixed doses were most commonly reported for the flexor digitorum profundus (23 studies); biceps brachii, flexor carpi ulnaris, and flexor digitorum superficialis, 20 studies each); flexor carpi radialis (19); brachioradialis (15); and pectoralis major (14). For the lower limb, doses were most commonly reported for the tibialis posterior (10 studies), and soleus, lateral, and medial gastrocnemius (8 studies each).
The dose vs muscle-volume plots suggested there was wide variation in practice regarding the AboBoNT-A dose given per muscle, with only a slight trend toward a relationship between AboBoNT-A dose and muscle size for both upper- and lower-limb muscles (Figure). When considering only studies of ≥50 patients, the range of mean/median doses was generally 100-200 U for small (<20 cm^3) and medium-sized (20-99 cm^3) muscles, and 200-250 U for large muscles (≥100 cm^3) of the upper limb; and 100-180 U for small muscles (<100 cm^3), 100-300 U for medium-sized muscles (100-399 cm^3), and 300-500 U for large muscles (≥400 cm^3) of the lower limb (all doses given in Speywood units).
Conclusions: AboBoNT-A doses reported in the literature varied considerably across muscles in patients with upper- or lower-limb spasticity, with only a moderate association with muscle volume. Input from clinical experts is warranted to inform recommendations for standardizing AboBoNT-A treatment initiation doses based on muscle size.
Funding: This study was sponsored by Ipsen.
Keywords: AbobotulinumtoxinA; Lower-limb spasticity; Upper-limb spasticity

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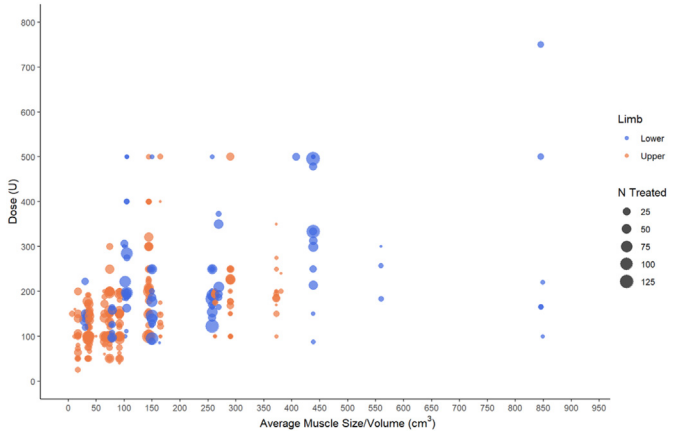

Fig. Mean/median AboBoNT-A dose* by average size of upper and lower limb muscles.

Comparison of Ultrasound and Electrical Stimulation Guidance for Botulinum Neurotoxin Injections: A Randomized Cross-Over Study

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Introduction: Botulinum toxin (BoNT) injection therapy is effective, established therapy for focal spasticity of the upper limb and is commonly used for upper limb focal dystonia. BoNT limb injections may be performed using anatomical landmarks, electromyography (EMG), electrical stimulation (E-stim), and ultrasound (US) guidance. A limited number of studies have attempted to compare these injection guidance techniques with inconclusive results.
Methods: Two-center, randomized, cross-over, assessor-blinded trial. Patients with focal hand dystonia or upper limb spasticity, on stable onabotulinumtoxinA (ona-BoNT-A) therapy for at least 2 previous injection cycles, were enrolled. Patients were randomized to either E-stim or US. Procedure-related discomfort at the end of the procedure and efficacy/adverse effects at one month after the procedure were assessed. At 3 months after the first procedure, the patients crossed over to the other guidance method, with similar repeat assessments. Repeated measures ANCOVA was used with linear mixed model covariate selection.
Results: Twenty subjects enrolled: 12 males, 10 upper limb spasticity and 10 dystonia. There was no difference between the two localization techniques in benefit or weakness after injection, as measured by both patient-reported visual analog scale (VAS, 0-100, least square (LS) mean 51.5 mm with US and 53.1 with E-stim) or clinician global impression of change (CGI). There was a significant difference in perceived discomfort, with E-stim perceived as more uncomfortable by patients, VAS LS mean 34.5 vs 19.9 for E-stim and US, respectively (P=0.0082). There was no difference in procedure duration between the two techniques. There were no serious adverse events.
Conclusions: E-stim and US guidance procedures yield similar benefit and weakness. E-stim was perceived as more uncomfortable by patients. Additional analyses, including the effects of each guidance method on procedure duration, correlation between muscle weakness and efficacy, and subgroup analyses by condition, are ongoing.
Keywords: Botulinum toxin; Electrical stimulation; Guidance; Ultrasound

IncobotulinumtoxinA in the Treatment of Musician's Focal Hand Dystonia: A Placebo-Controlled, Crossover Trial

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Introduction: The ability to reduce abnormal muscle activation in musicians' upper limb dystonia without causing debilitating weakness presents a significant technical challenge. Prior trials of botulinum toxin (BoNT) in musicians' dystonia have yielded variable results.
Methods: We conducted a double-blind, placebo-controlled, randomized, crossover study of incobotulinumtoxinA vs placebo in BoNT-naïve and -experienced patients. All patients were professional musicians with focal upper-extremity, task-specific dystonia affecting performance on their instrument. BoNT or placebo was administered at initial visit, with booster injections at weeks 2 and 4 at the discretion of the patient and injector. Cycle 2 began at week 12 with similar crossover visit schedules and repeat injections of either BoNT or placebo.
The injector and evaluator chose injection patterns and doses based on clinical opinion of the patient's musical performance and dystonic severity. Patients were evaluated at each visit with videotaping during performance, Medical Research Council (MRC) rating, dynamometry, and subjective patient assessment by visual analogue scale (VAS). Blood specimens were drawn at baseline and at final visit to test for the presence of neutralizing antibodies. Video segments were randomized to order, and two independent expert raters were given score sheets using a seven-point Clinical Global Impressions (CGI) scale and asked to rate dystonia severity and musical performance for each video segment compared to a reference tape of the patient at enrollment.
The primary outcome measure was the change in blinded dystonia rating at week 8 compared to baseline. Secondary outcome measures included patient-rated questionnaires, motor strength testing utilizing the MRC scale, dynamometry of the finger/wrist/elbow flexors to document any treatment-induced weakness, and patient VAS rating of overall dystonia severity.
Results: Twenty-one patients were randomized (BoNT-naïve:13; BoNT-experienced: 8). Nineteen patients completed both cycles; 2 participants discontinued after cycle 1 and have been excluded from the analysis. Eleven patients received placebo in cycle 1 and active drug in cycle 2 (PA). Ten patients received active drug in cycle 1 and placebo in cycle 2 (AP).
P value was based on the Cochran-Mantel-Haenszel test using the ridit score option.
Analysis of the primary outcome measure for cycle 1, week 8 in comparison to baseline video dystonia rating revealed superiority of active BoNT-A vs placebo (P=0.04), and overall musical performance (P=0.027).

Neutralizing antibody testing remains in progress. Mild weakness by dynamometry was seen, although clinically significant weakness was rare.
Conclusions: The placebo-controlled, double-blind, crossover design, with further blinded ratings of randomized, ordered videos by two expert raters, sets a high standard for efficacy. Despite the small sample size, this preliminary study demonstrated statistically significant efficacy of BoNT injections in musicians' dystonia. Patients were offered treatment on conclusion of the trial in open-label fashion, and the booster paradigm clearly allowed optimization of muscle and dose selection. This trial suggests that tailoring the use of BoNT to fit specific needs of elite performers yields clinically meaningful results and may inform future studies.
Keywords: Botulinum toxin; Focal dystonia; Musicians' dystonia

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A Phase 3, Open-Label, Multicenter Trial to Evaluate the Long-Term Safety and Efficacy of Repeat Treatments of DaxibotulinumtoxinA for Injection in Adults With Isolated Cervical Dystonia

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Introduction: The objective of this study was to evaluate the long-term safety of multiple continuous treatments of DaxibotulinumtoxinA for Injection (DAXI) in subjects with cervical dystonia (CD). DAXI is a novel botulinum toxin type A formulation with a proprietary peptide excipient. ASPEN-1, a Phase 3 double-blind, placebo-controlled study demonstrated efficacy and extended duration of effect in CD. We report results of open-label safety and efficacy from a large, multicenter, Phase 3, open-label trial of up to 4 continuous treatments of DAXI.
Methods: Adults with moderate-to-severe CD were recruited from study centers in the US, Canada, and Europe, who were enrolled in ASPEN-1. Additional botulinum toxin (BoNT) treatment-naïve or -experienced adult subjects not enrolled in ASPEN-1 were also enrolled. At baseline, using predefined criteria, the investigator selected an open-label dose of DAXI 125 U or 250 U for the subject based on clinical factors, CD severity, and BoNT treatment history. The investigator identified muscles for injection based on clinical presentation (eg, head, neck, and shoulder position, localization of pain, and muscle hypertrophy). The volume injected per muscle for each dose level was within a predefined dose range per muscle. During each treatment cycle subjects had visits at Weeks 4, 6, 12, and every 4 weeks thereafter up to Week 52 or end of study to assess safety and tolerability. Retreatment was based on loss of 80% of peak treatment effect, or subject request and investigator judgment.
Results: Of the 387 subjects screened, 358 subjects were enrolled: 272 (76.0%) rollover from ASPEN-1 and 86 (24.0%) de novo. Topline safety and efficacy results will be presented.
Conclusions: Topline safety and efficacy results will be presented.
Funding: The study was funded by Revance Therapeutics, Inc.
Keywords: Botulinum toxin type A; Cervical dystonia; DaxibotulinumtoxinA; Treatment

Characterization of Botulinum Neurotoxin Type A Local Muscular vs Antispastic Action in Rats

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Introduction: Botulinum toxin type A (BoNT-A) has been widely used as therapy for spasticity and dystonia, presumably due to its long-term effect within the injected muscles. However, beneficial effects of BoNT-A do not necessarily correlate with the degree and duration of peripheral flaccid paralysis. The aim of the present study was to examine this clinical finding by characterizing the antispastic activity of BoNT-A in relation to its lasting muscular effects in experimental animals.
Methods: Rats were injected into the gastrocnemius muscle with different BoNT-A doses (5, 2, and 1 U/kg). To assess the contribution of central BoNT-A actions, in separate experiments, BoNT-A 5 U-/kg and 2-U/kg treatments were combined with lumbar intrathecal (i.t.) BoNT-A–neutralizing antiserum. The extent and duration of flaccid paralysis was monitored by digit abduction score (DAS) assay, while muscle atrophy was assessed by estimation of lower leg cross-sectional area. After complete DAS recovery, the animals were injected into the BoNT-A–pretreated muscle with low dose intramuscular tetanus toxin (TeNT) to induce local hind-limb rigidity (49-62 days [d] after BoNT-A injection) and assessed for resistance to ankle dorsiflexion. At the end of the experiments (up to 78 d post BoNT-A), the presence of muscular BoNT-A-cleaved synaptosome-associated protein of 25 kDa (SNAP-25) was examined by immunohistochemistry.
Results: BoNT-A evoked a dose-dependent impairment of DAS that recovered after 2-5 weeks. Muscle atrophy, resulting in approximately 35% reduction of leg cross-sectional area after 2 and 1 U/kg doses, and slightly higher reduction (approximately 47%) after the 5-U/kg dose, showed no signs of recovery by the end of the experiment. TeNT-evoked hypertonia was similarly reduced by 5 and 2 U/kg doses, and less prominently in rats treated with the lowest BoNT-A dose (1 U/kg) or higher-dose BoNT-A + i.t. antitoxin. Reduction of TeNT-evoked rigidity in 1 U/kg and BoNT-A + antitoxin groups seemingly correlated with the extent of BoNT-A–mediated muscle atrophy. The presence of products of the toxin's enzymatic activity in neuromuscular junctions was evident up to 78 days after all BoNT-A doses and not affected by the antitoxin.
Conclusions: Muscular injections of BoNT-A induce a shorter, dose-dependent flaccid paralysis, as well as long-term muscle atrophy and toxin enzymatic activity in injected muscle. Although muscular effects of BoNT-A persist for up to 78 days after its peripheral injection, antitoxin experiments suggest that central actions of BoNT-A contribute to its lasting antispastic effect.
Funding: Croatian Science Foundation (project ID: UIP-2019-04-8277). Antibody to cleaved SNAP-25 and BoNT-A antiserum were kindly provided by Dr. Thea Sesardic (National Institute for Biological Standards and Control, UK).
Keywords: Anti-spastic action; Atrophy; Botulinum toxin type A; Flaccid paralysis; TeNT-evoked muscle hypertonia

Botulinum Neurotoxin Type A Prevents Ultraviolet-Induced Hyperpigmentation: A Randomized Controlled Trial

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Introduction: Hyperpigmentation disorders are common dermatological conditions that can have substantial impact on quality of life. Recently, botulinum neurotoxin type A (BoNT-A) has been shown to be protective against UVB (type B ultraviolet rays)-induced hyperpigmentation in in vitro and animal models[1]. This prospective, double-blind, randomized, controlled trial[2] was carried out to investigate the effect of treatment with BoNT-A on subsequent UVB-induced hyperpigmentation in human subjects.
Methods: Fifteen healthy subjects older than 18 years old were enrolled in the study. Four separate areas of the abdomen were each randomized to receive intradermal injections of either 0.3 mL BoNT-A (IncobotulinumtoxinA; Merz Pharmaceuticals GmbH, Frankfurt am Main, Germany) at dilutions of 1:2.5, 1:5, 1:7.5, or normal saline (control). Fourteen days after the injections, experimental sites were irradiated by local broadband UVB to induce hyperpigmented spots. The lightness index was measured at each experimental site using a colorimeter, and hyperpigmentation scores were assessed using a 10-point visual analogue scale by a blinded physician and by study subjects.
Results: All subjects completed the study and were included in the analysis. One week following UVB irradiation, BoNT-A treated sites of all dilutions had a significantly lower degree of hyperpigmentation compared to the control site, as assessed by both the mean lightness index, and by blinded physician and subject assessment. No difference was observed between BoNT-A dilutions using the mean lightness index.
Conclusions: Intradermal BoNT-A was protective against subsequent UVB-induced hyperpigmentation in human subjects.
Funding: None to declare.
Keywords: Clinical trial; Efficacy; IncobotulinumtoxinA; Hyperpigmentation

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Botulinum Toxin in Aesthetic Medicine

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Protocols and standardized treatments play an increasingly important role in the development of aesthetic medicine as a medical field. A reference guide that the aesthetic clinician can consult during daily practice is a practical approach and a valuable tool for their clinic that will benefit their patients (van Loghem, 2020). The guide includes background information about botulinum toxin, including mechanism of action, an overview of the available products, reconstitution, and patient consultation. It emphasizes practical issues, such as injection patterns, relevant anatomy, and avoiding and treating complications. This guide also introduces an incobotulinumtoxinA protocol that uses only half the recommended dose of other cited protocols. This results in a reduced duration of treatment effect, so patients are treated more frequently (every 2-3 months instead of every 4). The overall effect is a non-blocked, more subtle, refreshed, and relaxed result. OnabotulinumtoxinA and abobotulinumtoxinA are also covered, including recommended doses and injection schemes. The protocols included are based on consensus recommendations and publications from professional organizations. UMA Institute physicians have adjusted the injection patterns and doses with the intent of optimizing efficacy and minimizing side-effect risk for the upper, middle, and lower third of the face and non-facial body areas.
Funding/Disclosures: Jani van Loghem serves as a consultant to Merz Aesthetics.
Keywords: Botulinum toxin type A; Hyperdilute; IncobotulinumtoxinA; Reference guide; Treatment protocols

Reference

van Loghem J. *Botulinum Toxin in Aesthetic Medicine.* Amsterdam, The Netherlands: UMA Institute; 2020.

