

Long-Term Treatment Response and Longevity of Different Botulinum Toxin Type A Formulations in Cervical Dystonia

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1 ABSTRACT (ENGLISH)

Cervical dystonia (CD) is a chronic neurological movement disorder requiring repeated, individualized treatment with botulinum neurotoxin type A (BoNT/A). Although BoNT/A is the established first-line symptomatic therapy, long-term response may change because of dose adaptation, evolving muscle involvement, altered injection strategy, formulation exposure, or immunogenicity. In routine practice, these mechanisms are difficult to distinguish because documentation is heterogeneous and neutralizing antibody testing is rarely available. The “Real-World Evidence of Longevity of BoNT/A in Cervical Dystonia” (RELY-CD) study was designed as a retrospective real-world study to evaluate long-term BoNT/A treatment dynamics in CD and to compare dose-effect development between complex-free (CF) and complex-containing (CC) BoNT/A formulations.

RELY-CD analyzed patients with CD treated with BoNT/A over up to 10 years. The study used routinely documented parameters, particularly dose per muscle and clinical effect. To quantify long-term treatment dynamics, the dose-effect parameter DEff was developed, combining dose development and clinical effect into one longitudinal measure. The primary comparison assessed clinically meaningful DEff worsening in treatment year 7 compared with treatment year 2 between CF and CC monotherapy. A DEff value ≥ 1.2 was predefined as clinically meaningful worsening. Patients switching from CC to CF formulations were analyzed separately.

A total of 250 patients were eligible for analysis. Most patients maintained stable or improved dose-effect development. At year 7, clinically meaningful DEff worsening occurred in 11.1% of CF monotherapy patients and 24.2% of CC monotherapy patients. In switchers, DEff worsening occurred in 24.8%; trajectory analyses indicated that worsening was already present before the switch and improved thereafter. Muscle-specific dose analysis showed that muscle-specific doses remained largely stable, and patient- and clinician-rated global impressions indicated sustained improvement.

RELY-CD demonstrates that long-term BoNT/A treatment in CD is generally durable but clinically dynamic. The lower proportion of clinically meaningful DEff worsening under CF compared with CC monotherapy suggests potential formulation-related differences in long-term treatment stability, while remaining subject to the limitations of retrospective real-world data. DEff provides a pragmatic framework for monitoring dose-effect development when standardized long-term outcomes and antibody testing are not routinely available. Future validation should assess DEff against standardized clinical outcomes, patient-reported duration of effect, treatment satisfaction, and neutralizing antibody testing.

2 ZUSAMMENFASSUNG (DEUTSCH)

Zervikale Dystonie (CD) ist eine chronische neurologische Bewegungsstörung, die eine wiederholte, individualisierte Behandlung mit Botulinumneurotoxin Typ A (BoNT/A) erfordert. Obwohl BoNT/A die etablierte symptomatische Erstlinientherapie ist, kann sich das Langzeitansprechen aufgrund von Dosisanpassungen, dynamischer Muskelbeteiligung, geänderter Injektionsstrategie, Formulierungsexposition oder Immunogenität verändern. In der Routineversorgung sind diese Mechanismen schwer voneinander zu unterscheiden, da die Dokumentation heterogen ist und Tests auf neutralisierende Antikörper nur selten verfügbar sind. Die Studie „Real-World Evidence of Longevity of BoNT/A in Cervical Dystonia“ (RELY-CD) wurde als retrospektive Real-World-Studie konzipiert, um langfristige BoNT/A-Behandlungsdynamiken bei CD zu untersuchen und die Dosis-Wirkungs-Entwicklung zwischen komplexfreien (CF) und komplexhaltigen (CC) BoNT/A-Formulierungen zu vergleichen.

RELY-CD analysierte Patienten mit CD, die über einen Zeitraum von bis zu 10 Jahren mit BoNT/A behandelt wurden. Die Studie verwendete routinemäßig dokumentierte Parameter, insbesondere die Dosis pro Muskel und den klinischen Effekt. Zur Quantifizierung langfristiger Behandlungsdynamiken wurde der Dosis-Wirkungs-Parameter DEff entwickelt, der Dosisentwicklung und klinischen Effekt in einem longitudinalen Maß kombiniert. Der primäre Endpunkt untersuchte eine klinisch bedeutsame DEff-Verschlechterung im Behandlungsjahr 7 im Vergleich zu Behandlungsjahr 2 zwischen CF- und CC-Monotherapie. Ein DEff-Wert $\geq 1,2$ wurde vorab als klinisch bedeutsame Verschlechterung definiert. Patienten, die von CC- auf CF-Formulierungen wechselten, wurden separat analysiert.

Insgesamt waren 250 Patienten in die Analyse aufgenommen worden. Die meisten Patienten zeigten eine stabile oder verbesserte Dosis-Wirkungs-Entwicklung. Im Jahr 7 trat eine klinisch bedeutsame DEff-Verschlechterung bei 11,1 % der Patienten unter CF-Monotherapie und bei 24,2 % der Patienten unter CC-Monotherapie auf. Bei Wechseln trat eine DEff-Verschlechterung bei 24,8 % auf; Trajektorienanalysen zeigten, dass die Verschlechterung bereits vor dem Wechsel vorhanden war und sich danach verbesserte. Die muskelspezifische Dosisanalyse zeigte, dass die muskelspezifischen Dosen weitgehend stabil blieben, und patienten- sowie klinikerseitig bewertete globale Eindrucksskalen wiesen auf eine anhaltende Verbesserung hin.

RELY-CD zeigt, dass die langfristige BoNT/A-Behandlung bei CD im Allgemeinen dauerhaft wirksam, jedoch klinisch dynamisch ist. Der geringere Anteil klinisch bedeutsamer DEff-Verschlechterung unter CF- im Vergleich zu CC-Monotherapie deutet auf mögliche formulierungsbezogene Unterschiede in der langfristigen Behandlungsstabilität hin, bleibt jedoch den Limitationen retrospektiver Real-World-Daten unterworfen. DEff bietet einen pragmatischen Rahmen zur Beobachtung der Dosis-Wirkungs-Entwicklung, wenn standardisierte Langzeitendpunkte und Antikörpertests in der Routineversorgung nicht verfügbar sind. Zukünftige Validierungen sollten DEff gegenüber standardisierten klinischen Endpunkten, patientenberichteter Wirkdauer, Behandlungszufriedenheit und Tests auf neutralisierende Antikörper prüfen.

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4 USE OF ARTIFICIAL INTELLIGENCE

Artificial intelligence tools were used in the preparation of this thesis in a supporting capacity. ChatGPT 4.0 (OpenAI) was used to support language editing, structural refinement, consistency checks, and the identification of unclear or repetitive wording. Perplexity AI was used to assist with navigation of the scientific literature and to identify additional potentially relevant references for manual verification. All scientific content, analyses, interpretations, conclusions, and final wording were created, verified, and approved by the author. No artificial intelligence tool was used to generate, analyze, or interpret study data.

5 VERSICHERUNG AN EIDES STATT

Ich versichere an Eides Statt, dass die Dissertation von mir selbständig und ohne unzulässige fremde Hilfe unter Beachtung der „Grundsätze zur Sicherung guter wissenschaftlicher Praxis an der Heinrich-Heine-Universität Düsseldorf“ erstellt worden ist.

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Unterschrift

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7 LIST OF ABBREVIATIONS

aboA.....	<i>abobotulinumtoxinA</i>
BoNT/A	<i>botulinum neurotoxin type A</i>
CC.....	<i>complex-containing</i>
CD.....	<i>cervical dystonia</i>
CF	<i>complex-free</i>
COL-CAP	<i>Collum-caput</i>
CSTF.....	<i>Complete Secondary Treatment Failure</i>
DEff.....	<i>dose-effect parameter</i>
GICS	<i>Global Impression of Change Scale</i>
HA/Hn	<i>hemagglutinin</i>
HC.....	<i>Heavy Chain</i>
Hc/HCR.....	<i>C-Terminal Receptor-Binding Region</i>
HN.....	<i>HC N-Terminal Translocation Region</i>
incoA	<i>incobotulinumtoxinA</i>
kDa.....	<i>kilodalton</i>
L/LL toxin	<i>large/very large BoNT progenitor toxin complex</i>
LC	<i>Light Chain</i>
M toxin	<i>medium-sized BoNT progenitor toxin complex</i>
MCIC.....	<i>Minimal Clinically Important Change</i>
NAbs	<i>Neutralizing Antibodies</i>
NAPs.....	<i>neurotoxin associated proteins</i>
ng.....	<i>nanogram</i>
NTNH	<i>Non-Toxic Non-Hemagglutinin</i>
onaA.....	<i>onabotulinumtoxinA</i>
PSTF.....	<i>Partial Secondary Treatment Failure</i>
RELY-CD	<i>Real-World Evidence of Longevity of BoNT/A in Cervical Dystonia</i>
TWSTRS.....	<i>Toronto Western Spasmodic Torticollis Rating Scale</i>

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10 INTRODUCTION

10.1 CERVICAL DYSTONIA: A CHRONIC DISORDER REQUIRING LONG-TERM THERAPY

Dystonia is a neurological movement disorder in which involuntary muscle contractions generate abnormal movements, postures, or both. Contemporary definitions emphasize that dystonic movements are often patterned, may include tremor, and are typically aggravated by voluntary action with overflow activation of other muscles (Albanese *et al.*, 2013). Within this spectrum, cervical dystonia (CD) is the most common adult-onset focal dystonia (Defazio *et al.*, 2013). It is characterized by involuntary contractions of cervical and shoulder-girdle muscles, leading to abnormal head and neck postures such as rotation, laterocollis, retrocollis, anterocollis, lateral shift, sagittal shift, or mixed patterns (Albanese *et al.*, 2023). Alleviating maneuvers (also: sensory tricks) are present in up to 90% of patients and are a key clinical



feature distinguishing cervical dystonia from other movement disorders (Albanese *et al.*, 2023). They typically involve light fingertip contact on the chin, cheek, or neck, although some patients benefit from non-contact gestures such as raising the arms or even imagining the movement, all of which can transiently improve dystonic posture (Albanese *et al.*, 2023). *Figure 1* depicts a sketch of an individual with cervical dystonia, showing a predominant laterocaput component and performing an alleviating maneuver.

Figure 1. Characteristic Presentation of Cervical Dystonia with Sensory Trick

Sketch depicting an individual with an abnormal, asymmetric head and neck posture typical of cervical dystonia. The light fingertip contact to the face illustrates a sensory trick, a commonly observed maneuver that can transiently modulate involuntary muscle contractions and partially correct the dystonic posture without force. Illustration by Prof. Alberto Albanese published in Albanese *et al.* (2023). Used with kind permission for academic purposes under CC BY-NC-ND 4.0.

The burden of CD extends beyond abnormal posture. Patients frequently experience pain, tremor, functional disability, embarrassment, impaired participation, and reduced quality of life (Comella and Bhatia, 2015; Girach, Vinagre Aragon and Zis, 2019). Pain is an important symptom because it may be one of the main reasons for seeking treatment and can remain clinically relevant even when postural abnormality improves (Avenali *et al.*, 2018; Rosales *et al.*, 2021). CD can also be accompanied by psychiatric comorbidities, including depression and anxiety, which may influence symptom perception, treatment satisfaction, and long-term disease management (Moraru *et al.*, 2002; Waeschle *et al.*, 2025). For this reason, CD should

not be framed narrowly as a focal motor disorder but as a chronic neurological condition requiring individualized, repeated, life-long, and patient-centered treatment (Erro *et al.*, 2023; Castagna, Jinnah and Albanese, 2024).

The diagnosis of CD is primarily clinical (Albanese *et al.*, 2023). Imaging and laboratory testing are usually not diagnostic in idiopathic adult-onset CD, although they may be required to exclude secondary causes in selected cases (Albanese *et al.*, 2023). Clinical assessment involves identifying the dystonic pattern, recognizing tremor and sensory tricks, differentiating dystonia from functional movement disorders or musculoskeletal conditions, and documenting disease severity over time (Jinnah *et al.*, 2013; Albanese *et al.*, 2023). This has direct therapeutic relevance. The clinical pattern determines which muscles are injected, how dose is distributed between superficial and deep muscles, whether guidance techniques are required, and how treatment success is evaluated at reinjection visits (Walker, 2003; Albanese *et al.*, 2015; Contarino *et al.*, 2017; Castagna and Albanese, 2019).

In everyday practice, the therapeutic goal is rarely complete normalization, but rather sustained and clinically meaningful symptom control, pain reduction, functional improvement, and acceptable tolerability across repeated treatment cycles (Colosimo *et al.*, 2020; Rodrigues *et al.*, 2020; Comella *et al.*, 2021). This long-term perspective is central to this thesis. CD usually persists throughout adult life, and the most effective symptomatic therapy, botulinum neurotoxin type A (BoNT/A), must be repeated in regular cycles (Simpson *et al.*, 2016; Contarino *et al.*, 2017; Walter *et al.*, 2020). Consequently, the clinical problem is not solely whether BoNT/A works in the short term, which has been well established, but how long-term response is maintained, how treatment should be adapted, and how waning response or resistance can be recognized in routine care.

10.2 BOTULINUM NEUROTOXIN TYPE A: MECHANISM AND FORMULATIONS

BoNT/A is a bacterial protein toxin produced by *Clostridium botulinum* and related clostridial species. In nature, BoNT/A is primarily relevant as an oral toxin (Pirazzini *et al.*, 2017; Kumar and Singh, 2025). After ingestion, the neurotoxin must survive the acidic and protease-rich environment of the gastrointestinal tract before it can cross the intestinal barrier and reach peripheral nerve terminals (Pirazzini *et al.*, 2017; Kumar and Singh, 2025). This natural route of intoxication explains why BoNT/A is produced by the bacteria not only as the 150 kDa core neurotoxin, but as part of larger progenitor toxin complexes containing non-toxic associated proteins, or complexing proteins (Pirazzini *et al.*, 2017; Kumar and Singh, 2025). These complexing proteins include non-toxic non-hemagglutinin (NTNHA) and, depending on the gene cluster, hemagglutinin (HA) proteins or OrfX-associated proteins (Pirazzini *et al.*, 2017; Kumar and Singh, 2025). Their biological role is mainly related to protection and transport

during gastrointestinal exposure, rather than to the intracellular pharmacological action of the neurotoxin itself (Pirazzini *et al.*, 2017; Kumar and Singh, 2025). Figure 2 shows the peptide structure of the 150 kDa neurotoxin and the three-dimensional structure of the toxin complex (Kumar and Singh, 2025).

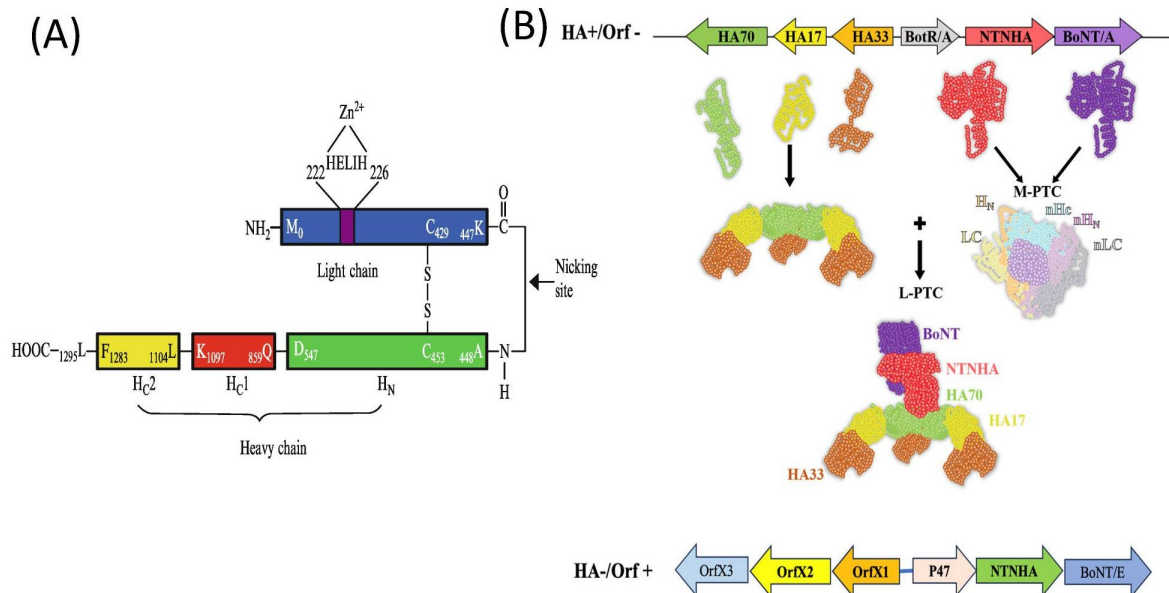


Figure 2. Molecular Composition of the Botulinum Neurotoxin Type A Complex

(A) Domain structure of botulinum neurotoxin type A (BoNT/A; 150 kDa), consisting of a light chain (LC; catalytic zinc-dependent endopeptidase) and a heavy chain (HC) composed of the receptor-binding domains HCC and HCN and the translocation domain HN. The LC and HC are linked by a disulfide bond and a proteolytic nicking site. (B) Schematic organization of the BoNT/A progenitor complex. In HA-positive (HA⁺/Orf⁻) complexes, BoNT/A associates with non-toxic non-hemagglutinin (NTNHA) and hemagglutinin proteins (HA70, HA17, HA33) to form M-PTC and L-PTC complexes. In HA-negative (HA⁻/Orf⁺) complexes, HA proteins are replaced by OrfX-associated proteins (OrfX1-3 and P47). Abbreviations: BoNT/A, botulinum neurotoxin type A; LC, light chain; HC, heavy chain; HN, translocation domain; HCN/HCC, N- and C-terminal receptor-binding domains of HC; NTNHA, non-toxic non-hemagglutinin; HA, hemagglutinin; PTC, progenitor toxin complex. Figure from Kumar and Singh (2025). Used with kind permission for academic purposes under CC BY-NC-ND 4.0.

Its mechanism of action is well understood at the neuromuscular junction (Pirazzini *et al.*, 2017). After binding to cholinergic nerve terminals, the toxin is internalized and its light chain cleaves the SNARE protein SNAP-25, thereby inhibiting acetylcholine release and producing a reversible chemodenervation of the injected muscle (Pirazzini *et al.*, 2017).

This mechanism explains the clinical usefulness of BoNT/A in CD, where the therapeutic goal is a temporary reduction of excessive dystonic muscle activity rather than permanent denervation (Pirazzini *et al.*, 2017). Intramuscular injection bypasses the gastrointestinal route of natural intoxication and delivers the therapeutically active neurotoxin directly to peripheral motor terminals (Pirazzini *et al.*, 2017). Recovery occurs gradually as functional neuromuscular transmission is restored, which explains why treatment must be repeated in cycles (Pirazzini *et al.*, 2017).

The distinction between natural toxin complexes and therapeutic BoNT/A formulations is important for interpreting formulation differences. In clostridial cultures, BoNT/A may occur in several complex sizes, including the medium-sized M complex of approximately 300 kDa, the large L complex of approximately 500–600 kDa, and the very large LL complex of approximately 900 kDa (Eisele *et al.*, 2011; Kumar and Singh, 2025). These complexes contain the 150 kDa neurotoxin together with NTNHA and, in HA-positive complexes, HA proteins such as HA70, HA17 and HA33 (Figure 2) (Pirazzini *et al.*, 2017; Kumar and Singh, 2025).

However, the therapeutic relevance of complexing proteins after intramuscular injection is disputed (Eisele *et al.*, 2011; Pirazzini *et al.*, 2017; Bigalke, Frevert and Dressler, 2026). Eisele *et al.* (2011) showed that purified BoNT/A complexes dissociate rapidly at neutral to slightly basic pH. At pH values greater than 7.0, release of the free 150 kDa neurotoxin occurred with a half-life of less than one minute (Eisele *et al.*, 2011). In contrast, BoNT/A complexes were stable at acidic pH values of 6.25 or lower (Eisele *et al.*, 2011). The same study showed that dilution and sodium chloride, both relevant to pharmaceutical preparation and reconstitution, promoted dissociation of higher molecular weight complexes (Eisele *et al.*, 2011). In reconstituted commercial BoNT/A products containing complexing proteins, most or all detectable neurotoxin was present in free 150 kDa form (Eisele *et al.*, 2011). These findings challenge the assumption that intact 900 kDa complexes persist after reconstitution or meaningfully restrict diffusion after injection (Eisele *et al.*, 2011).

Therapeutic BoNT/A preparations therefore differ not in the fundamental intracellular mechanism of action, but in formulation characteristics such as the presence or absence of complexing proteins, excipients, drying process, storage conditions, and biological potency units (Pirazzini *et al.*, 2017; Bigalke, Frevert and Dressler, 2026). Several BoNT/A formulations are commercially available and widely used for CD in Europe, including incobotulinumtoxinA (incoA), onabotulinumtoxinA (onaA), and abobotulinumtoxinA (aboA) (Carr, Jain and Sublett, 2021; Walter *et al.*, 2025). These products are not interchangeable. A 1:1 conversion is commonly accepted for incoA and onaA, whereas conversion between aboA and incoA or onaA is more variable, with ratios around 1:3 frequently used in clinical practice (Frevert and Dressler, 2010; Scaglione, 2016; Waeschle *et al.*, 2025). The RELY-CD protocol explicitly allowed center-specific conversion ratios, reflecting that dosing practice is influenced by local experience rather than by one universally accepted conversion factor (Waeschle *et al.*, 2025). Table 1 compares onaA, aboA, and incoA in terms of vial content and commercial availability of formulations (Bigalke, Frevert and Dressler, 2026).

Table 1. Overview of the Three Major BoNT/A Formulations Available in Europe

Product	Botox®	Dysport®	Xeomin®
Generic name	onabotulinumtoxinA	abobotulinumtoxinA	incobotulinumtoxinA
Manufacturer	Abbvie/Allergan, USA	Ipsen, France	Merz, Germany
Toxin type	Type A complex	Type A complex	Type A 150 kDa neurotoxin
Packaging	50 MU, 100 MU, 200 MU	300 MU, 500 MU	50 MU, 100 MU, 200 MU
Excipients	0.5 mg HSA, 0.9 mg NaCl	0.125 mg HSA, 2.5 mg Lactose	1 mg HSA, 4.7 mg Sucrose
Formulation	Vacuum dried powder	Lyophilizate	Lyophilizate
150 kDa Neurotoxin protein [ng/100 MU]	0.73	0.65*	0.44
Storage	2-8 °C	2-8 °C	Room temperature

*The neurotoxin protein value for Dysport® should be interpreted in the context of product-specific biological units; abobotulinumtoxinA units are not directly interchangeable with onabotulinumtoxinA or incobotulinumtoxinA units. Abbreviations: BoNT/A, botulinum neurotoxin type A; HSA, human serum albumin; kDa, kilodalton; MU, mouse units; NaCl, sodium chloride. Content adapted from Bigalke, Frevert and Dressler (2026). Used with kind permission for academic purposes under CC BY-NC-ND 4.0.

10.3 CLINICAL APPLICATION OF BONT/A

10.3.1 Treatment in CD

BoNT/A is the established first-line symptomatic treatment for CD (Castelão *et al.*, 2017; Albanese, Di Giovanni and Lalli, 2019). BoNT/A reduces excessive dystonic muscle activity and can improve abnormal posture, pain, tremor, and disability (Evidente *et al.*, 2013; Pirazzini *et al.*, 2017; Rodrigues *et al.*, 2020).