Effect of Botulinum Toxin Type A on Fibroblast Contraction

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Introduction: Reports indicate intradermal administration of botulinum toxin type A (BoNT/A) can induce a dermo-lifting effect.[1] How BoNT/A achieves this lifting effect remains unclear, but fibroblast contraction has been proposed as one possible mechanism.[2] The aim of this study[3] was to determine the effect of BoNT/A on dermal fibroblast contraction.
Methods: Normal human dermal fibroblasts were mixed with onabotulinumtoxinA (ONA), abobotulinumtoxinA (ABO), prabotulinumtoxinA (PRABO), incobotulinumtoxinA (INCO), and letibotulinumtoxinA (LETI) in dilutions of BoNT/A used in real-world clinical practice. To assess fibroblast contraction, 50 fibroblasts per dilution were photographed and the length of these cells was measured every 2 hours from baseline (0 hours) to 12 hours post-treatment.
Results: Fibroblast length was not significantly decreased by incubation with ONA at any of the tested timepoints or dilutions. Incubation with ABO at a dilution ratio of 1:7 notably decreased fibroblast lengths after 2 hours and significantly decreased fibroblast lengths after 10-12 hours. Incubation with PRABO at 1:7, 1:8, 1:9, and 1:10 dilution decreased fibroblast length, most rapidly at 1:7 and 1:8 dilutions. Incubation with INCO at 1:8, 1:9, and 1:10 dilution resulted in nearly immediate decreased fibroblast lengths, while incubation of INCO at 1:7 dilution decreased fibroblast lengths after 2-4 hours. Incubation with LETI decreased fibroblast lengths at all dilutions except 1:9, with near-immediate effects at 1:6, 1:7, 1:8, and 1:10; 1:4 dilution with LETI decreased fibroblast length from 1 hour.
Conclusions: Different commercial preparations of BoNT/A caused different fibroblast contractions in vitro. Product selection and dilution used may affect the clinical outcomes of intradermal injection of BoNT/A for face lifting. Considering that pan-facial toxin injections require a total dosage of 50-60 U, in the author's opinion 1:6 is the most practical dilution for clinical use. Using fewer than 50 U for pan-facial treatments produces no visible lifting effects at 2 weeks, even if some lifting is observed immediately post-injection. While higher dilutions for LETI and PRABO were found to cause fibroblast contraction, most of these dilutions were greater than 1:6 and are, therefore, expected to be clinically effective only in the short-term. INCO was the only toxin that significantly shortened fibroblasts almost immediately at all dilutions investigated. Further work is needed to establish INCO's capacity for significant and near-immediate fibroblast contraction in clinical settings, but this could potentially result in rapid onset of lifting effects that may coincide with the entire duration of efficacy.
Funding: RW is a paid consultant for Merz Asia Pacific Pte. Ltd.

Keywords: Fibroblast contraction; IncobotulinumtoxinA; Lifting effect

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Treatment Considerations for Bilateral Lower Extremity Spasms in a Spinal Cord Injury Patient

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Introduction: Among the common comorbid conditions associated with spinal cord injuries, spasms rank among the most painful and restrictive. Spasms may be innate in nature—a daily occurrence that spinal cord injury (SCI) patients must learn to live with, utilizing an oral, injectable, or infusion medication regimen. Alternately, new onset spasming may be a symptom of a new insult. In this case, an uncommon spasm pattern is targeted using chemodenervation of atypically injected muscle groups for therapeutic relief.
Methods/Case Review: A 70-year-old male veteran with T8 American Spinal Injury Association (ASIA)-A paraplegia presented with bilateral lower extremity spasms increasing in frequency and severity for three months.
The patient utilized a series of oral agents for pain and spasticity, along with an intrathecal baclofen pump, resulting in full control of pain and spasms for many years. The patient denied any acute injuries or other changes in the level of care of his spinal cord injury. The patient reported an increase in oral medication regimen and baclofen pump daily dosing over the course of the three months, yielding no benefit. Thus, full investigative measures, including imaging, labs, and serial physical examinations, were initiated by health care providers after all medications (oral and intrathecal) were returned to previous, baseline doses.
In the interim, providers performed chemodenervation of atypically injected muscle groups with abobotulinumtoxinA to provide symptomatic relief during extensive ongoing work-up and evaluation.
The patient reported resolution of spasms thereafter, but with a severe increase of neuropathic pain.
A diagnosis of severe stenosis of the spinal canal with complete effacement of the traversing cerebrospinal fluid at the L4-L5 level was found on a lumbar spine magnetic resonance imaging scan. The patient subsequently underwent L4 laminectomy and reported improvement of neuropathic pain and residual spasming.

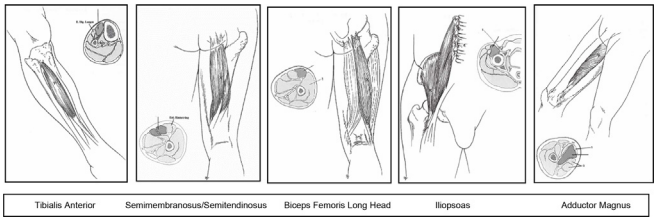

Fig 1. Anatomy of muscles injected.

Muscle Injected	Units
Right tibialis anterior	150
Right hip adductors	250
Right hamstrings	250
Right iliopsoas	100
Muscle Injected	Units
Left tibialis anterior	150
Left hip adductors	250
Left hamstrings	250
Left iliopsoas	100

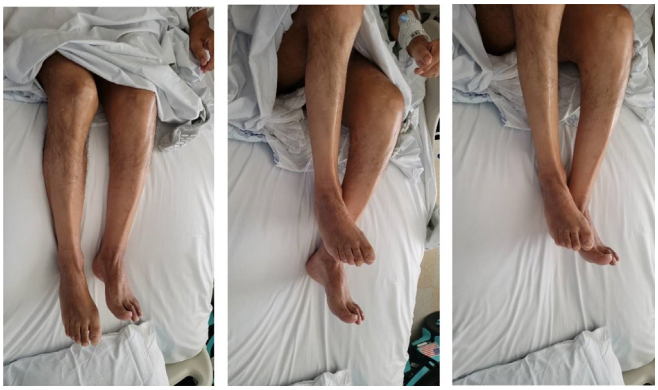

Fig 2. Patient's flexion-oriented spasm pattern.

Results: Chemodenervation possesses great potential in the medical field outside of its FDA-approved use for treatment of spasticity in upper and lower extremity muscle groups. While flexion-focused muscle groups of the lower extremities are not within the scope of approved use, alongside spasms themselves, this patient benefitted from treatment for a short time during ongoing evaluation.
Conclusions: Trainees may find solace and familiarity with the tried-and-true chemodenervation treatments and injection sites for their patients. Experienced botulinum toxin injectors may be more open to expanding their scope of practice. No matter the level of training, patient-focused care is ultimately of the utmost importance for all providers.
Keywords: Atypical pattern; Atypical targets; Off-label; Spasm; Spinal cord injury

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Subcutaneous Chemodenervation With Botulinum Toxin Type A for Amputees With Residual Limb Hyperhidrosis: A Case Series

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Introduction: Many limiting factors affect an amputee's ability to utilize a

prosthesis. Hyperhidrosis or increased sweating in the residual limb is a significant barrier to prosthetic use. Application of botulinum toxin to treat hyperhidrosis in the amputee population is not FDA-approved, and adequate, in-depth research of this treatment strategy is limited yet warranted. In this case series, three patients with lower extremity amputations undergo subcutaneous botulinum toxin type A injections for hyperhidrosis of their residual limbs and are evaluated 90 days after.

Methods:

Step 1:

We identified three appropriate patients from the James A. Haley Veterans' Affairs Hospital's Spasticity Clinic previously receiving subcutaneous botulinum toxin type A injections for hyperhidrosis.

Step 2:

Patients were consented prior to procedure. The botulinum toxin type A agent was diluted with normal saline. Each patient's residual limb was cleaned with an alcohol prep pad. Botulinum toxin type A was evenly administered subcutaneously in an approximately 1 cm x 1 cm grid-like pattern throughout the residual limb (Figure). Prior to leaving, patients were asked to rate their hyperhidrosis using the Hyperhidrosis Disease Severity Scale (HDSS).

Step 3:

Patients were instructed to resume normal activities with residual limb and scheduled to return to clinic for 90-day follow-up appointment for re-assessment.

Step 4:

Upon return to clinic 90 days after, patients were asked two standard questions:

1. "What percent reduction in sweating have you observed?"
2. "How much longer, on average, are you able to wear your prosthesis daily?"

Patients were again asked to rate their hyperhidrosis using the HDSS.

Results: At 90-day follow-up, an average decrease of 2 points in the HDSS was observed. Additionally, patients reported a 65% reduction in sweating and an average of 1.6 to 2.6 hours of increased time in the prosthetic limb (Table).

Conclusions: Hyperhidrosis related to prosthetic use represents a major barrier to comfort, safety, and overall prosthesis utilization. Although subjective measures remain the mainstay of measuring efficaciousness, efforts towards providing a safe, reliable, and sustainable treatment for hyperhidrosis continue. Chemodenervation remains an uncharted and unproven resource for amputees, yet one worth continued investigation.

Keywords: Amputee; Chemodenervation; Hyperhidrosis; Residual limb

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Table
Results

	Initial HDSS Score	Follow-Up HDSS Score	Percent Improvement	Increase of Time in Prosthesis
Patient 1	4	2	75%	2-3 hours
Patient 2	3	1	50%	1-2 hours
Patient 3	3	1	70%	2-3 hours

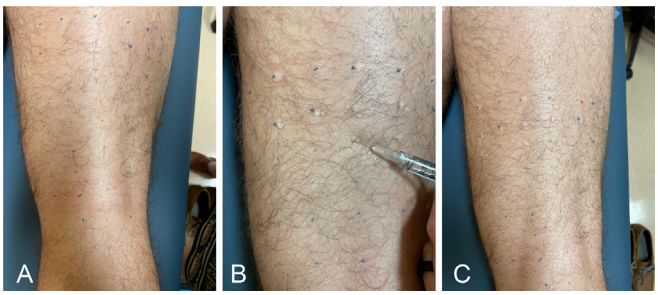

Fig. A. 1 cm x 1 cm grid pattern template on residual limb. **B.** Subcutaneous injections are performed. **C**. Subcutaneous wheals are noticeably visible thereafter.

Hyperhidrosis Disease Severity Scale.

	Extent of Excessive Sweating–Related Impairment of Daily Activities	Severity Rating
1	My sweating is never noticeable and never interferes with my daily activities	Minimal
2	My sweating is tolerable but sometimes interferes with my daily activities	Mild
3	My sweating is barely tolerable and frequently interferes with my daily activities	Moderate
4	My sweating is intolerable and always interferes with my daily activities	Severe

Evaluation Following the Switch from AbobotulinumtoxinA to IncobotulinumtoxinA of Dystonia Service in an Outpatient Setting

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Introduction: The Oxford University hospital policy on botulinum toxin brand changed and this required a switch from abobotulinumtoxinA to incobotulinumtoxinA in the Dystonia Service.

Different models of change were considered which resulted in the decision to make a rapid switch using a published dose ratio (Grosset, 2015). This was done cautiously to reduce the potential for adverse events and possible treatment failures. Presented here is an evaluation of the switch experience from this novel situation, which could benefit future cohort-level treatment decisions

Methods: Patients were presented with written information outlining the rationale for switching and were verbally briefed before the last injection of abobotulinumtoxinA.

A dose ratio of 4:1 was deployed on the basis of available evidence.

The outcomes were evaluated retrospectively using a sequential sample of 100 patients. Diagnosis, dose, time to next injection, response rating, and patient-reported adverse events for 2 pre-switch cycles and 2 post-switch cycles was recorded.

The patient's response to treatment in combination with the clinician's

assessment was given a rating of very good (VG), good (G), partial (P), and no response (N), and was recorded on the clinic form.
The standard reinjection interval schedule is 10 to 14 weeks. Patients who had any injection cycle >16 weeks were excluded as the delays were due to nonclinical reasons.
Results: Seventy-eight patients were analysed. Patients were informed of the intention to switch formulations immediately before their final abobotulinumtoxinA injection. At the following injection visit, there was a 17% decline in patients reporting a positive response to the new product. After this initial drop, the response to treatment improved for subsequent injection cycles. After 2 post-switch injections of incobotulinumtoxinA, the dose was maintained or reduced in 54% of patients and increased in 46% of patients. Dose ratios ranged from 2.4:1 to 5:1 and patients with a diagnosis of either Meige syndrome or cervical dystonia and dystonic head tremor were most likely to have had their doses changed over the 2 post-switch cycles.
The percentage of treatment responses rated very good and good pre-switch were: 1st cycle, 88%; 2nd cycle, 71% and post-switch: 1st cycle, 72%; 2nd cycle, 74%.
One patient reported difficulty with swallowing after the first incobotulinumtoxinA injection. Subsequent EMG-guided injections caused no further adverse events.
Conclusions: This study suggests a structured switch from abobotulinumtoxinA to incobotulinumtoxinA could be achieved without significant adversity to patients.
A dose ratio of 4:1 is an appropriate starting point; however, doses need to be individually tailored during subsequent visits.
Overall, the switch was well tolerated and most patients reported no change in treatment response. The drop in treatment response seen in cycle 2 coincided with when patients were informed of the future change in treatment and could be due to the psychological response to change, as no other explanation was found.
Funding: A grant from Merz Pharma UK was provided for medical writing support.
Keywords: AbobotulinumtoxinA; Dystonia; IncobotulinumtoxinA; Toxin switch

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Ultrasound-Guided Nerve Blocks to Improve Muscle Selection for Spasticity Management