The therapeutic effect of an injection typically begins within days, reaches a peak after several weeks, and then gradually wanes as neuromuscular transmission recovers (Pirazzini *et al.*, 2017). For sustained benefit, patients require repeated injections, commonly at intervals of approximately 10 to 16 weeks, although real-world schedules vary according to symptom recurrence, local practice, patient preference, access to care, reimbursement, and product labels (Poliziani, Koch and Liu, 2016; Walter *et al.*, 2020; Waeschle, Stark and Albrecht, 2022; Castagna, Jinnah and Albanese, 2024). This cyclic pattern distinguishes BoNT/A therapy from other neurological treatments. Each cycle is both a therapeutic intervention and a reassessment point, at which the clinician reviews the effect of the previous injection and adjusts the next injection plan (Albanese *et al.*, 2015). *Figure 3* illustrates a standard response curve in CD following BoNT/A injections, including quantifiable reinjection thresholds, range of minimal clinically important change (MCIC), and range of suboptimal effect. Long-term treatment is therefore dynamic. Clinicians adjust muscle selection, dose distribution, injection guidance, and sometimes formulation over many years (Haussermann *et al.*, 2004; Henrique Camargo *et al.*, 2011; Bentivoglio *et al.*, 2017).

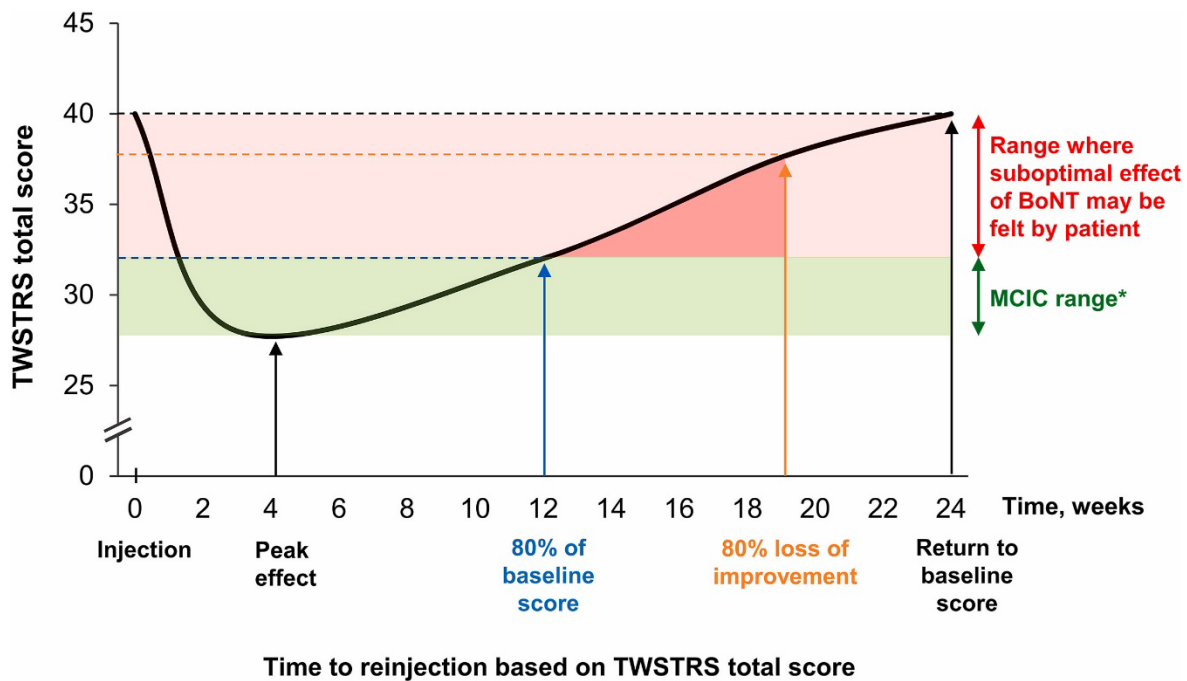


Figure 3. Cyclic Treatment Response and Re-Injection Paradigm in Cervical Dystonia

Schematic representation of the typical temporal profile of response to botulinum toxin injection in cervical dystonia, illustrating onset within days, peak effect after several weeks, and gradual decline as neuromuscular transmission recovers. TWSTRS total score is used as effect measure. The figure highlights the cyclic nature of treatment, with repeated injection intervals (approximately 10–16 weeks) serving both to maintain symptom control and to reassess and adapt subsequent treatment strategies based on prior response. Abbreviations: TWSTRS, Toronto Western Spasmodic Torticollis Rating Scale; BoNT, Botulinum Neurotoxin; MCIC, Minimal Clinically Important Change. Figure from Castagna, Jinnah and Albanese (2024). Used with kind permission for academic purposes under CC BY-NC-ND 4.0.

These adjustments may represent successful optimization. A dose increase may be clinically appropriate when the dystonic pattern evolves, when additional or larger muscles require treatment, when previous dosing was insufficient for the degree of abnormal muscle activity, or when symptom severity increases; therefore, dose escalation must be interpreted in the context of clinical adaptation rather than automatically as evidence of reduced responsiveness (Albanese *et al.*, 2015; Albrecht *et al.*, 2019; Hefter *et al.*, 2019; Waeschle *et al.*, 2025). However, dose escalation can also reflect reduced responsiveness (Hefter, Spiess and Rosenthal, 2014; Hefter, Rosenthal and Moll, 2016; Moll, Rosenthal and Hefter, 2018; Carr, Jain and Sublett, 2021; Rahman and Carruthers, 2024). Distinguishing adaptive dose optimization from emerging treatment failure is one of the central clinical and methodological challenges addressed by this thesis.

10.3.2 Treatment in other conditions

Beyond cervical dystonia, botulinum neurotoxin therapy is used in several neurological, autonomic, urological, dermatological, ophthalmological, pain-related, gastrointestinal and aesthetic indications (Dressler, 2012; Ayoub, 2025; Monash *et al.*, 2025). Its therapeutic rationale depends on the target tissue and includes reduction of skeletal muscle overactivity, inhibition of glandular secretion, relaxation of smooth muscle, and modulation of selected pain-related neurotransmitter pathways (Dressler, 2012; Ayoub, 2025). Regulatory status differs by

formulation, country and indication, and several reported applications remain off-label despite clinical use or emerging evidence (Martina *et al.*, 2021; Ayoub, 2025). Representative indications for these versatile BoNT/A applications beyond CD are summarized in *Table 2*.

Table 2. Selected clinical applications of botulinum neurotoxin therapy beyond cervical dystonia

Field	Example indication(s)	Therapeutic rationale / clinical use
Ophthalmology	Strabismus, blepharospasm, hemifacial spasm	Weakens overactive ocular or periocular muscles (Dressler, 2012; Ayoub, 2025).
Neurology	Focal dystonias, tics, tremor	Reduces involuntary muscle activity (Dressler, 2012; Ayoub, 2025).
Spasticity	Adult and pediatric limb spasticity	Reduces pathological muscle tone (Dressler, 2012; Ayoub, 2025).
Chronic migraine	Chronic migraine prevention	Reduces headache burden in chronic migraine (Ayoub, 2025; Monash <i>et al.</i> , 2025).
Urology	Neurogenic detrusor overactivity, overactive bladder	Reduces bladder overactivity (Dressler, 2012; Ayoub, 2025).
Autonomic / dermatology	Primary axillary hyperhidrosis	Reduces eccrine sweat secretion (Dressler, 2012; Martina <i>et al.</i> , 2021).
Salivary disorders	Sialorrhea / hypersalivation	Reduces salivary secretion (Dressler, 2012; Monash <i>et al.</i> , 2025).
Pain	Neuropathic pain, myofascial pain	May reduce pain-related neurotransmitter signaling (Dressler, 2012; Martina <i>et al.</i> , 2021; Ayoub, 2025).
Gastroenterology	Achalasia, sphincter of Oddi spasm, gastroparesis	Reduces sphincter or smooth-muscle hyperactivity (Dressler, 2012; Monash <i>et al.</i> , 2025).
Dermatology beyond hyperhidrosis	Raynaud phenomenon, Hailey-Hailey disease, hidradenitis suppurativa, flushing, oily skin	Investigated for effects on sweating, vasomotor symptoms, pain or glandular activity (Martina <i>et al.</i> , 2021).
Aesthetic medicine	Glabellar lines, lateral canthal lines, dynamic facial wrinkles	Relaxes selected facial muscles (Dressler, 2012; Ayoub, 2025; Monash <i>et al.</i> , 2025).

10.4 PRECLINICAL EVIDENCE DESCRIBING IMMUNOGENICITY OF BoNT/A

Preclinical studies provide an important mechanistic basis for understanding why repeated exposure to botulinum neurotoxin type A (BoNT/A) may, in some patients, lead to an immune response against the therapeutic protein. Although clinical immunogenicity ultimately depends on patient-related, treatment-related and formulation-related factors, experimental studies allow the antigenic properties of the neurotoxin molecule and its associated proteins to be examined under controlled conditions. These data are particularly relevant for distinguishing immune recognition of the 150 kDa neurotoxin itself from immune responses directed against neurotoxin-associated or complexing proteins, and for assessing whether such proteins may merely contribute additional antigenic load or also enhance the immune response through adjuvant-like effects. The following section summarizes the preclinical evidence on BoNT/A immunogenicity, with particular emphasis on the structural domains of the neurotoxin, the immunological contribution of neurotoxin-associated proteins, and the implications of these findings for long-term therapeutic use.

10.4.1 Structural prerequisites of BoNT/A immunogenicity

BoNT/A is a bacterial protein antigen and therefore contains multiple potential B-cell and T-cell recognition regions. The toxin is synthesized as a single-chain protein of about 150 kDa

and is proteolytically activated to a di-chain molecule consisting of an approximately 50 kDa light chain (LC) and an approximately 100 kDa heavy chain (HC) linked by a disulfide bond (Atassi and Dolimbek, 2004; Baldwin *et al.*, 2005; Zhao *et al.*, 2012). The LC is the catalytic zinc endopeptidase, whereas the HC contains an N-terminal translocation region (HN) and a C-terminal receptor-binding region (Hc/HCR) (Baldwin *et al.*, 2005; Garcia-Rodriguez *et al.*, 2011; Zhao *et al.*, 2012). The receptor-binding domain mediates neuronal targeting, the translocation domain supports delivery of the LC across the endosomal membrane, and the LC cleaves SNARE proteins after cytosolic delivery (Baldwin *et al.*, 2005; Garcia-Rodriguez *et al.*, 2011).

The disulfide-linked di-chain architecture is relevant not only for toxicity but also for antigenicity. In early experiments with purified type A and type E toxins, reduction of disulfide bonds before formalin toxoid preparation reduced subsequent antitoxin responses in guinea pigs (Sugiyama, Dasgupta and Ohishi, 1974). This result supports the cautious interpretation that conformational integrity can influence the quality of the antibody response, although it does not by itself define the exact epitopes responsible for neutralization (Sugiyama, Dasgupta and Ohishi, 1974).

Subtype variation adds a further layer of immunological complexity. Kozaki, Nakae and Kamata (1995) compared a BoNT/A associated with infant botulism in Japan with classical type A neurotoxin and found that several HC-reactive monoclonal antibodies distinguished the two toxins, whereas LC-reactive antibodies were more broadly reactive. In a later subtype-focused study, A1- and A2-derived light-chain antigens differed from A3 in proteolytic activity and immunoprotective efficacy, with A1-LC and A2-LC providing broader mouse protection than A3-LC (Liao *et al.*, 2025).