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Introduction: There is a need to improve the way we address interventional spasticity management. Twenty-five percent of physicians felt their national label prevented adequate spasticity management due to the amount of botulinum toxin (BoNT) allowed per injection cycle.[1] The optimal care of spasticity should include identification of those muscles that are primarily responsible for each spastic pattern. The differentiation between a treatable deformity due to spasticity and a fixed contracture is often not possible on physical examination alone. Genet noted that diagnostic nerve blocks (DNB) could improve muscle selection for BoNT injection or nerve selection for neurectomy.[2] Diagnostic nerve blocks have also proven helpful in distinguishing between muscle spasticity and tendon retraction. DNB with lidocaine have been used for decades, particularly in France, where the 2019 French Clinical Guidelines outlined their importance in spasticity management.[3] The classical approach involved localization using surface anatomy followed by percutaneous stimulation of the nerve. This blind approach is challenging due to the variability of individual nerve anatomy and the proximity of blood vessels to the nerve. Munin et al demonstrated that less fluid is used when ultrasound (US) guidance is utilized in injecting BoNT for spasticity.[4] We propose using ultrasound to improve the speed of the procedure, reduce the volume of injected anesthetic, and modernize the technique.
Methods: There are few published guides that describe motor nerve localization, with much of the literature on ultrasound-guided nerve blocks being present in the anesthesia literature. Building on the available surface anatomy texts used in spasticity management, we applied the principle of the nerves being found adjacent to the blood vessels. In addition, Doppler ultrasound was used to confirm the location and electrical stimulation (E-Stim) was applied at low amplitudes of 0.8 milliseconds to avoid muscle stimulation.
Results: Scanning of each key peripheral nerve—lateral and medial pectoral, thoracodorsal, median, ulnar, radial, femoral, obturator, sciatic, and tibial—for spasticity, in addition to use of surface landmarks and confirmation with E-Stim produces a consistent method for finding each nerve. Doppler flow imaging improved the ability to find the structures in patients with fibrosis. The blood vessels are more readily avoided with this method. Video images of each nerve were collected, and labelled diagrams of each nerve and its branches were made.[5] We have created a library of images for publication and education (Figure).
Conclusions: DNB can be a rapid procedure with the use of US guidance. The use of Doppler and electrical stimulation allows for exquisite localization. This procedure enables a prediction of how spasticity interventions, such as BoNT, may reduce spasticity for the patient. The use of ultrasound-guided DNB can improve spasticity outcomes through optimal muscle selection, as well as differentiate a treatable deformity from contracture. The clinical application of this difference is shown to allow for increased dosing into a target muscle vs the need for surgery or neurotomy to manage a contracture. Creation of a guide to nerve anatomy based on US mapping will enable dissemination of the technique.
Keywords: Doppler; Lidocaine; Nerve block; Spasticity

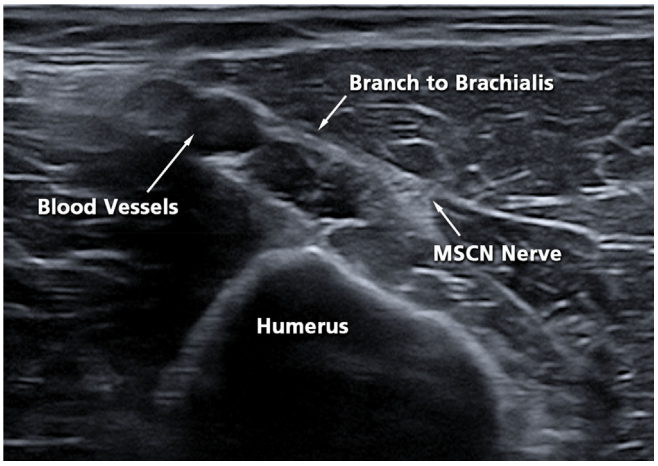

Fig. The musculocutaneous (MSCN) nerve to the brachialis muscle from the medial arm.

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Benefit of Multiple IncobotulinumtoxinA Injections for Pain Reduction in Adult Patients With Limb Spasticity: An Analysis of Pooled Data From Phase 2 and 3 Studies

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Background: Repeated treatment with incobotulinumtoxinA (incoA) has shown pain-relieving benefits in patients (pts) with limb spasticity in individual studies.
Objective: To assess pain relief after multiple incoA injections in a large sample of pts with limb spasticity–associated pain (SAP) using pooled data from six phase 2/3 studies (four placebo-controlled).
Methods: Adults with upper limb SAP received up to 4 incoA injections administered at 12- to 14-week intervals (injection cycles [ICs] 1-4; total observation period up to 56 weeks). Only IC 1 was placebo-controlled. Pain severity was assessed at control visits (CVs; 4 weeks after each injection) using the Disability Assessment Scale (DAS; pain scores ranging from 0 [no pain] to 3 [severe pain]). The proportions of pts with a response (defined as a reduction by ≥1 point in the DAS score from baseline to each CV) and with a complete response (DAS pain score=0) were assessed at every CV. Data were descriptively analysed. As placebo-treated pts in IC 1 received incoA in subsequent cycles, they contributed data to the appropriate incoA CVs 1-4.
Results: Five hundred seventeen pts with SAP at baseline were included in this analysis (517, 389, 347, and 184 pts at CVs 1-4, respectively). Response rates increased over time, being 53.0%, 62.7%, 66.9%, and 71.7% at CVs 1-4, respectively. Likewise, the proportion of pts with complete response increased over time, being 27.7%, 37.8%, 41.5%, and 42.9% at CVs 1-4, respectively.
Conclusions: In pts with upper limb SAP, treatment response rates were sustained and showed a cumulative effect over 56 weeks after multiple incoA injections, with a complete pain relief in >40.0% of pts. Results support the use of incoA in reducing upper limb SAP in affected adults.
Funding: This research was supported by Merz Pharmaceuticals GmbH.
Keywords: IncobotulinumtoxinA; Limb spasticity; Pooled analysis; Repeated treatment; Sustained pain relief

Pain Reduction in Adult Patients With Limb Spasticity Following a Single IncobotulinumtoxinA Injection: An analysis of Pooled Data From Phase 2 and 3 Studies

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Background: IncobotulinumtoxinA (incoA) has shown pain-relieving benefits in patients (pts) with limb spasticity in individual studies; data from sizeable pt cohorts are lacking.
Objective: To assess pain relief in a large cohort of incoA-treated pts with spasticity-associated pain (SAP) using pooled data from mostly placebo-controlled phase 2/3 studies.
Methods: Pain severity was assessed with the Disability Assessment Scale (DAS; 0-3) in adults with upper limb SAP. A ≥1 point reduction in the DAS pain score from baseline (BL) to 4 weeks was defined as response. Between-treatment group response rates (overall and by BL pain severity – DAS mild, moderate, severe) and the proportion of pts with complete pain relief (DAS pain score=0) at 4 weeks after 1 injection of incoA or placebo were analyzed using χ^2 test. Overall, between-group response rate differences were analyzed using logistic regression (presented as odds ratio [OR] and 95% confidence interval [CI]).
Results: Five hundred forty-four (incoA: 415, placebo: 129) pts reported SAP at BL. At 4 weeks, a significantly higher proportion of incoA- vs placebo-treated pts achieved a response (52.1% vs 28.7%; $P<0.0001$). IncoA-treated pts were more likely to achieve pain response vs placebo-treated pts (OR 2.6 [95% CI: 1.6-4.2]; $P<0.0001$). Irrespective of BL pain severity, significantly higher response rates were observed with incoA vs placebo at 4 weeks ($P<0.02$ all comparisons). Complete pain relief was achieved by significantly more incoA- vs placebo-treated pts at 4 weeks (27.1% vs 12.4%; $P=0.0006$).
Conclusion: Pts receiving incoA vs placebo are significantly, by 2.6 times, more likely to achieve reduced upper limb SAP, irrespective of baseline pain severity, at 4 weeks post-injection, thus supporting use of incoA in this setting.
Funding: This research was supported by Merz Pharmaceuticals GmbH.
Keywords: IncobotulinumtoxinA; Limb spasticity; Pain relief; Pooled analysis

Content Validation of a Post-Stroke Spasticity Risk Classification System: Cognitive Interviews With Clinicians

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Introduction: To improve early identification and assessment of patients at risk of developing post-stroke spasticity (PSS), Global Spasticity Experts collaborated to develop the PSS Risk Classification System. This tool provides clinicians with a framework and criteria for screening PSS patients to identify appropriate treatment referrals, such as referral for botulinum toxin treatment, in a timely manner. This study aimed to validate the content of the initial version to ensure its relevance and appropriateness for clinicians in referring PSS patients to a specialist.
Methods: Cognitive interviews were conducted with clinicians who treat PSS to assess the PSS Risk Classification System and its domains: a) urgent referral, b) routine referral, and c) periodic monitoring. Verbatim transcripts were analysed using qualitative data analysis software (ATLAS.ti) to

assess clinicians' understanding of the tool. Eligible clinicians did not inject onabotulinumtoxinA to treat spasticity, and did not participate in the development of the PSS Risk Classification System.

Results: Thirteen clinicians (US n=5; Canada n=8) were interviewed and expressed favourable impressions and deemed the tool fit for purpose with some revisions. Recommended revisions were minor and focused on language to increase clarity and relevance through simple text revisions and addition of verbiage, as well as revisions to layout and presentation for print/digital versions.

Conclusions: Clinician feedback supports the relevance and appropriateness of the PSS Risk Classification System. Clinicians requested that this tool be available in multiple formats, and available to occupational therapists, physiatrists, and primary care providers. Next steps include implementing revisions and confirming inter-rater reliability of the updated tool in the real world.

Keywords: Early identification; Post-stroke spasticity

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TOXINS 2022 Abstracts Part II

Protocol for the SMART Study: Effectiveness and Safety of SMART AbobotulinumtoxinA Therapy in Patients With Chronic Post-Stroke Upper Limb Spasticity in a Real-World Setting

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Introduction: Current DGN (German Society for Neurology) guidelines recommend botulinum toxin type A (BoNT-A) injections for patients with focal spasticity. However, only about 10% of patients with disabling spasticity after stroke are treated according to these guidelines. The SMART (spastic muscle palpation by anatomic landscape for BoNT-A injection to reduce muscle tone) injection concept is designed for abobotulinumtoxinA (aboBoNT-A, Dysport®) treatment in pre-selected patients living with simple patterns of adult upper limb spasticity (ULS) with mainly passive goal setting. Using this approach, the injection can be simplified, making BoNT-A treatment more accessible, especially in the practices of office-based neurologists.

The study will assess the effectiveness of aboBoNT-A SMART therapy for ULS using the Disability Assessment Scale (DAS) for the Principal Target of Treatment (PTT), Modified Ashworth Scale (MAS) in the Primary Target Muscle Group (PTMG), and evaluating pain and quality of life. The use of aboBoNT-A SMART therapy and its safety in a real-world setting will also be described.

Methods: The SMART study (ClinicalTrials.gov Identifier: NCT05224349) is a prospective, multicenter, observational study that will include 116 patients suffering from chronic post-stroke ULS. It will cover two injection cycles and will be conducted in Germany. The primary endpoint will be the change in DAS scores in the PTT for the upper limb at Visit 3 (12-16 weeks after injection at Visit 1) vs baseline.

Results: Full results of the SMART study will be available in 2024.

Conclusion: The SMART study will be the first prospective observational study to evaluate the effectiveness and safety of aboBoNT-A SMART therapy for ULS and it aims to provide proof of concept for the SMART injection for ULS.

Funding: The SMART study is funded by Ipsen.

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Keywords: AbobotulinumtoxinA; Clinical trial; Observational study; Real-world evidence; Upper limb spasticity

Reference

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Botulinum Neurotoxin Type A Directly Affects Sebocytes and Modulates Oleic Acid–Induced Lipogenesis

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Introduction and Objectives: Excess sebum (seborrhea) results in oily skin and is associated with large pore size and acne. Studies in healthy, seborrheic volunteers have reported that intradermal injection of commercial preparations of botulinum neurotoxin type A (BoNT/A; onabotulinumtoxinA [onabotA], abobotulinumtoxinA [abobotA], and incobotulinumtoxinA [incobotA]) reduced sebum production, and thus, skin oiliness and pore size.[1-5] The mechanism for these effects has not been fully elucidated; however, several theories involving direct or indirect effects of BoNT/A on neuronal cells and/or dermal cells (eg, sebocytes) have been proposed. In the present study, we evaluated the direct effect of BoNT/A complex, a commercial preparation of BoNT/A (onabotA), and BoNT/A variants on sebocyte lipogenesis using in vitro sebocyte cell models.

Methods: Human primary sebocytes or human immortalized sebocyte cells (SEB-1)[6] were treated with a lipogenesis stimulator (oleic acid [OA]) alone or with onabotA (100 units in 0.25 mL) or BoNT/A complex (5 pM-100 nM). Sebocyte differentiation and sebocyte lipid content was assessed using the lipid droplet fluorescence assay. To examine the mechanism of action of BoNT/A on sebocytes, SEB-1 cells were also treated with native 150-kDa BoNT/A, recombinant wild type and mutant variants of BoNT/A binding domain, or the selective fibroblast growth factor receptor (FGFR) 1-3 inhibitor BGJ398.

Results: Picomolar concentrations of BoNT/A complex, onabotA, and BoNT/A variants modulated sebocyte lipogenesis. Specifically, BoNT/A inhibited lipogenesis in OA-stimulated primary sebocytes and SEB-1 cells. BoNT/A had no effect on unstimulated primary sebocytes and increased lipogenesis in unstimulated SEB-1 cells. Wild type BoNT/A binding domain also induced lipogenesis in unstimulated SEB-1 cells, while mutant variants of BoNT/A binding domain, with either no binding to gangliosides or reduced binding to synaptic vesicle glycoprotein 2 (SV2), had no or reduced effect, respectively. Pretreatment with BGJ398 attenuated the lipogenic effects of BoNT/A and BoNT/A binding domain.

Conclusions: The data support clinical observations of BoNT/A modulating clinical sebum levels and suggest that this occurs, at least in part, via a direct effect involving the BoNT/A binding domain and sebocyte cell surface receptors.