10.4.2 Immunogenicity of neurotoxin domains and epitopes

The Hc/HCR domain is the most consistently protective single antigen in vaccine-oriented experiments (Baldwin *et al.*, 2005; Liu *et al.*, 2020). Baldwin *et al.* (2005) expressed recombinant HCR/A1 and HCR/E, immunized mice and rabbits, and showed that HCR/A1 induced neutralizing activity against BoNT/A1 and protected against the BoNT/A2 Kyoto F infant strain. Liu *et al.* (2020) compared BoNT/A functional-domain antigens and found that all tested antigens induced humoral responses but that Hc and the catalytic domain HN-L were the only constructs producing strong protection, with Hc showing the most effective protective potency in dose-dependent experiments.

The HN and LC regions are also important antibody targets. Kozaki *et al.* (1989) generated papain-induced fragments of type A neurotoxin and showed that fragments corresponding to the remaining portion of the HC competed for binding of radiolabelled neurotoxin to synaptosomes, whereas the LC plus HN fragment did not inhibit binding in that assay. Atassi

and Dolimbek (2004) mapped HN-domain antibody-binding regions using antitoxin antibodies from several host species and identified multiple continuous regions on the HN surface rather than a single dominant HN epitope. Garcia-Rodriguez et al. (2011) identified rare human monoclonal antibodies that bound multiple BoNT serotypes and localized their epitope to the tip of the HN, providing functional support for the immunological relevance of this region.

The LC is immunogenic despite being an intracellular effector domain. Dolimbek et al. (2011) mapped LC-recognition regions of blocking antibodies from mouse, horse and chicken antisera and found host-dependent but partly overlapping LC antigenic regions. Atassi et al. (2012) showed in BALB/c and SJL mice that LC-directed antibody and T-cell responses were genetically restricted and that B-cell and T-cell recognition regions could coincide or differ. Oshima, Aoki and Atassi (2014) further demonstrated that mice primed with inactivated toxin mounted T-cell responses to selected LC peptides, with strain-dependent response profiles. These findings are relevant because anti-drug antibody formation requires T-cell help even when the clinically measured endpoint is a binding or neutralizing antibody response (Atassi et al., 2012; Oshima, Aoki and Atassi, 2014).

Human immunoresistance data identify a limited but heterogeneous set of clinically relevant recognition regions. Dolimbek et al. (2007) used sera from 28 cervical dystonia patients who had become unresponsive to BoNT/A and whose sera protected mice in a mouse protection assay; the most frequently recognized HC regions included N25, C9/C10, C31, C15, C20, N16, N22 and N4, although not every serum recognized every peptide. Atassi et al. (2011) analyzed sera from 28 immunoresistant cervical dystonia patients and found prominent LC reactivity against L11, L14 and L18, with smaller subsets recognizing L27 and L29. The patient-to-patient heterogeneity in both studies is consistent with host-dependent MHC control of immune recognition and cautions against assuming a single universal immunodominant epitope for all treated patients (Dolimbek et al., 2007; Atassi et al., 2011).

Neutralizing monoclonal antibody experiments confirm that functional neutralization can target more than one domain. Wu et al. (2001) identified two monoclonal antibodies that protected mice against lethal BoNT/A challenge, with one antibody recognizing the LC and the other recognizing the HC. Mullaney, Pallavicini and Marks (2001) mapped neutralizing single-chain antibody epitopes to regions within the BoNT/A binding domain and interpreted these as conformational rather than simple linear epitopes. Mazuet et al. (2010) showed that several monoclonal antibodies neutralized BoNT/A and that two selected antibodies recognized different epitopes, but the same study also showed subtype-related differences in neutralizing efficiency against A1, A2 and A3. These antibody studies reinforce that neutralization depends on epitope location, conformation, antibody affinity and subtype context rather than on total antibody amount alone.

10.4.3 Neurotoxin-associated proteins, HA-33/Hn-33 and adjuvant-like effects

Clostridial BoNT/A can be produced as part of larger progenitor toxin complexes rather than as isolated 150 kDa neurotoxin. Zhao et al. (2012) described BoNT complexes containing the neurotoxin and auxiliary non-toxic proteins, with M toxin (medium-sized BoNT progenitor toxin complex) composed of BoNT and non-toxic non-hemagglutinin (NTNH) and larger L/LL toxin forms also containing hemagglutinin (HA/Hn) components. Kukreja et al. (2009) described BoNT/A complex as containing neurotoxin-associated proteins including Hn-33 and emphasized that these proteins protect BoNT/A in the gastrointestinal tract. The biological role of complexing proteins in oral intoxication should therefore be distinguished from their relevance to injected therapeutic preparations, where the proteins may still contribute antigenic material but are not required for neuronal pharmacological action (Kukreja *et al.*, 2009; Zhao *et al.*, 2012).

The strongest direct evidence for accessory-protein antigenicity comes from comparative immunochemical experiments. Kukreja et al. (2009) immunized rabbits with BoNT/A complex, purified BoNT/A, LC, HC, neurotoxin associated proteins (NAPs) and recombinant Hn-33, and used ELISA to compare the resulting immunoreactivity. In that study, antibodies raised against the whole BoNT/A complex reacted about 60-fold more strongly with complex than with purified neurotoxin and about 35-fold more strongly with Hn-33 or NAPs than with purified neurotoxin. Kukreja et al. (2009) also reported that antibodies raised against purified BoNT/A reacted similarly with purified neurotoxin and complexed neurotoxin, suggesting that the neurotoxin can be antigenic in both forms when it is the immunizing antigen.

Bryant, Cai and Singh (2013) refined the accessory-protein conclusion with indirect ELISA, competitive ELISA and immunoblotting. In competitive ELISA, approximately 50% inhibition of anti-complex antibody binding required about 8 ng of BoNT/A complex but about 50 ng of purified BoNT/A, indicating stronger competition by the complex than by purified neurotoxin. Bryant, Cai and Singh (2013) also found that BoNT/A complex and Hn-33 competed nearly identically for anti-complex and anti-Hn-33 antibodies, which supports the interpretation that Hn-33 is a major antibody target within the complex. Their immunoblot experiments further supported Hn-33 as the most immunoreactive component in the complex system, although the authors also noted possible residual toxin in NAP preparations.

HA-33/Hn-33 antigenicity has also been shown with recombinant protein. Sayadmanesh et al. (2013) expressed and purified recombinant HA-33/A and reported high antibody titres in mice after immunization. This study supports the conclusion that HA-33/A is antigenic, but it does not prove that HA-33/A causes neutralizing antibodies against the therapeutic neurotoxin. Therefore, HA-33/Hn-33 should be described as an antigenic complex component and a

plausible adjuvant-like contributor, not as a proven direct cause of clinical secondary non-response (Kukreja *et al.*, 2009; Bryant, Cai and Singh, 2013; Sayadmanesh *et al.*, 2013).

Animal repeated-dose experiments provide the most relevant support for formulation-dependent immunogenicity. Torii *et al.* (2014) compared LL toxin-derived subtype A1 with purified neurotoxin-derived A1 and A2 preparations in animals and detected neutralizing antibodies in the A1LL-administered group but not in swine and rabbits administered multiple doses of BoNT/A2. Torii *et al.* (2014) also found that BoNT/A2 retained more inhibitory activity than BoNT/A1 in animals with A1LL-induced neutralizing antibodies and in assays using sera from humans immunized with botulinum toxoid vaccine. These data support the hypothesis that purified neurotoxin preparations, especially subtype BoNT/A2 in that experimental context, can be less immunogenic or less affected by A1-directed antibodies than complex-derived A1 preparations (Torii *et al.*, 2014).

The term adjuvant effect should be used carefully. Kukreja *et al.* (2009) proposed that Hn-33 and other NAPs could potentially be employed as adjuvants because complex-associated proteins elicited stronger antibody responses than purified neurotoxin in their experimental system. Bryant, Cai and Singh (2013) concluded that Hn-33 was primarily responsible for much of the antibody response to BoNT/A complex, but their data measured binding antibodies rather than clinical resistance after therapeutic injection. Torii *et al.* (2014) provides stronger functional support because neutralizing antibodies were detected after repeated administration of A1LL but not after repeated BoNT/A2 administration in their animal experiments. In summary, complexing proteins, particularly HA-33/Hn-33, are antigenic and may enhance or redirect immune responses in some experimental settings, while direct proof of a universal clinical adjuvant effect remains incomplete (Kukreja *et al.*, 2009; Bryant, Cai and Singh, 2013; Torii *et al.*, 2014).

10.4.4 Robustness and limitations of the preclinical evidence

The preclinical evidence is strongest where antigen definition is clear and where antibody binding is paired with functional protection or neutralization. Studies such as Wu *et al.* (2001), Mullaney, Pallavicini and Marks (2001), Baldwin *et al.* (2005), Mazuet *et al.* (2010), Zhao *et al.* (2012), Torii *et al.* (2014) and Liu *et al.* (2020) robustly link antibody recognition to neutralization, protection, subtype specificity or domain function. Studies such as Kukreja *et al.* (2009), Bryant, Cai and Singh (2013) and Sayadmanesh *et al.* (2013) are highly relevant for component antigenicity but should be interpreted more cautiously for clinical neutralizing-antibody risk because they primarily measure binding-antibody responses.

The literature also contains sources which deserve recognition that inform on BoNT immunogenicity but are not directly relevant in the context of this work. Solomon *et al.* (1971) addressed somatic antigen relationships among proteolytic and nonproteolytic *C. botulinum*

strains, which is taxonomically relevant but not directly informative for therapeutic BoNT/A neurotoxin immunogenicity. Diagnostic assay papers and computational modelling papers can provide context, but they do not replace direct immunization, neutralization or protection experiments when making claims about preclinical immunogenicity (von Berg *et al.*, 2019; Rahman *et al.*, 2025, 2026). Rahman *et al.* (2025) and Rahman *et al.* (2026) are useful for hypothesis generation on HLA binding and long-term immunogenicity modelling, but their immunogenicity conclusions remain *in silico* and therefore require experimental validation. Sutphin *et al.* (2009) observed frequency-dependent anti-botulinum antibody production in a murine model after repeated BCB2024 BoNT/A administration, but the study measured IgG binding antibodies and did not distinguish neutralizing from non-neutralizing antibodies.

10.4.5 Implications for long-term BoNT/A treatment in cervical dystonia

The mechanistic literature supports a biologically plausible pathway from repeated BoNT/A exposure to binding antibodies, neutralizing antibodies and secondary treatment failure. BoNT/A contains antigenic regions on the LC, HN and Hc domains, and antibodies directed against binding, translocation or catalytic regions can interfere with toxin action in experimental systems (Mullaney, Pallavicini and Marks, 2001; Wu *et al.*, 2001; Dolimbek *et al.*, 2007; Atassi *et al.*, 2011; Garcia-Rodriguez *et al.*, 2011; Zhao *et al.*, 2012). Complexing proteins, especially Hn-33/HA-33, add antigenic material to some formulations and can dominate binding-antibody responses to the complex in laboratory models (Kukreja *et al.*, 2009; Bryant, Cai and Singh, 2013; Sayadmanesh *et al.*, 2013). Repeated exposure, formulation composition, dose, injection frequency, subtype sequence and host immunogenetics are therefore all plausible contributors to immunogenic risk (Dolimbek *et al.*, 2007; Sutphin *et al.*, 2009; Atassi *et al.*, 2011; Torii *et al.*, 2014; Rahman *et al.*, 2025).

These conclusions fit the RELY-CD framework but should not be overinterpreted. A decline in dose-effect relationship during long-term cervical dystonia therapy may be compatible with immunogenicity, but it can also arise from disease progression, changes in muscle selection, dose redistribution, altered injection technique, treatment interval changes, or documentation heterogeneity. The preclinical literature justifies considering immunogenicity as one mechanistic explanation for partial or complete secondary treatment failure, but it does not allow a retrospective clinical dataset to diagnose neutralizing antibodies without appropriate laboratory confirmation.