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Keywords: BoNT/A; OnabotulinumtoxinA; Sebocytes; Seborrhea

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Preclinical Assessment of OnabotulinumtoxinA for the Treatment of Mild Traumatic Brain Injury–Related Acute and Persistent Cephalic Allodynia

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Introduction: We investigated possible effects of onabotulinumtoxinA (onabotA) in a murine model of acute and persistent post-traumatic headache.
Methods: Mild traumatic brain injury (mTBI) was induced with a weight drop method. Periorbital and hind paw cutaneous allodynia were measured for 14 days. Mice were then exposed to bright light stress, and allodynia was reassessed. OnabotA (0.5 units) was injected subcutaneously over the cranial sutures at different post-injury time points. Experiments were conducted during the light cycle in accordance with the ARRIVE reporting guidelines and with approval of the Mayo Clinic Institutional Animal Care and Use Committee.
Results: After mTBI, mice exhibited periorbital and hind paw allodynia that lasted for approximately 14 days. Allodynia could be reinstated on days 14-67 by exposure to stress only in previously injured mice. OnabotA administration at 2 hours (h) after mTBI fully blocked both transient acute and stress-induced allodynia up to day 67. When administered 72 h post-mTBI, onabotA reversed acute allodynia, but only partially prevented stress-induced allodynia. OnabotA administration at day 12, when initial allodynia was largely resolved, produced incomplete and transient prevention of stress-induced allodynia. The degree of acute allodynia correlated positively with subsequent stress-induced allodynia.
Conclusions: mTBI induced transient cephalic allodynia followed by long-lasting sensitization and persistent vulnerability to a normally innocuous stress stimulus, respectively modeling acute and persistent post-traumatic headache. Administration of onabotA following the resolution of acute cephalic allodynia diminished the emergence of persistent cephalic allodynia, but the effects were transient, suggesting that underlying persistent mTBI-induced maladaptations were not reversed. In contrast, early onabotA administration fully blocked both acute cephalic allodynia as well as the transition to persistent cephalic allodynia, suggesting prevention of neural adaptations that promote vulnerability to chronification of pain. Additionally, the degree of acute allodynia was predictive of risk of chronic allodynia in this animal model.
Funding: These studies were funded by a grant from Abbvie to F.P.
Keywords: Acute post-traumatic headache; Botulinum toxin; Concussion; Mild traumatic brain injury (mTBI); Persistent post-traumatic headache; Post-traumatic headache

Treatment of Upper Facial Lines With OnabotulinumtoxinA Results in Long-Lasting Efficacy and Patient Satisfaction

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Introduction: A 1-grade improvement in facial line severity with onabotulinumtoxinA, such as a change on the Facial Wrinkle Scale (FWS) from severe to moderate, can have a meaningful impact on the treated individual, leading to high patient satisfaction. This study investigated the relationship between patient satisfaction and efficacy with onabotulinumtoxinA for treating upper facial lines (UFL), using data from four phase 3 registration studies.
Methods: A sub-analysis of pooled data from four pivotal phase 3 UFL clinical trials assessed the relationship between composite (investigator- and subject-assessed) ≥1-grade improvement on the FWS and patient satisfaction with onabotulinumtoxinA treatment in responders across the studies. Subanalyses of studies 098 (up to day 150) and 099 (up to day 120) assessed ≥1-grade improvement in the FWS at each visit with onabotulinumtoxinA 24 units (U) (n=528) or placebo (n=529) for crow's feet lines (CFL). Studies 142 and 143 (both up to day 180) assessed ≥1-grade improvement in the FWS at each patient visit with onabotulinumtoxinA 40 U (20 U forehead lines [FHL], 20 U glabellar lines [GL]; n=608) or 64 U (study 143 only; 20 U FHL, 20 U GL, and 24 U CFL; n=313), or placebo (n=257). Patient satisfaction was assessed using follow-up items 4 and 5 of the Facial Lines Satisfaction Questionnaire (studies 142 and 143) and the subject assessment of satisfaction of appearance (studies 098 and 099).
Results: Composite ≥1-grade improvement in facial line severity measured via the FWS was sustained following a single onabotulinumtoxinA treatment for up to 5 months in nearly 45% (FHL)/>30% (CFL) of subjects, and for 6 months in >25% of subjects (FHL only). These improvements in appearance corresponded with high patient satisfaction, which lasted up to 6 months. In the 2 FHL studies, improvements were specifically durable for up to 6 months; more than 80% of responders reported that they were *Mostly* or *Very satisfied* with the effect of treatment with onabotulinumtoxinA, and *Mostly* or *Very satisfied* with the natural effect of treatment with onabotulinumtoxinA. In the CFL studies, responders also reported high satisfaction with their appearance; over 30% of responders reported being *Satisfied* or *Very satisfied* at 5 months post-treatment (study 098).
Conclusions: A 1-grade improvement in UFL severity is clinically meaningful and accompanied by high patient satisfaction across four phase 3 clinical trials. Simultaneous treatment of all 3 UFLs (FHL, GL, CFL) with onabotulinumtoxinA leads to lasting, clinically meaningful improvements in UFL severity and patient satisfaction up to 6 months.
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Keywords: Forehead; OnabotulinumtoxinA; Patient-reported outcome measures; Patient satisfaction; Perception**;** Personal satisfaction

Characteristics and Treatment Response to OnabotulinumtoxinA of Patients From CD-PROBE With Anterocollis and Retrocollis

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Introduction: CD-PROBE included patients with anterocollis (AC) and retrocollis (RC) treated with onabotulinumtoxinA (onabotA). This study describes baseline characteristics and treatment response of patients with these often neglected yet disabling cervical dystonia (CD) subtypes.
Methods: CD-PROBE was an observational, multicenter, prospective study designed to identify real-world outcomes in patients with CD after onabotA treatment. The study included patients from 82 US sites with a CD diagnosis who were candidates for onabotA therapy, new to treatment, or not previously treated in a clinical trial in ≥16 weeks, and who had completed 3 onabotA treatments. Treatment benefit was measured using the Patient's Global Impression of Change (PGI-C) Scale, Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS), Cervical Dystonia Impact Profile (CDIP-58), Clinician's Global Impression of Change (CGI-C), and physician assessments of CD severity.
Results: Patients with AC (n=59) and RC (n=55) were older and more disabled with a shorter time to diagnosis and treatment than patients with laterocollis (LC) or torticollis (TC). From PGI-C responses, 58.4% of RC vs 47.8% of AC patients reported being much/very much improved by onabotA post-injection 3 (final visit). CGI-C responses similarly improved over this timeframe; 82.6% of RC and 60.9% of AC patients reported being much/very much improved. The mean total TWSTRS score decreased for AC and RC patients from injection 1 (46 [AC]; 40 [RC]) to final visit (36 [AC]; 32 [RC]); CDIP-58 scores also decreased over time. The proportion of patients with physician-assessed severe CD decreased from injection 1 (28.8% [AC]; 21.8% [RC]) to final visit (21.7% [AC]; 4.3% [RC]); most patients shifted to lower symptom severity. OnabotA dose generally increased per visit, with AC patients having lowest doses (153.5-195.4 units [U]) and RC patients the highest (184.0-213.4 U). Adverse event of dysphagia was reported in 4 AC and 7 RC patients. No new safety signals were identified.
Conclusions: CD-PROBE data indicates that real-world treatment with onabotA relieves CD symptoms in patients with anterocollis and retrocollis, as evaluated by patient and physician assessments.
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Marjan Sadeghi is an employee of AbbVie and may hold AbbVie stock.
Marc Schwartz has served as a biostatistical consultant for AbbVie.
Joseph Jankovic has received research and training grants from, and has served as a paid consultant to, AbbVie.
Shyamal Mehta is a consultant for AbbVie, Abbott, and Adamas Pharmaceuticals.
Keywords: Anterocollis; Cervical dystonia; OnabotulinumtoxinA; Retrocollis

Masseter Prominence Reduction, Treatment Satisfaction, and Self-Perceived Lower Face Appearance After OnabotulinumtoxinA: Results From a Randomized, Controlled, Phase 2b Trial

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Introduction: Primary results of this phase 2b trial demonstrated the efficacy of a single treatment of onabotulinumtoxinA (onabotA) in reducing the severity of Masseter Muscle Prominence (MMP). Safety data and additional secondary/exploratory endpoint outcomes assessing the efficacy of onabotA from the clinician and patient perspectives are reported here.
Methods: This 6-month, multicenter, double-blind, phase 2b study involved adults with bilateral MMP of grade 4 or 5 as assessed by investigators and participants using the MMP Scale (0=minimal and 5=very marked) or MMPS-Participant (MMPS-P; 0=not at all pronounced and 5=very pronounced), respectively. Eligible patients were randomly assigned (1:1:1) to receive onabotA 72 units (U), 48 U, or placebo on day 1. The following efficacy outcomes were evaluated at day 180: the proportion of participants who achieved investigator- and participant-assessed ≥2-grade improvement in MMPS and MMPS-P (more stringent than the clinically-relevant treat-to-target level of grade 3 or less), the proportion of participants satisfied/very satisfied with the treatment effect on the Lower Facial Shape Questionnaire-Treatment Satisfaction (LFSQ-TXSAT), and the change from baseline in the Participant Global Impression of Bother with lower face appearance. Adverse events were monitored at every visit up to day 180.
Results: A total of 145 participants were included in the modified intent-to-treat population (placebo, n=46; onabotA 48 U, n=53; onabotA 72 U, n=46). Participants were mostly female (90%) and White (76%), with a mean age of 39.3 years. The majority (77.3%) completed the study, with COVID-19 being a common reason for discontinuation (12/13 participants discontinued for *other reasons*). At the end of the study, a ≥2-grade improvement in MMP severity was achieved by a significantly greater proportion of subjects treated with onabotA 72 U and 48 U compared to placebo (MMPS: 47.8% and 35.8% vs 10.9%, $P<0.005$; MMPS-P: 76.1% and 73.6% vs 47.8%, $P<0.01$). A significantly higher proportion of participants were satisfied/very satisfied with the onabotA effect on the LFSQ-TXSAT vs placebo (82.6% and 69.8% for 72 U and 48 U, respectively, vs 32.6% for placebo; $P\leq0.0002$). Furthermore, participants in the onabotA groups were significantly less bothered with their lower face appearance at day 180 than those treated with placebo (-2.0 and -2.1 for onabotA 72 U and onabotA 48 U, respectively, vs. -1.4 for placebo; $P\leq0.002$). The incidence of treatment-emergent adverse events (TEAEs) was similar between the onabotA 72 U and 48 U groups, and the majority were mild and resolved spontaneously. The most frequent TEAE was nasopharyngitis (3.9%, onabotA; 0%, placebo), followed by upper respiratory infection, injection site pain, muscular weakness at the injection site, mastication disorder, and facial paresis (each 2.9%, onabotA; 0%, placebo).
Conclusion: OnabotA demonstrated sustained efficacy in reducing MMP over 6 months in both dose groups, as assessed by investigators and

participants. Participants were less bothered with the appearance of their lower face and were satisfied with onabotA treatment. No clear dose-response relationship was observed between onabotA 72 U and 48 U. OnabotA was found to be safe and well tolerated, and both dose groups showed similar safety profiles.

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Keywords: Botulinum toxin; Lower face; Mandible; Neuromodulator; Patient-reported outcome measures

Botulinum Neurotoxin Accelerates the Resolution of Bacterial Skin Infections in Mice

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Introduction: Bacterial skin and soft tissue infections (SSTIs) are important causes of human suffering, morbidity, and mortality.[1,2] We previously found that neuro-immune signaling regulates host defense.[3] Here we investigate the efficacy of onabotulinumtoxinA (onabotA [BOTOX®]), a botulinum neurotoxin type A (BoNT/A) commercial product, as a treatment for gram-positive and gram-negative bacterial skin infections by infecting mice with methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus pyogenes* (Group A *Streptococcus*, GAS), or *Pseudomonas aeruginosa* (PA), bacteria representing frequent skin pathogens in humans.[1] We measure infection resolution and immune cell influx over time.

Methods: Male and female mice were infected via subcutaneous injection with MRSA, GAS, or PA. Mice were treated with either onabotA or vehicle 2 days post-infection after formation of bacterial abscesses. Body weight and abscess and lesion sizes were recorded daily for 14 days. To quantify bacterial load, skin tissue was homogenized and plated on agar medium to enumerate colony-forming units (CFU). To determine the effect of onabotA on immune responses, leukocytes were analyzed at different time points by flow cytometry of skin digested and stained for CD45, Ly6C, Ly6G, CD11b, CD11c, CD206, CD117, EpCam, and I-A/I-E.

Results: Subcutaneous injection of onabotA significantly decreased the size of abscesses and dermonecrotic lesions caused by three major human pathogens (MRSA, GAS, and PA) and accelerated the resolution of infection compared to vehicle-treated mice. OnabotA had differential effects on bacterial load depending on pathogen. Furthermore, onabotA treatment significantly shifted the kinetics of immune cell recruitment in the skin, in particular decreasing the neutrophil population at later time points.

Conclusions: For the first time we show that nonclinical treatment with a BoNT/A product improves both gram-positive and gram-negative invasive skin infection. In mice, onabotA reduced the size of skin abscesses and lesions. We observed that injection with onabotA inhibited the recruitment of immune cells that are known to contribute to tissue damage. We also found that onabotA treatment accelerated the resolution of inflammation. The mechanisms by which onabotaA improved outcomes in this preclinical model, as well as its potential as a clinical therapeutic, warrants further investigation.

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Keywords: Abscess; Bacterial infection; Botulinum toxin; Lesion

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Efficacy and Safety of 2 Doses of OnabotulinumtoxinA for the Treatment of Masseter Muscle Prominence: 6-Month Results From a Randomized, Phase 2b Placebo-Controlled Study

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Introduction: Evaluate efficacy and safety of onabotulinumtoxinA compared with placebo for treating masseter muscle prominence (MMP).

Methods: Adults with bilateral grade 4 (*Marked*) or 5 (*Very marked*) MMP on the 5-grade clinician-assessed MMP Scale (MMPS) received onabotulinumtoxinA 72 units (U), 48 U, or placebo. Primary efficacy endpoint was participants achieving MMPS grade $\leq$3 at day 90. Participants with grade $\leq$3 for the MMPS and Participant MMPS (MMPS-P), and participants achieving grade $\geq$2 in the Participant Self-Assessment of Change (PSAC), were evaluated until day 180.

Results: Of 150 participants randomized, 34 (22.7%) discontinued (12 [8.0%] due to COVID-19). The modified intent-to-treat population included 145 participants (mean age, 39.3 years; mean BMI, 24.1 kg/m^2; 89.7% female; 75.9% White). MMPS and MMPS-P responder rates were higher for onabotulinumtoxinA 72 U and 48 U than placebo at days 90 (MMPS: 91.3% and 90.6% vs 21.7%; MMPS-P: 93.5% and 96.2% vs 47.8%; both P<0.0001) and 180 (MMPS: 71.7% and 56.6% vs 26.1%; MMPS-P: 87.0% and 86.8% vs 60.9%; both P<0.01). More onabotulinumtoxinA 72 U and 48 U participants achieved PSAC grade $\geq$2 than placebo at days 90 (73.9% and 90.6% vs 21.7%; P<0.0001) and 180 (76.1% and 66.0% vs 28.3%; P<0.001). Treatment-related adverse events occurred in onabotulinumtoxinA 72 U and 48 U groups (12.2% and 9.4%) vs none in placebo; the majority were mild in severity.

Conclusions: A single treatment of onabotulinumtoxinA (72 U and 48 U) reduced MMP and improved lower face contour for up to 6 months. Both dose groups demonstrated favorable safety profiles.

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Keywords: Botulinum toxin; Lower face; Mandible; Neuromodulator; Patient-reported outcome measures.

Unravelling the Inhibitory Activity of Botulinum Toxins on the Enteric Nervous System

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Introduction: Botulism is a rare, mainly foodborne, neuroparalytic syndrome caused by the ingestion of foods contaminated with botulinum toxin (BoNT), one of the most poisonous biological substances known. The syndrome is caused by the inhibition of acetylcholine release at the neuromuscular junction, leading to the characteristic descending flaccid paralysis and, in worst cases, death by respiratory failure.[1,2] The cellular and molecular mechanisms of action of BoNTs on somatic motor neurons has been largely characterized. In natural botulism, the toxin is adsorbed through the intestinal wall with constipation as one of the first symptoms, but little is known about the possible action of BoNTs on the enteric nervous system (ENS).[3,4] The ENS, which is also called the "second brain", is a very complex subdivision of the autonomic nervous system, having a central role in the control of enteric motility, secretion, blood flow, and response to infections and a considerable impact on many aspects of host health.[5,6] Therefore, we investigated the action of BoNTs on the wide variety of neurons present in the ENS.
Methods: We reproduced a model of foodborne botulism in mice (CD1 and C57BL/6 strains), feeding them with sublethal doses of BoNT/A or BoNT/B from clostridial cultures.[7] We then used our home-made antibodies with specificity against BoNT protein cleavage products to detect the proteolytic activity of the toxins using immunofluorescence. To evaluate the effects of the intoxication on animal physiology, we measured mice peristalsis, hind limb muscle functionality, and ventilation rate using the charcoal-pellet transit assay, compound muscle action potential, and a home-developed ventilation assay, respectively. Furthermore, to evaluate the possible effects on enteric neuroimmune crosstalk, we used mice models of infection with *Salmonella typhimurium* and *Shigella flexneri* and colony-forming unit counts from spleen, liver, and Peyer's patches.
Results and Conclusions: We showed for the first time the proteolytic activity of BoNTs in enteric cholinergic and non-cholinergic neurons for both BoNT/A and BoNT/B. Moreover, we identified a dose of BoNTs that leads to a significant slowdown in peristalsis with no systemic signs of botulism, indicating that the ENS is an important first site of action for these toxins. This preliminary result raises many valid questions about the effects of BoNTs on gut physiology, which are usually underestimated. For this reason, we are now investigating the possible effects on enteric neuroimmune crosstalk, and we intend to further investigate the effects on gut microbiota composition, both of which are fundamental for host protection against infection.[8,9] By doing so, we propose to shed new light on the interaction between the toxin and this very complex nerve network, considering the intestine not just as a route of entry for the toxin, but also its first important site of action.
Keywords: Botulinum neurotoxin; Enteric nervous system; Foodborne botulism; Neuroimmune crosstalk

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Antigen-Binding Fragments From Purified Human Monoclonal Antibodies Open to the Intrathecal Therapy of Tetanus

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Introduction: Tetanus neurotoxin (TeNT) is the causative agent of tetanus, a life-threatening disease characterized by neurogenic muscle rigidity and spasticity. These effects are caused by TeNT activity in inhibitory interneurons within the spinal cord. Although tetanus can be prevented using a highly effective vaccine, a worldwide clinical practice in emergency rooms is the administration of anti-TeNT immunoglobulins (TIG), which are used for both prophylaxis of tetanus development in wounded patients and to treat patients already experiencing tetanus symptoms. TIG neutralizes TeNT in peripheral body fluids before it enters peripheral nerves and is retrotransported to the spinal cord. Intrathecal administration of TIG to block TeNT at its site of action would be more effective, but this approach is limited by the low level of anti-TeNT antibodies present in TIG and the amount of protein that can be safely injected into the cerebrospinal fluid. All of these drawbacks can be overcome by highly purified human monoclonal antibodies (humAbs), which are emerging as superior therapeutics against several diseases.
Methods: By screening immortalized memory B cells pooled from the blood of immunized human donors, we isolated TT104 and TT110, two humAbs that display an unprecedented neutralization ability against TeNT. We produced the antigen-binding fragments (Fab) derivatives of TT104 and TT110 and determined the epitopes they recognize using cryo-electron microscopy. We also performed a battery of biochemistry, imaging, and cell biology experiments to define the molecular basis of TT104 and TT110 interference with the TeNT mechanism of neuron intoxication. Lastly, we used experimental animal models of local and generalized tetanus to assess the neutralization ability of humAbs and Fabs via the intramuscular and intrathecal administration routes.
Results: TT104 and TT110 bind two epitopes required for TeNT binding to target neurons and light chain translocation inside the neuronal cytosol, respectively. The identification of these epitopes sheds new light on TeNT activity in neurons. In mouse bioassay experiments, the combination of

TT104 and TT110 humAbs displays a prophylactic activity comparable to TIG when injected long before TeNT. In addition, the combination of TT104-Fab and TT110-Fab prevents tetanus in post-exposure experiments when injected within the first 6 hours after TeNT, again with an efficacy comparable to TIG. Crucially, none of these treatments can prevent tetanus when administered more than 12 hours after TeNT inoculation, while it can be prevented by the TT104-Fab and TT110-Fab combination administered via the intrathecal route. Most importantly, experiments in rats show that intrathecal Fabs administered 24 hours after TeNT reduce the severity and duration of tetanus symptoms to a greater degree than intramuscular TIG.

Conclusions: TT104 and TT110 humAbs meet all the requirements to improve the current prophylaxis and therapy of human tetanus and are ready for clinical trials. TT104- and TT110-Fab derivatives open to an effective intrathecal therapy of symptomatic tetanus.

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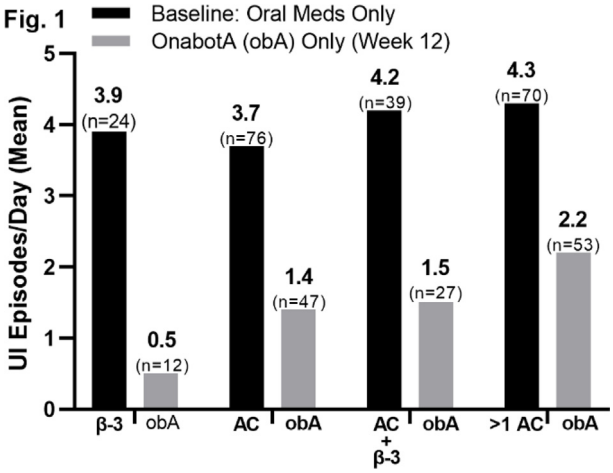
Keywords; Fab; Human monoclonal antibody; Intrathecal administration; Spasticity; Tetanus; Tetanus neurotoxin



OnabotulinumtoxinA Improves Idiopathic Overactive Bladder Symptoms in Patients Refractory to Oral Medications

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Introduction: There is a paucity of data comparing the efficacy of onabotulinumtoxinA (onabotA) treatment between patients treated with one oral overactive bladder (OAB) medication to those treated with more than one. This real-world study examines urinary incontinence (UI) episodes and the treatment benefit of onabotA in patients who are refractory to one or more oral medications.

Methods: A prospective, observational study (ClinicalTrials.gov Identifier: NCT02161159) enrolled adult patients with OAB symptoms inadequately managed by oral medications. Patients were naïve to botulinum toxin for OAB; efficacy and safety analyses were conducted on those that received ≥1 dose of onabotA. Adverse events (AEs) and adverse drug reactions (ADRs) were recorded for up to 12 months after onabotA treatment. We analyzed UI episodes at baseline for all patients taking oral medications (β-3 adrenergic agonist [β-3] and/or an anticholinergic [AC]) for OAB. Only patients taking oral medications before, but not after onabotA and who had ≥1 diary entry at the indicated timepoint were included in analyses of UI episodes after onabotA at 1 and 12 weeks and treatment benefit scores (TBS) at 12 weeks.

Results: Baseline UI episodes were similar in patients treated with one versus more than one oral medication; reductions in UI at week 12 post-onabotA did not differ based on the number of prior oral medications (Fig. 1). UI was significantly reduced (**P*=<.001) in as little as 1 week after onabotA for all prior oral treatment groups (β-3, -3.3*, n=16; AC, -1.8*, n=53; β-3 + AC, -2.2*, n=28; >1 AC, - 1.7*, n=52). Of the 233 patients who reported TBS at week 12, 88% were improved or greatly improved after onabotA. In the safety population (N=504), 57 AEs were reported in 38 patients (7.5%); 9 were serious. Urinary retention, as determined by the treating physician, was reported in 5 patients (1.0%); 1 was severe. Symptomatic urinary tract infection was reported in 2 patients (0.4%).

Conclusions: Treatment with onabotA led to significant reductions in UI episodes, with no significant improvement in patients who had been on more than one oral medication as compared to only one oral before onabotA treatment. Given these results, clinicians may want to consider onabotA treatment earlier as opposed to cycling through oral medications.

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Keywords: OnabotulinumtoxinA; Overactive bladder; Urinary incontinence

Individualized OnabotulinumtoxinA Treatment of Upper Limb Spasticity in US Clinical Practices: Analysis of Practice Patterns From the ASPIRE Study

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Introduction: The United States (US) label for onabotulinumtoxinA (onabotA) was recently expanded to include treatment of 8 additional muscles associated with adult upper limb (UL) spasticity. ASPIRE, an international, observational study, examined onabotA utilization and effectiveness in treating spasticity over a 2-year period. We analyzed data from ASPIRE to characterize utilization patterns within muscle groups consistent with the updated US label in patients with UL spasticity.
Methods: This post hoc analysis included adult patients from US centers who received treatments to UL muscles and total onabotA dose (≤400 units [U]) injected per treatment session consistent with the US label.
Results: Of 443 US patients, 336 had UL spasticity and 45 were injected with ≤400 U of onabotA in selected muscles that are now consistent with the newly approved label. Mean age (SD) was 51.3 (16.0) years, 26 (58%) were male, and 23 (51%) were onabotA-naive. Postures treated included clenched fist (71.1%), flexed wrist (55.6%), flexed elbow (73.3%), thumb in palm (20.0%), intrinsic plus hand (20.0%), and pronated forearm (31.1%). The majority of patients (80.0%) had >1 abnormal posture. Of a total of 38 adverse events (AEs) recorded, 1 treatment-related AE (muscular weakness) and no treatment-related serious AEs were reported.

Table
Summary of Muscles Injected With OnabotA for Each Clinical Presentation in US Patients.

Clinical Presentation/ Posture	Patients who received onabotA injections only in muscles consistent with label prior to July 2021, n*	Patients who received onabotA injections in muscles consistent with expanded label July 2021, n†
Clenched fist (n=32)	30	32
Flexed wrist (n=25)	25	25
Flexed elbow (n=33)	4	33
Thumb in palm (n=9)	3	9
Intrinsic plus hand (n=9)	0	9
Pronated forearm (n=14)	0	14

Note: The sum of the patient numbers exceeds 45 because patients may have been treated for >1 posture.
* Biceps brachii, flexor carpi radialis, flexor carpi ulnaris, flexor digitorum profundus, flexor digitorum superficialis, adductor pollicis, and flexor pollicis longus.
† All muscles listed in above footnote plus brachioradialis, brachialis, pronator teres, pronator quadratus, lumbricals, interossei, flexor pollicis brevis, and opponens pollicis.

Conclusions: This ASPIRE post hoc analysis provides real-world evidence of the opportunity to educate on the expanded label for onabotA with attention to elbow, forearm, and finger postures and the ability to treat, on-label, multiple UL postures more holistically.
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Conflicts of Interest/Disclosure; Gerard E. Francisco, Wayne Feng, Michael C. Munin, Kenneth Ngo, Marc Schwartz, and Alberto Esquenazi are investigators for AbbVie. Marjan Sadeghi and Aleks Zuzek are employees of AbbVie.
Keywords: OnabotulinumtoxinA; Spasticity; Upper limb

Evaluation of PREEMPT Fixed-Dose, Fixed-Site, and Follow-The-Pain Treatment Paradigms in the PREDICT Study

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Introduction: The phase 3 PREEMPT clinical trials established the safety and efficacy of 155-195 units (U) of onabotulinumtoxinA in adults with chronic migraine (CM). The objective was to analyze the real-world effectiveness and safety of 155 U, 156-195 U, and 195 U of onabotulinumtoxinA in patients with CM from the PREDICT study.
Methods: PREDICT (ClinicalTrials.gov Identifier: NCT02502123) was a Canadian 2-year, prospective, observational study in adults with CM. Patients received onabotulinumtoxinA approximately every 12 weeks (≤7 treatment cycles [Tx]) per the Canadian product monograph. The primary endpoint was mean change from baseline in Migraine-Specific Quality of Life (MSQ) at Tx4. Headache days (daily headache diary) and physician and patient satisfaction were evaluated throughout the study. This analysis stratified the safety population (≥1 onabotulinumtoxinA dose) into 3 groups (155 U,156-195 U, and 195 U) by the dose received on ≥3 of the first 4 treatment cycles.
Results: Of 184 patients that received ≥1 onabotulinumtoxinA dose, 68 received 155 U, 65 received 156-195 U, and 13 received 195 U on ≥3 treatments. Baseline characteristics were similar between groups. Baseline mean (SD) headache days/month 21.6(6.4) 155 U; 20(7) 156-195 U; and 21.7(6) 195 U decreased over time (Tx4: -7.1[6.7] 155 U; -6.5[6.7] 156-195 U; -11.2[6.4] 195 U versus baseline). Improvements in all MSQ domains were observed across groups at Tx4 and the final visit. Physicians rated most patients as improved, and the majority of patients were satisfied at the final visit (80.8% 155 U; 83.6% 156-195 U; 90% 195 U). Treatment-emergent adverse events (TEAEs) were reported in 18/68 patients (26.5%) in the 155 U group, 41/65 (63.1%) in the 156-195 U group and 10/13 (76.9%) in the 195 U group; treatment-related TEAEs were 9(13.2%), 10(15.4%) and 3(23.1%) respectively; serious TEAEs were 0, 3(4.6%) and 1(7.7%), none were considered treatment-related.
Conclusion: Consistent with the PREEMPT clinical trials and the REPOSE observational study, long-term treatment with 155 U, 156-195 U, and 195 U onabotulinumtoxinA in PREDICT was safe, generally well-tolerated, and effective in the treatment of CM. No new safety signals were identified.
Support: Allergan (prior to its acquisition by AbbVie).
Disclosures: Corrie Graboski has served on a Scientific Advisory or Data Safety Monitoring Board for Allergan/AbbVie, Lundbeck, and Nova Pharm. She has served on a Speakers' Bureau for Allergan/AbbVie, Eli Lilly, Novartis, and Nova Pharm and has received research support from Amgen/Novartis. The institution of Corrie Graboski has received research support from Allergan/AbbVie.
May Ong-Lam has served on a Scientific Advisory or Data Safety Monitoring Board for Lundbeck and Spectrum Therapeutics and also on a Speakers' Bureau for Spectrum Therapeutics. The institution of Dr. Lam has received research support from Spectrum Therapeutics. Werner J. Becker has served as a consultant for AbbVie, Lundbeck, Eli Lilly, Novartis, and Teva. Dr. Becker has served on a Speakers' Bureau for Teva.
Julia Ma has received personal compensation for serving as an employee of AbbVie.
Dr. Ma has received stock or an ownership interest from AbbVie.
Katherine Sommer has received personal compensation for serving as an employee of AbbVie. She has received stock or an ownership interest from AbbVie.
Ian Finkelstein has served as a consultant for Allergan, Aralez, Eli Lilly, and Novartis.
Dr. Finkelstein has served on a Scientific Advisory or Data Safety Monitoring Board for Allergan, Eli Lilly, Novartis, and Aralez. He has also served on a Speakers' Bureau for Allergan, Novartis, and Aralez.
Keywords: Chronic migraine; OnabotulinumtoxinA; Prospective study