10.5 LONG-TERM BoNT/A EXPOSURE AND THE RISK OF IMMUNOGENICITY

Because BoNT/A is a foreign bacterial protein, repeated injections can stimulate an immune response (Murphy, Weaver and Janeway, 2017; Carr, Jain and Sublett, 2021). Antibodies may bind to the neurotoxin without clinical consequences, but neutralizing antibodies (NAbs) can

reduce the biological activity of BoNT/A (U.S. Food and Drug Administration, 2014; Carr, Jain and Sublett, 2021). Clinically, this may appear as shortened duration of benefit, reduced peak effect, need for increasing doses, patient-reported loss of response, partial secondary treatment failure (PSTF), or, more rarely, complete secondary treatment failure (CSTF) (Dressler, 2004; Hefter, Rosenthal and Moll, 2016; Walter *et al.*, 2020). Immunogenicity is therefore a major long-term concern in patients with chronic conditions requiring life-long BoNT/A exposure. The general principle of development of clinical resistance against biologic drugs is illustrated in *Figure 4* (Carr, Jain and Sublett, 2021).

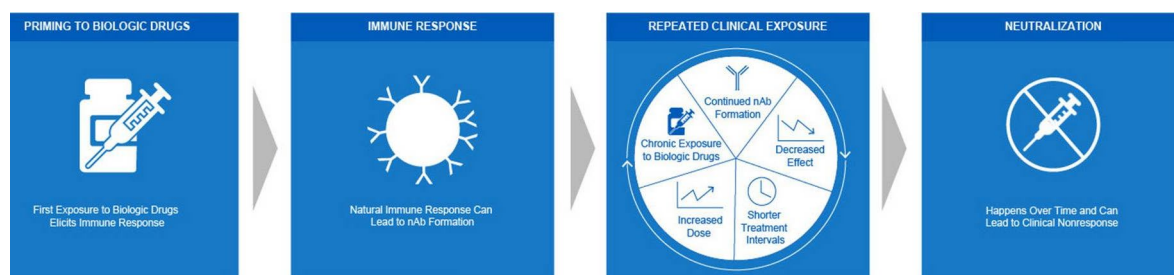


Figure 4. Development of clinical nonresponse following repeated exposure to biologic drugs

Repeated exposure to biologic therapies may induce neutralizing antibodies (nAbs), which can progressively reduce treatment efficacy and contribute to clinical resistance. Early indicators may include the need for higher doses or shorter treatment intervals, followed in some patients by partial or complete nonresponsiveness. Abbreviation: nAb, neutralizing antibody. Figure from Carr, Jain and Sublett (2021). Used with kind permission for academic purposes under CC BY-NC-ND 4.0.

The clinical interpretation of NABs is complex. Some patients with clear secondary nonresponse have no detectable NABs, even with sensitive assays, whereas some NAB-positive patients continue to experience partial benefit (Kranz *et al.*, 2008; Hefter *et al.*, 2019; Walter *et al.*, 2020). This imperfect correlation reflects several factors: limitations of antibody assays, non-immunological causes of waning response, differences between partial and complete failure, dose compensation, and clinical heterogeneity (Benecke, 2012; Bellows and Jankovic, 2019). In long-term treated CD patients who were still responding to BoNT/A, antibody-positive patients had significantly worse clinical severity and were treated with higher doses than antibody-negative patients (Hefter *et al.*, 2019). This suggests that immunogenicity may impair treatment even before complete failure becomes obvious.

Dose is a consistent risk factor across studies. In a large cross-sectional study of 596 BoNT/A-treated patients across neurological indications, 83 patients (13.9%) had measurable NABs, and the probability of developing NABs increased with single and cumulative dose as well as formulation (Albrecht *et al.*, 2019). Walter *et al.* (2020) similarly identified high dose per treatment and formulation-related factors as risk factors for antibody-induced complete secondary failure. These observations are highly relevant for CD, where treatment often involves relatively high doses in neck muscles and continues for many years (Carr, Jain and Sublett, 2021; Walter *et al.*, 2025).

Formulation composition may also influence immunogenicity. The three major BoNT/A preparations differ in foreign protein content (Frevert and Dressler, 2010; Frevert, 2015). IncoA contains the therapeutically active 150 kDa neurotoxin without complexing proteins, whereas onaA and aboA contain additional clostridial proteins, including HA-33/Hn-33 as the dominant antigenic complexing protein (Kukreja *et al.*, 2009; Bryant, Cai and Singh, 2013; Frevert, 2015). The natural biological role of complexing proteins is to protect the toxin during gastrointestinal passage, but this pathway is bypassed during intramuscular therapeutic injection (Frevert and Dressler, 2010; Fujinaga, Sugawara and Matsumura, 2013; Pirazzini *et al.*, 2017). The clinical benefit of these proteins is therefore disputed, while their potential contribution to immunogenicity remains relevant (Frevert and Dressler, 2010, 2016; Carr, Jain and Sublett, 2021).

Recent systematic evidence strengthens this concern. Walter *et al.* (2025) reported that, across included studies, secondary treatment failure and NAb positivity were more frequent after treatment with aboA or onaA than with incoA in CD and spasticity, and no patients treated exclusively with incoA were found to have developed immunogenic secondary treatment failure or persistent NABs in the reviewed evidence. Although such comparisons are limited by differences in populations, dose, indication, treatment duration, and definitions, they provide a strong rationale for examining formulation-specific long-term dose-response patterns in real-world CD treatment.

Despite the biological importance of NABs, antibody testing is not routinely available in most settings (Waeschle, Stark and Albrecht, 2022; Waeschle *et al.*, 2025). Sensitive assays such as the mouse hemidiaphragm assay are specialized, expensive, time-consuming, and not feasible for broad routine monitoring (Benecke, 2012; Bellows and Jankovic, 2019; Waeschle *et al.*, 2025). Therefore, physicians must infer possible reduced responsiveness from treatment records, patient reports, and dose development (Hefter *et al.*, 2019; Waeschle, Stark and Albrecht, 2022; Waeschle *et al.*, 2024). This practical diagnostic gap motivated the development of a real-world approach that does not require routine laboratory confirmation of antibodies but still captures the core clinical phenomenon of concern: worsening effect despite treatment adaptation.

10.6 REAL-WORLD MANAGEMENT: INDIVIDUALIZED INJECTIONS AND HETEROGENEOUS DOCUMENTATION

The practical management of CD depends on repeated clinical judgement. Injection plans are individualized according to the dystonic pattern, tremor, pain, side effects, previous response, and patient preference (Marion *et al.*, 2016; Simpson *et al.*, 2016; Contarino *et al.*, 2017). The same total dose may have different implications depending on how it is distributed across

muscles. For example, increasing the dose in the splenius capitis may reflect persistent rotation, whereas adding the obliquus capitis inferior may reflect recognition of a deep rotatory component (Su *et al.*, 2022). A simple total-body dose trajectory cannot fully capture such changes. Muscle-specific analysis is therefore necessary to understand long-term treatment evolution.

However, real-world documentation is heterogeneous (Waeschle, Stark and Albrecht, 2022; Waeschle *et al.*, 2024). Some centers routinely collect TWSTRS or Tsui scores, whereas others use global impression scales, visual analogue scales, Likert scales, or unstructured clinical notes. Quality-of-life instruments are used even less consistently. The first feasibility study preceding RELY-CD surveyed 49 centers caring for 5,322 CD patients and showed that efficacy, dose per muscle, duration of effect, and safety/tolerability were commonly recorded, but quality-of-life outcomes were not routinely collected in most centers (Waeschle, Stark and Albrecht, 2022; Waeschle *et al.*, 2024). This study also found marked regional differences in awareness of possible resistance signs and in the use of resistance testing.

The second feasibility survey of 38 centers in Canada, Poland, and Spain confirmed this heterogeneity (Waeschle *et al.*, 2024). Although 71% of centers used at least one efficacy scale, the specific scale varied substantially. The 7-point Global Impression of Change Scale was most commonly reported, TWSTRS use differed strongly between countries, and quality-of-life questionnaires were used by only 21% of centers (Waeschle *et al.*, 2024). These findings have two important implications. First, retrospective long-term studies must be designed around data elements that are found in routine records. Second, a multicenter real-world study cannot rely on one universal efficacy scale unless it excludes large numbers of otherwise informative patients.

The feasibility studies therefore provided the methodological bridge to RELY-CD. They identified two parameters that were both clinically meaningful and routinely available: dose per muscle and at least one established efficacy measure. The challenge was then to combine these parameters in a way that could accommodate different scales while preserving within-patient consistency. This led to the dose-effect concept used in RELY-CD (Waeschle *et al.*, 2025).

10.7 MUSCLE-SPECIFIC INJECTION PATTERNS AND LONG-TERM DOSE DEVELOPMENT

10.7.1 Muscle selection as a core element of individualized BoNT/A treatment

Treatment with BoNT/A in CD is not defined by the total injected dose alone, but by the selection of clinically relevant muscles and the distribution of dose between them. CD may present with rotation, laterocollis/caput, retrocollis/caput, anterocollis/caput, lateral or sagittal

shifts, tremor, pain, or mixed patterns, and these clinical patterns reflect different combinations of superficial and deep cervical muscles (Tiderington *et al.*, 2013; Albanese *et al.*, 2015; Finsterer *et al.*, 2015; Contarino *et al.*, 2017). *Figure 5* illustrates the collum-caput (COL-CAP) concept which supports the muscle selection for treatment based on head or neck positions (Reichel, Stenner and Jahn, 2009). Therefore, injection planning requires identification of the dystonic pattern, translation of this pattern into a muscle hypothesis, and repeated reassessment of the previous injection response (Albanese *et al.*, 2015; Castagna and Albanese, 2019). In this context, the same total dose may represent very different therapeutic strategies depending on whether it is concentrated in a small number of large superficial muscles, distributed across several cervical and shoulder-girdle muscles, or shifted toward deeper muscles that may require guidance techniques (Walker, 2003; Contarino *et al.*, 2017; Su *et al.*, 2022).

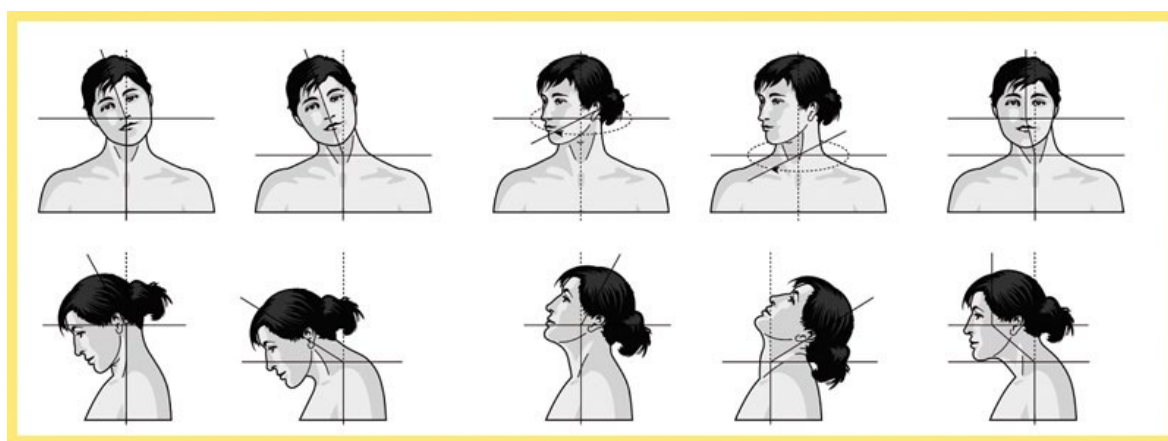


Figure 5. Schematic of cervical and cranial dystonias

Upper row , from left to right: laterocaput, laterocollis, torticaput, torticollis, lateral shift. Lower row, from left to right: anteropaput, anteropollis, retrocaput, retrocollis, anterior sagittal shift. Figure from Reichel, Stenner and Jahn (2009). Used with kind permission by the publisher (License number 6300301385417).