Botulinum Neurotoxin X Lacks Potency in Mice and in Human Neurons

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Introduction: Botulinum neurotoxins (BoNTs) are the most potent toxins known to man and are associated with the potentially lethal paralytic disease called botulism. Produced by *Clostridium botulinum*, these toxins are capable of infecting humans and animals in several ways, including through consumption of contaminated food products, infection of anaerobic wounds, or colonization of infant intestinal tracts. *C botulinum* strain 111 was isolated from an infant botulism case in Japan, where serological testing revealed production of botulinum neurotoxin type B (BoNT/B).[1] Decades after the sample was taken, bioinformatic analysis of the genome revealed that strain 111 encodes both a plasmid-borne BoNT/B2 as well as a chromosomal putative novel BoNT, which was named BoNT/X.[2] BoNT/X shares up to 30% amino acid similarity with serotypes A-G and, importantly, shows no cross-reactivity with antibodies raised against these serotypes.[3] While catalytic activity of the light chain (LC) to human and vertebrate soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) has been demonstrated, it was unknown if BoNT/X is capable of causing human or vertebrate botulism. In this study we investigated the biologic activity of recombinant and endogenously produced BoNT/X in mice and in neuronal cell models.
Methods: Recombinant wild type un-tagged BoNT/X and recombinant wild type un-tagged nontoxin-nonhemagglutinin (NTNH)-BoNT/X complexes were expressed in an atoxic *C botulinum* expression host, and toxicity was examined in neuronal cell models and in mice. To characterize the potential toxicity of endogenously produced BoNT/X, strain 111 underwent plasmid curing to remove the plasmid carrying the *bont/b2* gene cluster, and culture supernatant as well as isolated BoNT/X were examined for toxicity in mice.
Results: BoNT/X was endogenously expressed in *C botulinum* strain 111 at levels of ~20 μg/mL. Neither recombinant BoNT/X nor endogenously produced BoNT/X resulted in deaths in mice injected intraperitoneally with up to 1 μg of toxin, although some mice injected with 1 μg of endogenously produced BoNT/X appeared to exhibit mild symptoms consistent with botulism but recovered. Intramuscular injections of endogenous, semi-purified BoNT/X resulted in apparent moderate hind limb paralysis at amounts of 750 ng. For other BoNT serotypes, intraperitoneal injections of ~5-10 pg are sufficient to cause fatal botulism in mice and similar amounts injected intramuscularly result in complete hindlimb paralysis.[4] Further, recombinantly produced BoNT/X resulted in vesicle-associated membrane protein (VAMP) cleavage in human neurons at low nanomolar concentrations. While this suggests that BoNT/X may be able to cause neurotoxicity at concentrations over 1 million-fold greater than other BoNTs, the mechanisms of neuronal cell entry and toxicity at these high concentrations of protein are unknown.
Conclusions: These studies indicate that *C botulinum* strain 111 does produce BoNT/X at levels consistent with production levels of other BoNTs in *C botulinum*. Neither culture supernatant of the plasmid-cured strain, nor isolated BoNT/X, nor recombinantly-produced BoNT/X holotoxin resulted in botulism in mice at amounts over 1 million-fold greater than amounts required for botulism by other BoNTs. These data were confirmed by cell-based assay, in which BoNT/X resulted in VAMP cleavage only at nanomolar amounts, concentrations at which even LC alone causes SNARE cleavage. Overall, these data indicate that BoNT/X is not a potent vertebrate neurotoxin like other BoNTs, yet they confirm the great relatedness of this protein to BoNTs on a functional level. Thorough and careful characterization of this new potential pathogen will be critical for implementing safe botulism prevention strategies, as well as providing a foundation for potential use of the toxin in pharmaceutical development.
Funding; CRDF Global, DAA9-20-66959-1, DAA3-20-66957.
Keywords: BoNT; BoNT/X; Botulinum neurotoxin; Botulism; Mice; Neuron

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Development and Characterization of a Human Cell-Based Assay for Relative Potency Testing of Botulinum Neurotoxin Serotype A

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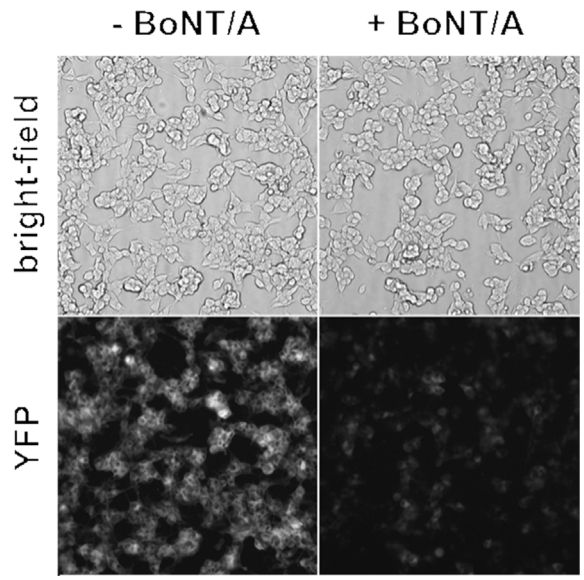
Introduction: Commercial release of any BoNT-based drug product requires a means to determine drug product potency to ensure manufacturing consistency and patient safety. The mouse median lethal dose (LD_{50}) bioassay has historically been the standard method for testing BoNT-containing samples, including drug products, since it tests all aspects of BoNT activity and is sensitive to the low concentrations of BoNT present in BoNT-based therapeutics. However, any future BoNT-based drug product release in the United States or European Union should be supported by a cell-based assay (CBA) to meet governmental requirements for minimizing or eliminating the need for animal-based assays. In addition to high sensitivity and testing of all BoNT cellular activities, the CBA's performance should also improve upon the mouse 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/A-containing drug products and compare it to an existing CBA.
Methods: We engineered a human cell line to stably express a fluorescent reporter that enables the sensitive detection of BoNT/A without further differentiation and used the cell line to develop a commercial assay known as the SapientCell™ CBA. The SapientCell cell line's expression profile of BoNT/A receptors synaptic vesicle protein 2 (SV2) and ganglioside was characterized and compared to the murine BoCell® cell line used in the BoCell® CBA. The SapientCell CBA's sensitivity was optimized for detection of BoNT/A while maintaining cell health. Finally, we qualified the CBA by testing its precision, accuracy, range, and linearity using BoNT/A-containing samples.



Results: The SapientCell CBA can detect BoNT/A with half maximal effective concentration (EC_{50}) values below 1 picomole (pM) with a linear assay range that spans greater than 2 logs. The CBA can be used to determine the relative potency of BoNT/A-containing samples with relative accuracies between 85% and 115% and a coefficient of variability of less than 10%.
Conclusions: The human-based SapientCell CBA method for determining the relative potency of BoNT/A-containing samples was successfully developed. We demonstrate that the SapientCell CBA differs from the existing murine-based BoCell® CBA in terms of its BoNT/A receptor expression profile and tolerance to common drug product excipients, while maintaining excellent overall assay robustness. The optimized SapientCell CBA method has the sensitivity required to determine the potency of BoNT/A-based drug products and offers excellent precision and accuracy over a range of sample potencies. The SapientCell CBA will be made available either as a service provided by BioSentinel or as a licensed cell line and method.
Funding; This project was internally funded by BioSentinel, Inc.
Keywords: Botulinum neurotoxin serotype A; Cell-based assay; Drug product testing; Relative potency

Quantification of a Clinical Assay for Botulinum Neurotoxins Through Mass Spectrometric Detection

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Introduction: Botulism is a paralytic disease due to the inhibition of acetylcholine exocytosis at the neuromuscular junction, which can be lethal if left untreated. Botulinum neurotoxins (BoNTs) are produced by the spore-forming bacterium, *Clostridium botulinum*, and some related clostridial organisms. A method has been developed for laboratory-confirming botulism, utilizing mass spectrometry (Endopep-MS), and recently, we have adapted the method to be used to quantify the amount of toxin present by serotype.
Methods: The Endopep-MS method detects BoNT in serum or stool based on the cleavage products of a peptide substrate, which mimics its native target. Toxin is extracted from the sample with high-affinity monoclonal antibodies and the cleavage of a serotype-specific peptide substrate determines the presence of BoNT, utilizing matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry detection. The amount of toxin can then be quantified by comparing the cleavage products to the intact substrate; the higher the ratio of cleavage products to intact substrate, the higher the level of toxin. Quantification is accomplished without the use of an internal standard.
Results: The Endopep-MS method has been developed as a high-throughput, rapid, and sensitive method for detection of botulinum neurotoxins in clinical samples. The data acquired has established linearity and determined the limit of detection in buffer and clinical matrices.
Conclusions and Discussion; The Endopep-MS method to laboratory-confirm botulism can be made quantitative without the addition of a separate internal standard.
The opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the Centers for Disease Control and Prevention.

Real-World Effectiveness of Ubrogepant for the Acute Treatment of Migraine in Combination With OnabotulinumtoxinA Preventive: Results From the COURAGE Study

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Introduction: Ubrogepant is an oral calcitonin gene–related peptide (CGRP) receptor antagonist approved for the acute treatment of migraine and may be used to treat breakthrough attacks by individuals using preventive medications. Limited information exists on the real-world effectiveness of ubrogepant when used in combination with onabotulinumtoxinA.
Methods: Using data collected via the Migraine Buddy application, this prospective, observational study evaluated real-world acute treatment effectiveness in people taking preventive treatment and ubrogepant 50 mg or 100 mg for acute treatment of migraine. Eligible participants were adults who reported ≥3 migraine attacks in the last 30 days, had treated ≥3 prior attacks with ubrogepant, and were concurrently taking an anti-CGRP monoclonal antibody (mAb), onabotulinumtoxinA, or both as migraine preventive treatment. Self-reported assessments were collected daily for approximately 30 days. Daily assessments included meaningful pain relief (MPR), defined as a reduction of headache pain to a meaningful degree or remaining pain free if none was reported at ubrogepant dosing, and return to normal function (RNF) after dosing. This analysis reports results from the first ubrogepant-treated attack and first 10 ubrogepant-treated attacks in the ubrogepant plus onabotulinumtoxinA group. The repeated attack endpoints were modeled via logistic generalized estimating equations to account for the correlated nature of the repeated attacks.
Results: A total of 122 participants reported using ubrogepant as acute treatment in combination with onabotulinumtoxinA (without an anti-CGRP mAb) as migraine preventive treatment. A median of 9 attacks (interquartile ratio [IQR], 6-12) per respondent were recorded. Participant characteristics are shown in the Table. For the first ubrogepant-treated attack at 2 and 4 hours post dose, MPR was achieved in 53.3% and 76.2% of participants, respectively. RNF was achieved by 25.4% and 45.9% of ubrogepant-treated participants at 2 and 4 hours post dose, respectively. For the first 10 ubrogepant-treated attacks, MPR was achieved in 44.8% and 72.9% of attacks at 2 and 4 hours post dose, respectively (Figure). RNF was achieved by 30.1% and 52.1% at 2 and 4 hours post dose, respectively, for the first 10 ubrogepant-treated attacks.
Conclusions: These findings support the real-world effectiveness of ubrogepant as an acute treatment in combination with onabotulinumtoxinA, providing evidence for a common treatment pattern. These

results also demonstrate the feasibility of using a novel, app-based design to evaluate the real-world effectiveness of migraine treatment. Evaluating the real-world effectiveness of ubrogepant in combination with other preventive treatments is an area of future research consideration.
Support: Allergan (prior to its acquisition by AbbVie).

Table
Participant Characteristics

Characteristic	OnabotulinumtoxinA[a] + Ubrogepant (n=122)
Age, mean (SD), y	40.4 (10.3)
Female, n (%)	117 (95.9)
Race, n (%)	
White/Caucasian	107 (92.2)
Latinx/Hispanic	4 (3.5)
Black/African American	2 (1.7)
Number of recorded attacks per respondent, median (IQR)	9 (6, 12)
Number of ubrogepant-treated attacks per respondent, median (IQR)	5 (3, 6)
PHQ-4,[b] mean (SD)	7.8 (3.0)
MIDAS grade, n (%)	
I (minimal)	3 (2.5)
II (mild)	3 (2.5)
III (moderate)	10 (8.2)
IVa (severe)	20 (16.4)
IVb (very severe)	86 (70.5)
Historical triptan response, n (%)	
Triptan insufficient responder	101 (84.2)
Triptan responder	14 (11.7)
Triptan naive	5 (4.2)
Ubrogepant dose, n (%)	
50 mg	55 (44.3)
100 mg	68 (55.7)
Number of ubrogepant taken, n (%)	
Attacks treated with ubrogepant, n/N[c] (%)	599/1054 (56.8)
1	494 (82.5)
2	105 (17.5)

IQR, interquartile ratio; MIDAS, Migraine Disability Assessment; PHQ-4, Patient Health Questionnaire-4; SD, standard deviation.
Unless otherwise specified, percentages are out of the number of participants in sample with non-missing responses.
[a] Excluding 2 missing responses, all participants reported that they received onabotulinumtoxinA injections in the forehead, side and back of head, and neck.
[b] PHQ-4 scale ranges from 0-12 with total scores as follows: normal (0-2), mild (3-5), moderate (6-8), severe (9-12).
[c] n=number of migraine attacks treated with ubrogepant, N=total number of migraine attacks.