This muscle-specific nature of treatment is important for interpreting long-term outcomes. For example, dose changes in key muscles such as the sternocleidomastoid, splenius capitis, trapezius, levator scapulae, semispinalis capitis, or obliquus capitis inferior may reflect different clinical decisions and different underlying dystonic patterns (Albanese *et al.*, 2015; Contarino *et al.*, 2017; Su *et al.*, 2022). A dose increase in one muscle may indicate persistent activity of that muscle, whereas a reduction in another muscle may reflect improved control, adverse effect avoidance, or recognition that the previous target was less relevant. Consequently, total dose is an incomplete marker of treatment intensity unless the underlying muscle distribution is also considered.

10.7.2 Dose adaptation as optimization rather than automatic treatment failure

Long-term BoNT/A treatment is dynamic because CD itself is clinically heterogeneous and because injection plans are refined over time. Dose escalation may occur when symptom

control is insufficient, when additional muscles become clinically relevant, when a dystonic pattern evolves, when the physician identifies previously untreated deep muscle involvement, or when treatment is intentionally optimized after an initially conservative dosing strategy (Albanese *et al.*, 2015; Marion *et al.*, 2016; Contarino *et al.*, 2017). Therefore, an increase in dose should not automatically be interpreted as reduced responsiveness or immunogenicity. Conversely, stable total dose does not necessarily imply stable treatment if the dose has been redistributed between muscles, if injection intervals have changed, or if the clinical effect has declined despite unchanged dosing.

This distinction is particularly relevant in retrospective real-world studies. In routine care, physicians usually adapt dose, muscle selection, and sometimes formulation according to the effect and tolerability of previous cycles (Marion *et al.*, 2016; Simpson *et al.*, 2016; Contarino *et al.*, 2017). Such iterative adaptation is a strength of individualized CD treatment, but it complicates the interpretation of long-term dose trajectories. A higher dose may reflect successful optimization, while the same dose increase may also be compatible with emerging treatment failure if clinical benefit worsens or duration of effect shortens at the same time (Hefter, Spiess and Rosenthal, 2014; Hefter, Rosenthal and Moll, 2016; Carr, Jain and Sublett, 2021). For this reason, dose development should be interpreted together with clinical effect and, where possible, at the level of individual muscles rather than only at the level of the total injected dose.

In this way, the analysis of muscle-specific dose development links routine injection practice to the broader question of long-term treatment stability. It helps distinguish adaptive dose redistribution from potentially unfavorable dose-effect development and prevents overinterpretation of total dose changes as direct evidence of treatment failure. This is important because the central clinical challenge in long-term CD care is not only whether BoNT/A remains effective, but how physicians maintain benefit through repeated, individualized adaptations across many treatment cycles.

10.8 BRIEF LITERATURE REVIEW: WHAT WE KNOW ABOUT LONG-TERM OUTCOMES IN CD

A substantial body of literature supports the long-term efficacy and safety of BoNT/A in CD. Earlier studies and clinical experience demonstrated sustained benefit over repeated injection cycles, acceptable tolerability, and high patient satisfaction in many cohorts (Evidente *et al.*, 2013; Colosimo *et al.*, 2020; Marsili *et al.*, 2021; Petracca *et al.*, 2023; McAllister *et al.*, 2025). These studies established that BoNT/A can remain effective over many years and that repeated injection is an appropriate long-term management strategy. They also showed,

however, that treatment is not uniform. Dose, injection interval, muscle selection, and patient-reported satisfaction vary over time and between patients.

Large observational studies provide particularly relevant real-world evidence. INTEREST IN CD2 followed patients treated with BoNT/A in routine practice over three years and showed continued clinical improvement with aboA, while also demonstrating that patient satisfaction at peak effect was consistently higher than at the end of the treatment cycle (Colosimo *et al.*, 2020). This illustrates a key point for long-term management: a patient may respond well at peak effect yet still experience significant waning before reinjection. Duration of effect and end-of-cycle control are therefore clinically important but may not be fully captured by a single severity score.

Very long-term single-center cohorts have described treatment over decades. Such studies show sustained benefit for many patients, but they also reveal changes in dose, intervals, adverse events, and satisfaction over time (Jochim *et al.*, 2019; Yahalom *et al.*, 2021; Petracca *et al.*, 2023). Their main strength is longitudinal depth; their main limitation is generalizability. They often reflect expert-center practice, may have incomplete representation of all modern formulations, and frequently lack a standardized method linking dose adaptation to clinical effect. Many rely on patient-reported benefit or global assessments and do not systematically analyze dose per muscle.

Long-term immunogenicity studies provide a different focus. These studies investigate and identify risk factors and formulation-related differences. However, these studies are single-center, assay-dependent, or enriched for patients with suspected nonresponse (Hefter, Spiess and Rosenthal, 2014; Hefter, Rosenthal and Moll, 2016; Albrecht *et al.*, 2019; Hefter *et al.*, 2019; Walter *et al.*, 2020). They demonstrate that antibody-related loss of response exists and matters clinically, but they do not fully solve the everyday monitoring problem. In routine practice, most patients are not tested longitudinally for NABs, and treatment decisions are made from clinical records, patient reports, and dose adjustments (Samadzadeh *et al.*, 2020). A recent meta-analysis by Walter *et al.* investigated NAB positivity and NAB-induced STF in all eligible studies published before April 2024 (Walter *et al.*, 2025). They found statistically significant differences between formulations in clinical dystonia. The incidence of STF was found to be lower in CD patients treated with incoA compared to onaA ($p=0.03$) or aboA ($p<0.001$). Similarly, there were significantly fewer NAB-positive CD patients detected with the mouse hemidiaphragm assay in patients treated with incoA compared to aboA ($p=0.01$) and onaA ($p=0.02$).

In summary, several high-quality long-term and observational studies have made important contributions to the understanding of long-term BoNT/A treatment in CD. High-quality clinical studies demonstrate sustained efficacy and safety of BoNT/A over repeated treatment cycles,

while immunogenicity studies provide robust evidence that neutralizing antibodies are clinically relevant in a subset of patients. However, these bodies of evidence usually address different parts of the long-term treatment problem. Some studies provide detailed efficacy data but include limited interpretation of immunogenicity or dose-effect relationships. Others focus on immunogenicity, but are often single-center, depend on specialized antibody assays, or are not designed to reflect routine monitoring in everyday practice. Studies that combine clinical response and immunogenicity frequently remain limited by single-center design or lack true long-term follow-up across multiple years of routine treatment. In addition, the feasibility studies show that standardized documentation of outcomes, dose, duration of effect, and patient-reported measures is inconsistent across centers. Thus, the gap is not a lack of high-quality evidence, but a lack of integration: long-term efficacy, immunogenicity risk, dose adaptation, and real-world documentation have rarely been studied together in a multicenter setting. This evidence base defines the gap addressed by RELY-CD.

10.9 THE RELY-CD STUDY FRAMEWORK AND THE DEFF CONCEPT

Against this background, RELY-CD was designed to translate a clinically familiar problem into an analyzable real-world framework: how can long-term treatment stability or deterioration be assessed when dose, muscle selection, formulation exposure, and outcome documentation all change over time? In routine CD care, reduced responsiveness is rarely observed as a single event. It usually emerges gradually through patterns such as increasing dose requirements, shorter duration of benefit, lower perceived efficacy, or worsening clinical scores (Marion *et al.*, 2016). These patterns are clinically meaningful, but difficult to compare systematically across centers because patients are treated individually and outcome measures differ between practices (Waeschle, Stark and Albrecht, 2022; Waeschle *et al.*, 2024).

The central idea of RELY-CD is therefore to evaluate treatment response as a relationship between dose and effect, rather than as dose or effect alone. This reflects everyday decision-making more closely than a single outcome score. A higher dose is not necessarily unfavorable if it results from appropriate optimization and leads to better or sustained symptom control (Hefter, Samadzadeh and Rosenthal, 2021). Similarly, a stable dose is not necessarily reassuring if the clinical effect declines. The clinically relevant question is whether the amount of treatment required to achieve a given effect changes over time.

The dose-effect parameter, DEff, was developed to capture this relationship using data that can realistically be extracted from routine records (Waeschle *et al.*, 2025). It combines information on dose development with information on clinical effect, thereby describing whether treatment remains stable, improves, or worsens over time. DEff was not conceived as a diagnostic test for antibody-mediated resistance. Rather, it is a structured marker of long-

term treatment dynamics. A worsening DEff indicates that the balance between administered dose and observed effect has become less favorable and should therefore prompt clinical interpretation. A visual representation of the DEff calculation is shown in *Figure 6A*. *Figure 6B* illustrates the impact of dose or effect change on the DEff.

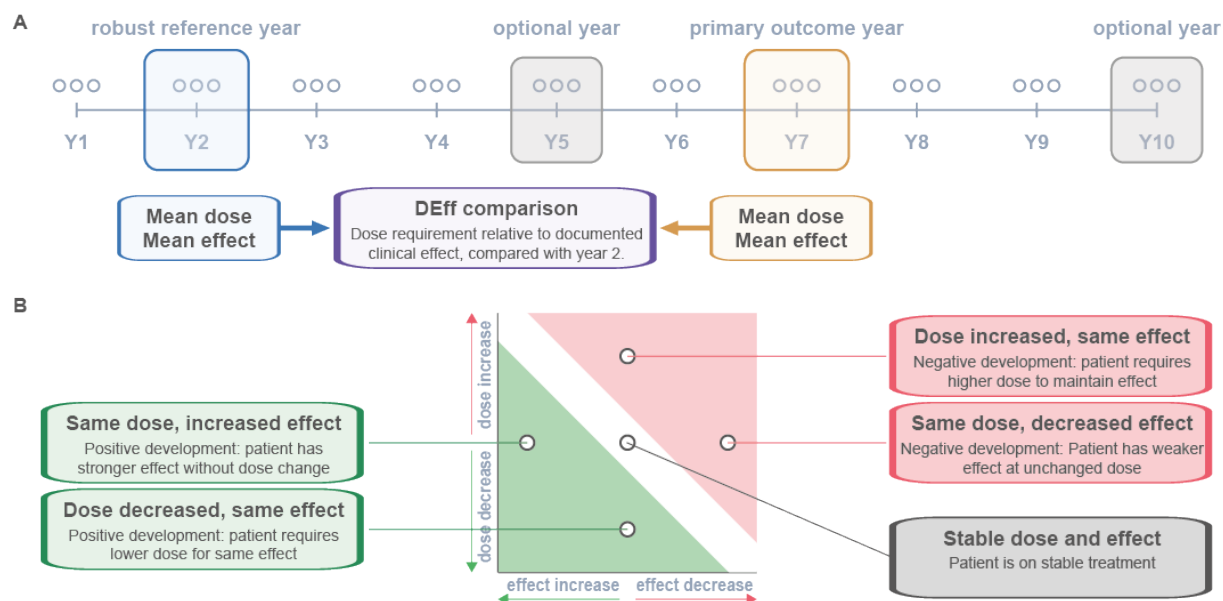


Figure 6. Illustration of the DEff Concept

A: The DEff is calculated using mean values of each visit in a treatment year (represented as circles). The quotient of dose change and effect change are multiplied to form the DEff. B: Different scenarios for dose and/or effect change and the impact on the DEff. Abbreviations: DEff, dose-effect parameter; Y, year.

This interpretation remains essential because a worsening dose-effect relationship can have multiple causes. It may reflect immunogenicity, but it may also result from progression or spread of dystonia, altered muscle involvement, technical factors, insufficient targeting of deep muscles, changes in patient expectations, comorbidity, psychological stress, or physical changes. The value of DEff lies in making such patterns visible and comparable in a multicenter real-world dataset, without assuming a single underlying mechanism.