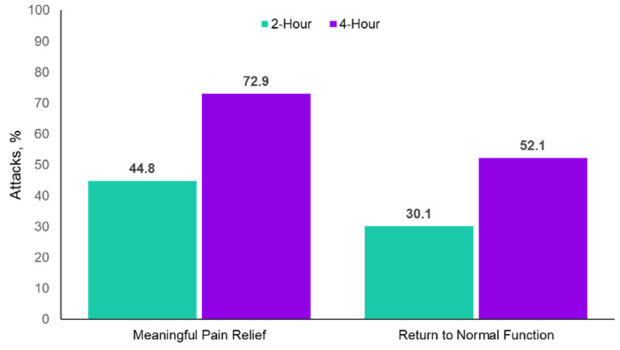

Figure. Meaningful pain relief and return to normal function in onabotulinumtoxinA participants 2 and 4 hours post ubrogepant administration (first 10 attacks)

Disclosures: Richard B. Lipton has received research support from the National Institutes of Health, the Food and Drug Administration, and the National Headache Foundation. He serves as consultant, advisory board member, or has received honoraria or research support from AbbVie/Allergan, Amgen, Biohaven, Dr. Reddy's Laboratories (Promius), electroCore, Eli Lilly, GlaxoSmithKline, Lundbeck, Merck, Novartis, Teva, Vector, and Vedanta Research. He receives royalties from *Wolff's Headache*, 8th edition (Oxford University Press, 2009), and Informa. He holds stock/options in Biohaven, Ctrl M, and Manistee.
Ella Engstrom was an employee of OPEN Health Group at the time of the study.
Daniel Serrano is an employee of OPEN Health Group.
Linda Davis serves as a consultant, advisory board member, or has received honoraria from AbbVie, Amgen, Eli Lilly, Novartis, and Teva.
Janette Contreras-De Lama and Katherine Sommer are employees of AbbVie and may hold AbbVie stock.
Susan Hutchinson serves as a consultant, advisory board member, or has received honoraria from AbbVie, Amgen, Biohaven, Currax, Eli Lilly, Impel, Lundbeck, Novartis, Teva, Theranica, and Upsher-Smith.
Keywords: Acute migraine therapy; CGRP; Migraine prophylaxis; Botox®

Diffusion, Spread, and Migration of Botulinum Toxin: An Update

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Introduction: It has been almost 10 years since our original review of the science on the characteristics of diffusion, spread, and migration of botulinum toxin (BoNT) (Ramirez-Castaneda J, et al, 2013). This update aims to critically review some of the more significant developments, updates, and available experimental and clinical literature since our 2013 publication on this topic and place it in a practical context.
Methods: As of April 2022, there has been 80 citations of our original publication. Each of these papers was carefully evaluated for their relevance to the topic of diffusion, spread, and migration. Further, PubMed advanced searches were conducted with the Boolean Operator "OR" to combine the search terms "diffusion", "spread", and "migration", and then combined using "AND" with the combined search results of every possible variation of botulinum toxin search terms.
Results: Of the 80 papers citing our original diffusion review, 10 were judged to be significant in addressing the diffusion/spread/migration topics, including reviews and original data-driven works. The combined search between "diffusion OR spread OR migration" and all possible variations of botulinum toxin terms resulted in 1,081 hits. Out of this group, only 17 papers met the criteria for experimental or clinical relevance to BoNT diffusion/spread/migration.
Conclusions: A systematic re-review of the experimental and clinical literature on the topic of BoNT diffusion, spread, and migration was performed. Analysis of the publications on this topic since our 2013 review further confirmed the primary conclusion drawn in 2013: there is a remarkable paucity of high-quality original research specifically addressing this important topic. This review presents and discusses all the relevant updates since 2013.
Keywords: Botulinum toxin, Diffusion, Spread, Migration

Reference

Ramirez-Castaneda J, Jankovic J, Comella C, Dashtipour K, Fernandez HH, Mari Z. Diffusion, spread, and migration of botulinum toxin. *Mov Disord*. 2013;28(13):1775-1783.

Cortical Rewiring Following Peripheral Injection of Botulinum Neurotoxin Type A

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This abstract is dedicated to the memory of Matteo Caleo.

Introduction: Botulinum neurotoxin type A1 (BoNT/A1) is a bacterial metalloprotease cleaving the SNARE protein SNAP-25 (synaptosomal-associated protein, 25kDa), which is responsible for synaptic vesicle fusion. The toxin blocks neurotransmission at cholinergic nerve terminals, mainly at the neuromuscular junction (NMJ). BoNT/A1 action is potent, specific, and long-lasting, yet reversible. These features underlie its wide use in human therapy for treating neurological conditions characterized by neuronal hyperactivity. Evidence suggests that a fraction of BoNT/A1 undergoes long-distance axonal transport and could mediate a direct effect on central circuits even outlasting toxin effects at the NMJ. We previously reported BoNT/A1-mediated cleavage of SNAP-25 in second-order neurons of the spinal cord and brainstem. Chronic silencing of presynaptic terminals in central circuits by BoNT/A1 could thus have an impact on cortical areas. Studies on patients with dystonia have indeed revealed a cortical effect of peripheral injection of BoNT/A1. Here, we aim to assess whether BoNT/A1 peripheral injections can influence motor cortical areas, affecting the morpho-functional physiology of cortical neurons connected with BoNT/A1-affected central nuclei.

Methods: Three-month-old Thy1-GFP mice, expressing GFP in layer V pyramidal neurons, were injected with BoNT/A1 (5 U/kg) in the whisker pad. Blockade of whisker movement was checked the day after to ensure the success of the injection. For the ex vivo analysis of spine morphology, animals were sacrificed 30 days after the injection and brains were collected and processed for confocal imaging. Z-stacks of second- and third-order dendrites were acquired, and morphological parameters were measured using Fiji image analysis software. Two-photon imaging of GFP-positive dendrites was performed through a 3-mm cranial window over the motor cortex at all imaging time points: before BoNT/A1 injection, and at 3,15, and 30 days post injection. Twenty-four-hour spine formation and elimination rates were quantified as the number of new or lost spines over the total number of spines. Spine density was quantified as the number of spines per micrometer (μm) of dendritic segment.

Results: Ex vivo analysis revealed a striking decrease in spine density in cortical motor areas 30 days after BoNT/A1 injection, while whisker paralysis lasted only around 10 days. Moreover, we observed an increase in stubby spines, known to be an immature spine type that could either be new or in the process of being eliminated. To understand the mechanism underlying spine loss, we then measured spine dynamics in awake mice longitudinally using two-photon microscopy. Imaging of apical dendrites in the motor cortex before and after BoNT /A1 injection revealed a decrease in spine density already 15 days after the peripheral insult, confirming our ex vivo data. Moreover, we observed a decrease in spine formation at day 3, and an increase in spine elimination at day 15 after BoNT/A1 injection.

Conclusions: Overall, our data reveal profound morphological changes in cortical neurons after intramuscular BoNT/A1 injection, which persisted longer than the peripheral effect at the NMJ. Our hypothesis is that cortical spine remodeling plays a key role in the therapeutic action of BoNT/A1 in neuropathologies and strongly contributes to the long-lasting benefits observed in patients.

Funding; Italian National Research Council (CNR Joint Lab 2018 Project, SAC.AD002.091).
Keywords: Axonal trafficking; Botulinum neurotoxin; Central plasticity; Dendritic spine; Two-photon imaging

Modulatory Effects on the Spread of Pathological Tau by Botulinum Neurotoxins

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Introduction: In tauopathies such as Alzheimer's disease (AD), tau aggregates are known to propagate across functionally connected neuronal networks, but the mechanisms underlying this process are poorly understood. Several lines of evidence support the hypothesis that tau release is dependent on neuronal activity. Pathological tau can be found in the extracellular space, inside synaptic vesicles, and other synaptic compartments, or in a free form. In this project we aim to test both in vitro and in vivo the effect of selected botulinum neurotoxins (BoNTs) and tetanus neurotoxin (TeNT) on the release of tau from synaptic terminals. BoNTs and TeNT enter synapses, where they cleave different synaptic SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins, thus impairing synaptic vesicle fusion and neurotransmitter release. Different BoNTs will allow us to determine whether the inhibition of synaptic vesicle release is effective in limiting tau spread, both in neuronal cultures in microfluidic chambers (MFCs) and in brain tissue.

Methods: In vitro: Primary neurons were cultured in the central part of three-compartment MFCs and transduced with lentiviruses expressing human tau (hTau) isoforms. Cells were then treated with BoNTs in the lateral chambers and stimulating agents in the central compartment. The content of hTau in the culture media was quantified with an enzyme-linked immunosorbent assay (ELISA).
In vivo: Adeno-associated viral vectors (AAVs) expressing hTau isoforms were injected in the vitreous, and the superior colliculus (SC) and lateral geniculate nucleus (LGN) areas were analysed through immunohistochemistry.

Results: In the in vitro assay, we found out that botulinum neurotoxin type A (BoNT/A) can decrease the release of the 1N4R mutant hTau (P301S), but not the wild-type form. Moreover, neuronal stimulation significantly increases the release of P301S hTau, while wild type hTau is not strongly affected. TeNT apparently increases hTau release, particularly of P301S hTau. The AAV constructs are correctly expressed in both primary neurons and in retinal ganglion cells (RGCs), and brain sections of SC and LGN were analysed. Using this method, we plan to validate the results obtained in neuronal cultures and determine the mechanisms of tau release by neurons, using BoNTs as tools.

Conclusions: Clostridial neurotoxins represent a powerful tool for the study of neurons and synapses. Using BoNT/A, we showed that hTau release is modulated by specific SNARE complex components and differs depending on the isoform. The use of other BoNT types in both in vitro and

in vivo models will help us to identify the SNARE proteins involved in tau release. This approach will provide novel insights on the mechanisms controlling tau release from synaptic terminals and identify novel molecular targets for the development of therapeutic interventions to treat tauopathies.
Funding: Alzheimer's Society PhD Studentship.
Keywords: Alzheimer's disease; BoNT/A; Secretion; Tau; Transmission

Development of an App-Based Patient-Centered Outcome (PCO) Measure for Dystonia

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Introduction: The main goal of this project is to provide data to establish clinical trial readiness in future clinical trials for novel treatments for dystonia. The first step is to develop a Patient-Centered Outcome (PCO) measure for three common focal dystonias: cervical dystonia (CD), blepharospasm (BSP), and laryngeal dystonia (LD). Botulinum neurotoxin (BoNT) is a first-line therapy for focal dystonia and results in significant improvement; yet, approximately one-third of patients discontinue use of BoNT, suggesting that the therapy may not fully address patient expectations. A PCO that can measure therapeutic response across motor, disability, and psychosocial domains in an app-based format that is sensitive to change and feasible for use on a frequent basis outside the clinic is sorely needed.
Methods: We used a modified iterative Delphi process based on Food and Drug Administration (FDA) guidance to develop content, select items for the PCO, improve the items using patient focus groups, and revise the items based on a large patient survey. We conducted content validation with our specialist panels. PCO items were first identified through use of the Dystonia Coalition Natural History Database (Kilic-Berkmen, et al, 2021). Data from 200 patients, each with BSP, CD, or LD, were included in the initial analysis for item generation. Prospective items were analyzed for their contribution to the overall severity scores on the clinical and patient-centered outcome scales. Items that were repetitive were excluded. Iterative meetings with specialist panels consisting of neurologists, otolaryngologists, ophthalmologists, speech language pathologists, and Patient Advocacy Group (PAG) representatives, as well as virtual focus groups of patients with BSP, CD, or LD were held. An online survey was conducted for each of the PCO categories with over 1,000 dystonia patients participating. Finally, a content validity ratio (CVR) was calculated based on the input of the specialist panel members. This process was repeated until the CVR showed good agreement of panel members as to the relevance and clarity of the items.
Results: We successfully developed PCO measures tailored for each dystonia subtype: CD, BSP, and LD. The PCO consists of 14-17 items covering three domains (motor, disability, and psychosocial measures). The PCO reflects the input of international specialist panels, more than 1,000 dystonia patients, and PAGs, and followed FDA guidance. The PCO will be used in an app-based format compatible with smartphones and tablets for ease of use.
Conclusions: The development of these PCO measures was accomplished during the COVID-19 pandemic using robust existing patient-centered data from previous Dystonia Coalition projects; active engagement with PAGs; and use of virtual focus groups and online surveys. Next steps include using the PCO to characterize the therapeutic response to BoNT over time, which will: 1) measure the peak effect size; 2) capture the up-and-down effect during BoNT treatment; and 3) prepare for a future adjunct clinical trial.
Funding; U54 NS116025/NS/NINDS NIH HHS/United States.
Keywords: Efficacy; Botulinum toxin; Dystonia; Outcomes

Reference

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Real-World Persistence and Costs Among Patients With Chronic Migraine Treated With OnabotulinumtoxinA or Calcitonin Gene–Related Peptide Monoclonal Antibodies: A Retrospective Claims Analysis Study

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Introduction: Persistence with oral migraine preventive medications (OMPMs) is reportedly low. Real-world data on treatment persistence and costs associated with recently approved preventive chronic migraine (CM) treatments are limited. This study evaluates real-world persistence rates and costs among patients with CM treated with onabotulinumtoxinA (onabotA [BOTOX®]) or calcitonin gene–related peptide monoclonal antibody (CGRP mAb).
Methods: This retrospective, longitudinal, observational study analyzed the IBM MarketScan® Commercial and Medicare Supplemental databases (7/1/2017-2/29/2020). Adults treated with either onabotA or CGRP mAbs (based on overall migraine ICD-10 codes) and having continuous coverage $\geq$6 months before and $\geq$12 months after treatment initiation were included. Persistence to index treatment was assessed at 6, 9, and 12 months, and all-cause and migraine-related costs were evaluated during the 12-month follow-up period. Persistence and costs were adjusted for potential confounders (demographics, comorbidities, OMPM use) using generalized linear model regression.
Results: Of 66,303 patients with onabotA or CGRP mAb claims, 2697 patients with CM met inclusion/exclusion criteria. In the total population, patients were primarily female (86%), and their mean age was 44 years, which was consistent among the individual CGRP mAbs. Persistence was higher among those treated with onabotA versus the combined CGRP mAbs group at 6 (67% vs 47%; $P<0.001$), 9 (51% vs 37%; $P<0.001$), and 12 (40% vs 27%; $P<0.001$) months. OnabotA and CGRP mAbs were associated with comparable 12-month all-cause ($16,681 vs $16,666) and migraine-

related ($8198 vs $8518) costs. Compared to CGRP mAbs, onabotA was associated with lower 12-month acute medication ($763 vs $1240; $P<0.001$), OMPM ($685 vs $993; $P<0.01$), and migraine-related inpatient ($224 vs $728; $P<0.01$) costs. Migraine-related emergency department costs were comparable between onabotA and CGRP mAbs ($149 vs $129). Findings were sustained after regression adjustment for confounders.

Conclusions: CM patients initiating onabotA treatment had higher persistence and comparable all-cause and migraine-related costs over 12 months compared to CGRP mAbs.

Support: Allergan (prior to its acquisition by AbbVie).

Keywords: Chronic migraine; Monoclonal antibody; OnabotulinumtoxinA

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Jennifer McVige has served as a consultant for Theranica and on a Scientific Advisory or Data Safety Monitoring Board for Lundbeck. Dr. McVige has served on a Speakers' Bureau for Allergan/AbbVie, Amgen, Biohaven, Eli Lilly, Teva, and Neurelis. The institution of Dr. McVige has received research support from Amgen, Eli Lily, Biohaven, Lundbeck, and the Dent Family Foundation. Dr. McVige has a non-compensated relationship as a member of a Neurology Board with the United Council for Neurologic Subspecialties that is relevant to American Academy of Neurology (AAN) interests or activities. Dr. McVige also has a non-compensated relationship as a Board Exam board member with the American Board of Psychiatry and Neurology that is relevant to AAN interests or activities.