RELY-CD therefore addresses the data gap by providing a framework that is both clinically pragmatic and analytically structured. It uses routinely documented treatment information, preserves the longitudinal nature of BoNT/A management, and focuses on the dynamic relationship that matters most in long-term care: whether increasing or ongoing treatment continues to translate into meaningful clinical benefit. This framework provides the rationale for the manuscripts included in this thesis and a schematic representation is shown in *Figure 7*.

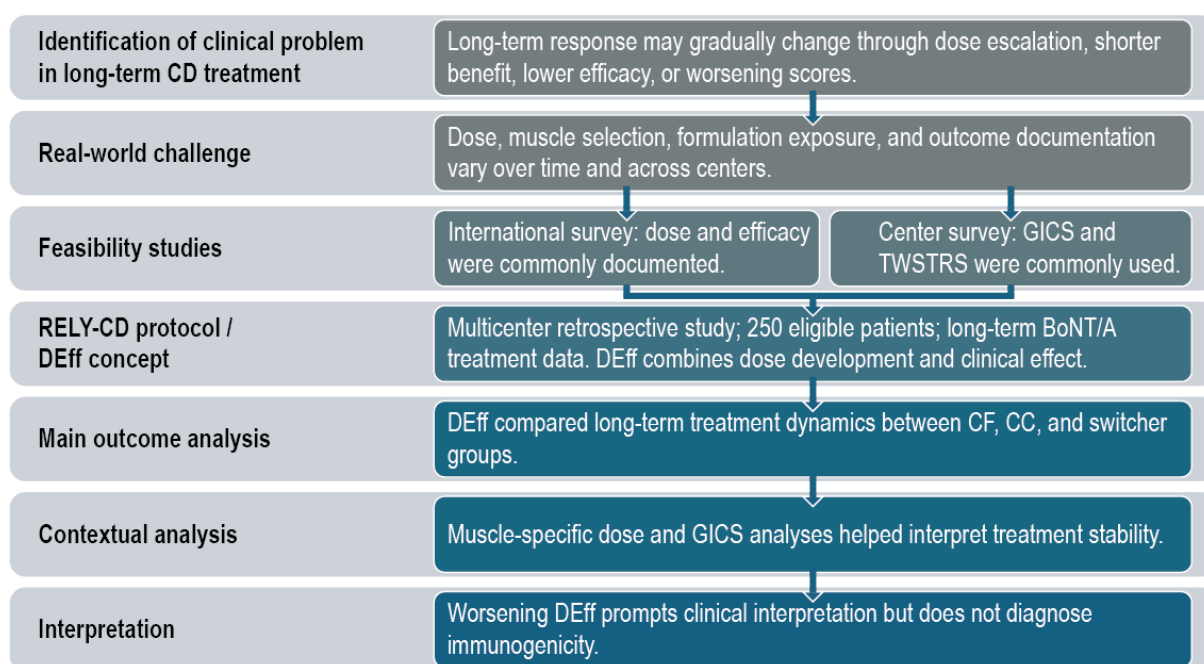


Figure 7. Schematic Representation of the RELY-CD Study Framework

The RELY-CD program links feasibility work, protocol development, and long-term outcome analyses into a structured real-world framework for assessing BoNT/A treatment stability in cervical dystonia. DEff combines dose development and clinical effect to describe long-term dose-effect dynamics, while complementary dose and GICS analyses contextualize treatment stability. DEff indicates a changing dose-effect relationship but does not diagnose a specific mechanism such as immunogenicity. Abbreviations: BoNT/A, botulinum neurotoxin type A; CC, complex-containing; CD, cervical dystonia; CF, complex-free; DEff, dose-effect parameter; GICS, Global Impression of Change Scale; TWSTRS, Toronto Western Spasmodic Torticollis Rating Scale.

11 RESEARCH AIMS AND HYPOTHESES

The publications included in this thesis follow a stepwise research framework. The overall objective was not only to describe long-term BoNT/A treatment in CD, but to develop a feasible real-world approach for evaluating long-term treatment stability, reduced responsiveness, and dose adaptation across centers. The central assumption underlying the project was that clinically relevant changes in treatment response may be detectable from routinely documented information if dose and effect are interpreted together.

The framework was developed in five consecutive steps. First, the feasibility of using real-world treatment documentation in CD had to be assessed. Second, the heterogeneity of outcome measures had to be characterized in more detail, because any multicenter retrospective study would depend on the scales actually used in clinical practice. Third, the RELY-CD protocol had to translate these feasibility findings into a structured study design and define the dose-effect parameter DEff. Fourth, the main RELY-CD analysis had to test whether DEff could identify long-term differences in dose-effect development between formulation groups. Fifth, the dose manuscript had to examine the underlying treatment patterns, especially muscle-specific dose adaptation and global impressions of change, in order to place the DEff findings into the context of routine clinical management.

First, it was hypothesized that routinely documented data from specialized CD centers in different countries would be sufficient to evaluate long-term BoNT/A treatment dynamics, despite heterogeneity in clinical documentation. This hypothesis was essential because RELY-CD was not designed as a controlled trial, but as a multicenter, multinational, retrospective real-world study based on routine treatment records. Due to the retrospective nature of the study, availability of certain clinical parameters was a requirement. A prospective study of this duration and magnitude would not have been feasible due to resource limitations.

Second, it was hypothesized that long-term treatment response in CD cannot be adequately described by clinical effect or dose alone. Instead, a combined dose-effect approach was expected to provide a more clinically meaningful representation of treatment stability or deterioration. The underlying assumption was that a dose increase may reflect appropriate treatment optimization, but that dose escalation together with stable or worsening clinical effect may indicate a less favorable long-term treatment course.

Third, it was hypothesized that true long-term follow-up is required to detect clinically relevant changes in dose-effect development. Short-term or single-cycle studies can demonstrate efficacy, but they are less suited to capture gradual changes that emerge after years of repeated BoNT/A exposure, dose adaptation, formulation exposure, and evolving injection patterns. Therefore, the comparison between later treatment years and a post-optimization reference period was central to the RELY-CD design.

Fourth, it was hypothesized that long-term dose-effect development may differ between BoNT/A formulation groups. Specifically, patients treated with complex-free incoA were expected to show a more stable dose-effect relationship over time than patients treated with complex-containing formulations, onaA or aboA. This hypothesis was based on the biological and clinical relevance of foreign protein exposure, immunogenicity, and secondary treatment failure in long-term BoNT/A therapy. In addition, patients switching from complex-containing to complex-free formulations were considered a clinically important group, because switching reflects real-world treatment decisions and may occur in the context of insufficient response, tolerability, availability, or physician preference.

Fifth, it was hypothesized that dose development would be muscle-specific rather than uniform across the total injected dose. Because CD treatment is individualized according to the dystonic pattern, changes in dose were expected to differ between commonly injected superficial and deep neck muscles. The dose manuscript therefore examined whether long-term dose adaptation, muscle selection, and patient- and clinician-rated global improvement could help contextualize the DEff findings and distinguish routine treatment optimization from potentially unfavorable dose-effect development.

Together, these hypotheses define the logic of the RELY-CD publication framework. The feasibility studies tested whether the required real-world data environment existed. The protocol defined the method for transforming heterogeneous routine documentation into a structured long-term analysis. The main manuscript tested the formulation-related dose-effect hypothesis over true long-term follow-up. The dose manuscript then examined the muscle-specific treatment adaptations underlying these long-term patterns.

12 OVERVIEW OF THE INCLUDED STUDIES

12.1 FEASIBILITY STUDIES

12.1.1 Characterization of routine botulinum toxin (BoNT) therapy and patient population in cervical dystonia

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Author contribution: B.W. contributed to the conceptualization, methodology, validation, investigation, data curation, writing of the abstract, creation of the poster, project administration, and funding acquisition.

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Characterization of routine botulinum toxin (BoNT) therapy and patient population in cervical dystonia

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Meeting: [2022 International Congress](#)

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Keywords: [Botulinum toxin: Clinical applications: dystonia](#), [Dystonia: Treatment](#)

Category: [Dystonia: Clinical Trials and Therapy](#)

Objective: We conducted a survey to characterize real-world treatment of CD patients with focus on treatment duration, clinical parameters and awareness of clinical resistance as a potential complication.

Background: Cervical dystonia (CD) is a life-long condition which requires repeated injections of BoNT for symptom management. Collection of clinical parameters can be heterogeneous in a real-world setting limiting analysis.

Method: 147 centers in 7 countries in Europe and Canada responded to an online survey of 15 questions. Parameters were CD patient population, BoNT formulation, routine clinical parameters and experience with clinical resistance.

Results: 49 centers completed the survey with a reported total of 5322 CD patients on follow-up. 39% received incobotulinumtoxinA, 35% onabotulinumtoxinA and 25% abobotulinumtoxinA. 58% of patients received the same formulation. Treatment duration was 5–10 years in 70% of patients. The most frequently recorded data points were efficacy (96%), dose (94%), duration of effect (88%) and safety/tolerability (88%). TWSTRS (65%), VAS (51%), and Investigator's Global Impression (43%) were the most commonly used scales. 71% of centers reported to not routinely collect data on quality of life (QoL). While all centers in Germany reported having observed signs of clinical resistance in their patients this was only the case for 36% of centers in Spain.

Conclusion: This international survey revealed a large proportion of patients receiving long-term BoNT treatment suggesting sustained patient satisfaction over several years. QoL related outcomes are sparsely used but mandatory for a modern patient-centric approach. Surprisingly, awareness of clinical resistance differs regionally which might ultimately affect treatment regimen and outcomes.

The abstract has been submitted to the ISPRM 2022 in Lisboa from 3rd-7th July 2022 and is currently under review.

12.1.2 Real-world use of clinical scales to assess botulinum toxin efficacy in cervical dystonia treatment

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Author contribution: B.W. contributed to the conceptualization, methodology, validation, investigation, data curation, writing of the abstract, creation of the poster, project administration, and funding acquisition.

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Deep Brain Stimulation Evoked Potentials (DBSEPs) In Children With Dystonia

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Introduction

Pallidal Deep Brain Stimulation (DBS) is an established therapy for dystonia in adults and children. Stimulation parameters, including selection of active electrode contacts, are currently determined empirically. Recording the cortical response to the pallidal stimulus, using a technique called Deep Brain Stimulation Evoked Potentials (DBSEPs)[1,2] could provide a more objective measure of optimal contact selection. This study reports on the feasibility of conducting this investigation in children with genetic/idiopathic or acquired dystonia in a UK national referral centre for paediatric neuromodulation.

Materials and Methods

Fifteen young people (age 7-19years, mean 13.71) with dystonia participated, all with bilateral pallidal DBS implanted at least 6 months previously. Dystonia was genetic/idiopathic in eight and acquired in seven. Scalp EEG was recorded with an ASA (ANT-Neuro) system, using 10-20 international electrode placement and sampling at 5kHz. Impedances were reduced below 10kOhm. Contacts from the left and right stimulating electrode were tested separately, using 6Hz bipolar stimulation, with the other side turned off. Pairs of electrode contacts were tested sequentially using combinations of cathodal stimulation with an adjacent anode. Voltage was 2V, with pulse-width maintained at the patient's therapeutic level. Each combination was recorded for 3 minutes, giving

>1000 stimuli per contact pair. Therapeutic settings were restored on study completion.

Offline, the DBS stimulus artefact was used to segment EEG data into epochs (10ms pre-stimulus, 150ms post-stimulus). Raw data were inspected manually and portions of data.