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Real-World Dosing Differences of OnabotulinumtoxinA and AbobotulinumtoxinA in Treatment of Upper Limb Spasticity

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Introduction: In the United States, three botulinum toxin type A (BoNT/A) products are approved by the US Food and Drug Administration (FDA) to treat upper limb spasticity (ULS). Each BoNT/A product is manufactured and tested via proprietary processes, resulting in unique clinical profiles (Brin, et al, 2014). To this end, FDA-approved labels of BoNT/As state that potency units of one product are not interchangeable and cannot be converted into units of another BoNT/A product. In this retrospective study, we compared doses of onabotulinumtoxinA (onabotA) and abobotulinumtoxinA (abobotA) utilized in the treatment of adult ULS.

Methods: De-identified medical records were obtained via survey of 101 unique health care professionals (HCPs) on adult patients diagnosed with post-stroke ULS per International Classification of Diseases, 10th revision (ICD-10), and treated with botulinum toxin using current procedural terminology (CPT) in the US. Two hundred fifteen patient charts were reviewed: 107 treated with onabotA and 108 with abobotA. Patients received at least 3 treatments of onabotA or abobotA in one spastic upper limb prior to March 2020. Doses injected per muscle were compared between BoNT/A products.

Results: Of the participating HCPs, 71% specialized in neurology, 21% in physiatry, and 8% in other specialties. There was no difference in the average injection intervals for patients treated with onabotA vs abobotA (102 vs 99 days; standard deviation: 72 vs 90, respectively). The average doses used varied across muscles injected, resulting in a range of dose ratios calculated ad hoc between the 2 BoNT/A products (Table 1).

Table 1
Doses of OnabotA or AbobotA Injected Into Muscles of Patients With Upper Limb Spasticity.

Muscle[a] (n=onabotA, abobotA)	Average OnabotA Dose (U) Injected (SD)	Average AbobotA Dose (U) Injected (SD)	OnabotA: AbobotA (Lower 95% CI, Upper 95% CI)
Biceps (n= 76, 72)	83.1 (47.9)	181.5 (105.6)	1:2.2 (1.8, 2.6)
Brachialis (n= 4, 29)	57.5 (29.9)	205.3 (103.8)	1:3.6 (2.3, 5.9)
Brachioradialis (n= 21, 27)	41.7 (24.0)	158.3 (82.3)	1:3.8 (2.8, 5.2)
Flexor carpi radialis (n= 59, 25)	34.9 (26.8)	113.0 (45.7)	1:3.2 (2.6, 4.1)
Flexor carpi ulnaris (n= 46, 27)	34.2 (23.8)	120.4 (68.0)	1:3.5 (2.7, 4.7)
Flexor digitorum profundus (n= 39, 20)	37.9 (17.0)	108.8 (53.8)	1:2.9 (2.2, 3.6)
Flexor digitorum superficialis (n= 39, 20)	36.3 (16.4)	118.8 (53.1)	1:3.3 (2.5, 4.1)
Flexor pollicis longus (n= 33, 10)	23.6 (10.5)	72.0 (38.0)	1:3.1 (2.1, 4.2)
Pronator quadratus (n= 4, 4)	32.5 (5)	92.5 (78.9)	1:2.9 (1, 5.2)
Pronator teres (n= 15, 20)	35.7 (22.0)	147.5 (61.2)	1:4.1 (2.9, 6.0)

[a] Only includes muscles injected when n≥4 patients for both onabotA and abobotA.

Conclusions: This study provides real-world evidence that there is no universal dose conversion ratio between abobotA and onabotA. The muscle-by-muscle comparison findings of this study suggest that fixed-dose conversion practices (at a syringe level) may lead to disrupted dosing of specific muscles, as individualized dose ratios ranged from 1:1 to 1:6. Our results support the FDA mandate that units of one BoNT/A product cannot be compared with or converted into units of any other BoNT/A product and, in clinical practice, fixed dose ratios are not apparent.

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Conflicts of interest: Ritu Singh, Aleksej Zuzek, and Mariana Nelson are employees of AbbVie and may hold AbbVie stock. Marc Schwartz has

served as a biostatistical consultant for AbbVie.
Keywords: AbobotulinumtoxinA; Botulinum toxin; OnabotulinumtoxinA; Real-world; Upper limb spasticity

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Cultured Human-Induced Pluripotent Stem Cell–Derived Motor Neurons as a Sensitive Model for BoNT Detection

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Introduction: Botulinum neurotoxins (BoNTs) primarily target peripheral motor neurons in the neuromuscular junction where they inhibit release of the neurotransmitter acetylcholine and thereby induce flaccid paralysis. Optimal models for the study of BoNTs are able to detect toxin activity with high sensitivity and require all steps of the cellular intoxication process. Currently, cell-based assays using multiple cell models, including optimized neuronal cell lines, primary neurons, and human-induced pluripotent stem cell (hiPSC)–derived neurons, are used to detect BoNT in vitro for potency determination and research applications. The high affinity of BoNTs for motor neurons in vivo in the neuromuscular junction makes hiPSC-derived motor neurons a physiologically relevant model for BoNT research and detection.
As the stem cell industry has grown, more sources of high-quality hiPSC-derived neurons have become available for use in the BoNT research field. Evaluations of several hiPSC-derived–neuronal cell models have shown differences in sensitivity to BoNTs.[1,2] A commercially available motor neuron cell model (Fuji Film Cellular Dynamics Inc) and an "in-house differentiated" motor neuron culture had greater sensitivity to BoNTs than other neuronal subpopulations; however, motor neuron sensitivity was dependent on hiPSC differentiation method.[3]
In this study, we have analyzed and compared additional commercially available hiPSC-derived neuronal cell types for detection of BoNT, further demonstrating high sensitivity of motor neuron cultures to BoNT/A (botulinum neurotoxin type A).
Methods: Cryopreserved hiPSC-derived GABAergic, glutamatergic, and spinal motor neurons were provided by BrainXell (Madison, WI). The motor neurons were produced using the high-yield differentiation protocol of Du et al.[4] Neurons were cultured according to the manufacturer's protocol. Cell cultures were exposed to purified BoNT/A1 after >28 days in culture. BoNT/A1 was added to culture wells in a series of dilutions. Cleaved versus uncleaved SNAP-25 (synaptosomal-associated protein, 25kDa) in cell lysates was determined by Western blot. Half maximal effective concentration (EC_{50}) values were determined using GraphPad Prism.
Results: The three neuronal cultures detected BoNT/A1 with varying sensitivity. Cultured motor neurons were found to have an EC_{50} value of 0.97 U/well (95% confidence interval [CI]: 0.5-1.9 U/well), GABAergic and glutamatergic cultures EC_{50} values of 3.4 U/well (95% CI: 1.6-7.2 U/well) and 3.42 U/well (95% CI: 1.2-16.9 U/well), respectively. Thus, motor neurons demonstrated the greatest sensitivity to BoNT/A1.
Conclusions: All three hiPSC-derived neuron cultures detected BoNT with sensitivity similar to previously examined hiPSC-derived neuron cultures, with the motor neurons showing the highest sensitivity. Sensitivity of the BrainXell motor neuron culture was similar to that reported for hiPSC-derived motor neurons using the same differentiation method.[3] Further study of the molecular mechanisms underlying the differential sensitivity of various hiPSC-derived motor neuron cultures has the potential to reveal neuron-specific factors governing differential neuronal cell entry by BoNTs.
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Keywords: BoNT detection; Botulinum neurotoxin; BrainXell; Human-induced pluripotent stem cells (hiPSC); Motor neuron

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COVID-19 mRNA Vaccination

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Introduction: Since the 1980s botulinum toxin type A (BTA) has been widely used in cosmetic as well as neurological cases and its use is expanding with new indications and improved availability.
The COVID-19 pandemic and the introduction of the new mRNA COVID-19 vaccines raise the issue of immunological response and sensitivity reactions.
There have been a few reports of reactions to facial fillers[1] and one report of a hypersensitivity reaction to an inactivated vaccine with BTA use[2] but none to our knowledge to mRNA vaccinations. We describe two cases of hypersensitivity reactions to BTA following mRNA COVID-19 vaccinations.
Case Report: A 45-year-old female (AB) receiving BTA injections for chronic migraine, received a Pfizer mRNA COVID-19 vaccine dose for the first time one day after receiving BTA injections. Another 66-year-old female (CD) receiving BTA injections for cosmetic use also received the first Pfizer mRNA COVID-19 vaccine one week prior to the BTA injection. The women are mother and daughter and were injected from the same onabotulinumtoxinA bottle containing 200 units diluted with 4 cc of normal saline.
AB received 160 units of onabotulinumtoxinA dosed according to the migraine protocol 3 months after the BTA injection. She reported pain in the injected areas and swelling in the same areas that started 2 days after being vaccinated. The pain and swelling lasted for one week. Three weeks after the first injection she received the second dose of the vaccine and half an hour later she again developed pain and swelling in the areas injected with BTA.
This was the first BTA injection for CD and she received 15 units of onabotulinumtoxinA for cosmetic reasons. She complained of pain and swelling in the BTA-injected areas that began the day after and lasted for a week before resolving.
There is no family history of angioedema. AB did not use NSAIDs or any other medications prior to the injection. CD is on chronic aspirin 100 mg. CD did not receive a specific evaluation. AB has normal esterase inhibitor C1 levels (25.4 to add units), normal C3 complement level (176.7 mg/dL), and slightly elevated C4 complement level (47.7 mg/dL, normal range up to 40 mg/dL). These results are not consistent with a diagnosis of hereditary angioedema.
Conclusion: We report a case of hypersensitivity reaction to botulinum neurotoxin injection after mRNA COVID-19 vaccination. The possibility of such reactions should be considered when planning BTA injections
Keywords: Botulinum toxin type A; COVID-19; Hypersensitivity; mRNA vaccine

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Selective Expression of the Protease of Botulinum Neurotoxin Type A1 in Nociceptive Neurons Persistently Blocks Neurotransmission

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Chronic pain is a leading health and socioeconomic problem, affecting more than 20% of the world's population. Currently, non-steroidal anti-inflammatory drugs and opioid analgesics are widely used for treatment of pain. However, due to their short half-lives and frequent association with serious side effects, an unmet need exists for long-lasting analgesics. SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein receptors) are required for neuropeptide release and noxious signal transducers' surface trafficking. Thus, selective expression of the SNARE-cleaving light-chain protease of botulinum neurotoxin type A1 (LCA) in peripheral sensory neurons could alleviate chronic pain. However, a safety concern associated with this approach is the lack of a sensory neuronal promoter to prevent the expression of LCA in the central nervous system. To address this, we exploited the unique characteristics of Pirt (phosphoinositide-interacting regulator of transient receptor potential channels), which is expressed in most nociceptive neurons in the dorsal root ganglia and trigeminal ganglia. For the first time, we identified a Pirt promoter element and cloned it into a lentiviral vector-driving GFP (green fluorescent protein) reporter gene expressed selectively in cultured mouse dorsal root ganglion neurons (mDRGs). Pirt promoter—driven LCA expression yielded rapid and concentration-dependent cleavage of SNAP-25 (synaptosomal-associated protein, 25 kDa) in the subpopulation of transient receptor potential cation channel subfamily V member 1 ($TRPV1^+$) mDRGs. Moreover, the transcripts of pain-related genes (TAC1 (tachykinin precursor 1); CALCB (calcitonin gene-related peptide 2); HTR3A (5-hydroxytryptamine receptor 3A); NPY2R (neuropeptide Y2 receptor); GPR52 (G protein—coupled receptor 52); SCN9A (voltage-gated sodium channel alpha subunit 9); TRPV1 and TRPA1 (transient receptor potential cation channel subfamily A member 1) in pro-inflammatory cytokine—stimulated mDRGs were downregulated by viral-mediated expression of LCA. Furthermore, viral expression of LCA yielded long-lasting inhibition of pain mediator release. Thus, for the first time, we show that selective expression of a SNARE-cleaving protease in peripheral sensory neurons attenuates pain-related gene transcription and neuropeptide release. The engineered Pirt-LCA virus may provide a novel means for long-lasting pain relief.

Keywords: Chronic pain; Gene therapy; Neuropeptide; Neurotoxin; Pirt; SNARE

Switching Adult Patients With Spasticity from OnabotulinumtoxinA to AbobotulinumtoxinA: A Real-World Data Analysis Across Three US-Based Treatment Centers

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Introduction: The therapeutic impact of botulinum toxin type A (BoNT-A) as first-line treatment for adult focal spasticity is well established across numerous therapeutic guidelines. However, relatively little real-world evidence exists to inform clinician decision-making when considering switching patients between BoNT-A products. This 5-year retrospective study aims to illustrate the safe transitioning of patients from onabotulinumtoxinA (ONA) to abobotulinumtoxinA (ABO) therapy in US centers that have considerable experience with BoNT-A usage in adults with spasticity.
Methods: Chart data from patients with upper limb spasticity (ULS), lower limb spasticity (LLS), or upper and lower limb spasticity (ULS+LLS) aged $\geq$18 years were collected from three US-based treatment centers. Eligible patients had $\geq$2 ONA treatment cycles before switching to ABO from September 2015 to September 2020; they were followed for three more ABO treatment cycles.
Results: A total of 88 patients (mean$\pm$SD age 44.9$\pm$19.6 years, 62.5% male) switched from ONA to ABO; in 84 (95.5%) patients, the switch was due to "medical need/effectiveness not achieved." Spasticity etiologies included stroke (25.0%), traumatic brain injury (13.6%), spinal cord injury (2.3%), adults with cerebral palsy (42.0%), multiple sclerosis (9.1%), and other etiology (8.0%). Fifty-one patients (58.0%) had bilateral spasticity, with a mean 5.0$\pm$2.2 muscles injected at each visit over the 5-injection-cycle treatment period. Across the 2 initial cycles prior to the switch, mean doses of ONA were 425.0$\pm$179.0 units (U), 477.1$\pm$132.8 U, and 454.2$\pm$56.0 U for ULS, LLS, and ULS+LLS, respectively; following the switch, mean ABO doses across three treatment cycles were 1005.1$\pm$439.2 U, 1006.2$\pm$334.2 U, and 1203.2$\pm$325.7 U, respectively. No adverse events (AEs) were reported following conversion.
Conclusions: In this real-world study, patients with ULS, LLS, or ULS+LLS were switched from ONA to ABO and continued for at least three treatment cycles without any reported AEs. Mean doses of both ONA and ABO were generally aligned with the upper ranges of their respective dosing guidelines. Further randomized controlled studies evaluating the efficacy and safety of ONA vs ABO are warranted.
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Keywords: AbobotulinumtoxinA; Adult lower limb spasticity; Adult upper limb spasticity; OnabotulinumtoxinA; Real-world evidence; Spasticity