Results

Clear DBSEPs were obtained in 12/15 patients from one or both stimulation sides. Peak latency was 20ms. The largest amplitude response was recorded over the ipsi-lateral central/centro-parietal region in each patient. Both the peak latency and topography were in keeping with adult literature [1]. In 3/15 patients no convincing DBSEP was obtained from stimulation of either side.

Discussion/Conclusion

This study confirms the feasibility and tolerability of this technique in children with DBS for dystonia. The findings are consistent with adult studies [1], confirming reproducibility of the method. Future work will investigate the potential clinical application of DBSEPs to inform an objective and individualised approach to DBS contact and parameter selection.

References

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Real-World Use Of Clinical Scales To Assess Botulinum Toxin Efficacy In Cervical Dystonia Treatment

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Introduction

Botulinum toxin type A (BoNT/A) is the first-line treatment in symptomatic management of cervical dystonia (CD) and repeated injections are required for life-long symptom control. Treatment can be complex and documenting the effect is an important tool to optimize patient outcomes. A previously conducted survey showed the heterogeneity of efficacy scales used in routine clinical practice. This follow-up survey further characterizes real-world use of different scales.

Materials and Methods

38 centers in Canada, Poland, and Spain completed an online survey including questions about number of CD patients, frequency of injection sessions, type and frequency of efficacy scales, type of quality of life (QoL) scales, and safety.

Results and Discussion

The centers reported a total of 5968 CD patients currently treated with BoNT/A. Average number of injection sessions per year was 3.8 (3.6, 3.7, and 4.0 in Poland, Spain, and Canada, respectively). 29 of 38 centers documented patient-reported waning or duration of effect, and the type of guidance technique used; 71 % documented patient BoNT/A treatment in other indications (including Aesthetics) and 84 % documented suspected cause of treatment issues such as lack of effect. 27 centers used at least one type of efficacy scale while 11 centers did not routinely assess efficacy using a scale. Most frequently used was a 7-point Global Impression of Change Scale (GICS, patient or physician), reported by 21 centers who used the GICS in 81 % of their patients. The Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) was used in 14 centers and application rate varied between countries (15 % and 69 % in Spain and Poland, respectively). Only one center in Canada reported use of the TWSTRS. Various other scales were reported including the Tsui scale, Visual Analogue and Likert scales. Only 21 % used a questionnaire to assess QoL (SF-36, EQ-5D, CDC24).

This survey confirms the previously reported heterogeneity of efficacy scales and further gives evidence of the variability within centers how efficacy scales are applied. While the importance of quantifying quality of life is increasingly recognized, use of standardized patient reported outcomes remains the exception.

Funding Source

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Device-Led Social Cognition Measurement In Cervical Dystonia (Proof Of Concept)

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Introduction

Patients with cervical dystonia (CD) suffer from motor and non-motor symptoms. The non-motor symptoms include sensory, psychiatric and cognitive features. We have previously published studies on deficits in social cognition in patients with CD. One aspect of this is difficulty in recognising facial expression. This can affect interpersonal relationships; patients' understanding of the emotions of others, and provide insights into disease mechanism.

Several physiological signals (heart rate, galvanic skin response, blood pressure) show abnormal responses in patients with social cognition deficits. Their measurement may have potential in the diagnosis of social cognition. A wearable device to assess responses in certain conditions, such as during conversations with others, can track physiological changes in social cognitive abilities. Such a device, with an externally facing camera, can provide feedback for accurate recognition of facial expressions.

Objective

1) Design a device that integrates physiological signals and a camera to aid in diagnosing social cognition deficits and provide feedback to participants. 2) To test the system on volunteers with cervical dystonia and healthy volunteers.

Methods

Our device integrates a heart rate sensor, galvanic skin response sensor, a blood oxygen sensor and a camera on a portable single-board computer. Data is transferred via Wi-Fi to a workstation for real-time processing. We tested the device with healthy and cervical dystonia volunteers under a range of facial expression recognition tasks from a standardised database. A convoluted

12.2 PROTOCOL AND DEMOGRAPHICS OF THE RELY-CD STUDY: ASSESSING LONG-TERM CLINICAL RESPONSE TO BOTULINUM NEUROTOXIN IN CERVICAL DYSTONIA

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<https://doi.org/10.3390/toxins17040180>

Study Protocol

Protocol and Demographics of the RELY-CD Study: Assessing Long-Term Clinical Response to Botulinum Neurotoxin in Cervical Dystonia

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Abstract: The RELY-CD study investigated the long-term clinical response to botulinum neurotoxin type A in cervical dystonia within a multicenter, real-world setting. This retrospective study focused on patients treated with complex-free (incobotulinumtoxinA) and complex-containing (onabotulinumtoxinA and abobotulinumtoxinA) BoNT/A formulations over an up to 10-year period. The novel dose–effect parameter “DEff” was introduced to quantify the relationship between dose adjustments and clinical outcomes, enabling the identification of partial treatment failures. The primary endpoint was a comparison of a clinically meaningful worsening in DEff in treatment year 7 compared to year 2 between complex-free and complex-containing botulinum neurotoxin type A. The RELY-CD study provides unique insights into long-term treatment patterns, clinical resistance phenomena, and the implications of formulation differences on treatment outcomes, addressing a critical gap in the literature on real-world botulinum neurotoxin type A application. The study methodology, including the definition and calculation of the novel DEff, as well as clinical baseline characteristics, are presented.

Keywords: cervical dystonia; botulinum toxin; immunogenicity; real-world evidence; long-term treatment; incobotulinumtoxinA; onabotulinumtoxinA; abobotulinumtoxinA

Key Contribution: The present study provides comprehensive data of long-term botulinum neurotoxin type A treatment in a multicenter real-world setting. We introduce the dose–effect parameter “DEff” for the recognition of partial treatment failure in most clinical settings.

1. Introduction

Botulinum neurotoxin type A (BoNT/A) is widely used in the treatment of various neurological disorders, including cervical dystonia (CD), a condition characterized by involuntary muscle contractions leading to abnormal postures and movements of the head and neck [1]. BoNT/A exerts its effect by binding specifically to cholinergic nerve terminals, entering the cell via endocytosis, translocating its light chain into the cytosol, and cleaving the SNARE protein SNAP-25, thereby inhibiting neurotransmitter release and causing prolonged neuromuscular paralysis [2]. The treatment of CD with BoNT/A is typically lifelong, requiring repeated injections to maintain symptom control [3]. However, the repeated administration of BoNT/A carries the risk of developing resistance, primarily due to the formation of neutralizing antibodies against the neurotoxin [3–5]. No association was found between the patient-related factors “age” and “sex” and the increased risk of antibody development [4]. Neutralizing antibodies are characterized by their ability to diffuse into the tissues, thereby binding to their target (neurotoxin) quickly and with a high affinity [6]. This resistance can lead to a reduction in the clinical efficacy of the treatment, necessitating higher doses or more frequent injections to achieve the same therapeutic effect [7–9].

Immunogenicity, the ability of a foreign substance to provoke an immune response, is a universally valid concept and known concern of biologic drugs [10]. This principle is particularly relevant in the context of therapeutic proteins, where current guidelines focus on the minimization of immunological drug resistance development [11]. BoNT/A, a potent neurotoxin produced by the bacterium *Clostridium botulinum*, is one such foreign protein with the potential for immunogenicity [2,12–14].

The three formulations of BoNT/A approved in CD by the European Medical Agency (EMA) differ in the foreign protein content; incobotulinumtoxinA (incoA, Xeomin®, Merz Pharmaceuticals GmbH, Frankfurt a.M., Germany) contains only the therapeutically active 150 kDa neurotoxin, while onabotulinumtoxinA (onaA, Botox®, AbbVie Ltd., Dublin, Ireland) and abobotulinumtoxinA (aboA, Dysport®, Ipsen Ltd., Paris, France) contain additional clostridial proteins known as complexing proteins [15–17]. All products contain non-therapeutically active additional excipients [15–17]. In a natural environment, clostridial bacteria and the toxin are ingested by the host [2,18]. The complexing proteins shield the toxin from degradation in the stomach and facilitate transport into the blood stream from the small intestine [2,18]. Uptake via the gastrointestinal tract involves passage through the protease-rich environment of the gut, facilitated by protective progenitor toxin complexes that aid in transcytosis across intestinal epithelial cells, whereas intramuscular injection delivers the active toxin directly to peripheral nerve terminals, where it is taken up via synaptic vesicle recycling [2]. In clinical application, the pathway through the gastrointestinal tract is bypassed by intramuscular injections. Hence, an additional clinical benefit of complexing proteins is disputed [19–21].

These complexing proteins may play a role in the immunogenicity of the formulations [13]. Preclinical studies show that complexing proteins increase the release of pro-inflammatory cytokines in neuronal cells [22], and some complexing proteins act as adjuvants [23]. A single-center clinical study has demonstrated the lower immunogenic potential of incoA compared to onaA and aboA by measuring neutralizing antibodies in patient blood samples in different indications [4]. Clinical implications, such as partial or complete secondary treatment failure, were demonstrated by two independent groups [5,7,24]. IncoA was found to exhibit the lowest immunogenic potential [5,7,24,25].

These clinical studies have relied on the sensitive and specific, yet not widely available, mouse hemidiaphragm assay (MHDA or MPN assay for “mouse phrenic nerve assay”) to measure neutralizing antibodies and identify partial or complete non-responders [26]. However, there is a notable absence of multicenter long-term studies in CD that detect clinical resistance phenomena in real-world settings. To bridge this gap, we designed the present study, introducing the novel dose–effect parameter (DEff).

Two feasibility studies were conducted to evaluate the availability of routinely collected clinical parameters in CD [27,28]. These studies identified muscle dose and the use of at least one well-established efficacy scale as key parameters routinely collected by most centers. Both the dose increase and reduced efficacy are cardinal signs of developing resistance [13,29]. Building on these findings, we developed the DEff, which calculates the change in dose per muscle and treatment effect from two different time points (average of treatment years), i.e., the DEff corresponds to a change in the treatment response following dose adjustment. This parameter aims to provide a more comprehensive assessment of the long-term clinical response to BoNT/A. Dose and efficacy parameters are also part of the definition from Hefter et al. of “partial secondary treatment failure”, including a systematic worsening of the treatment effect despite dose adjustments [29].

Here, we present the protocol for the international real-world study “Real-World Evidence of Longevity of BoNT/A in Cervical Dystonia” (RELY-CD). It elaborates the novel DEff parameter and presents the demographics and clinical characteristics at baseline. The RELY-CD study aims to provide valuable insights into the long-term clinical response to the three EMA-approved BoNT/A products in patients with CD, with a particular focus on the differences between complex-containing and complex-free formulations.

2. Results

2.1. Data Collection and Treatment Groups

Between July 2023 and May 2024, a total of 270 cervical dystonia patients from 13 study sites were registered in the eCRF: 9.6% at one German site, 16.0% at seven Spanish sites, and 74.4% at six sites in Poland. A total of 250 patients were eligible for the analysis. The breakdown into the different analysis sets and product groups is shown in Figure 1.

Of the 128 monotherapy patients, i.e., patients only ever treated with either CC or CF BoNT/A products, 27 (21.1% of monotherapy group) were treated with incoA, 51 with onaA (39.8% of monotherapy group), and 50 with aboA (39.1% of monotherapy group) at baseline.

2.2. Formulation-Switching Subgroup: Switchers

The switchers were separated into the following three different types within the switcher group: 6 patients (4.9% of the switcher group) were switched from CF to CC, 104 patients (85.2% of the switcher group) were switched from CC to CF, and 12 (9.8% of the switcher group) underwent multiple switches between CC and CF formulations. The conversion ratio between incoA and onaA was 1:1, the conversion ratio between either incoA and aboA or onaA and aboA was applied according to chart entry of the respective

patient. The mean (median) conversion ratio of incoA/aboA at the different sites was 1:3.3 (1:3). If the conversion ratio was unknown, the median (1:3) was applied.

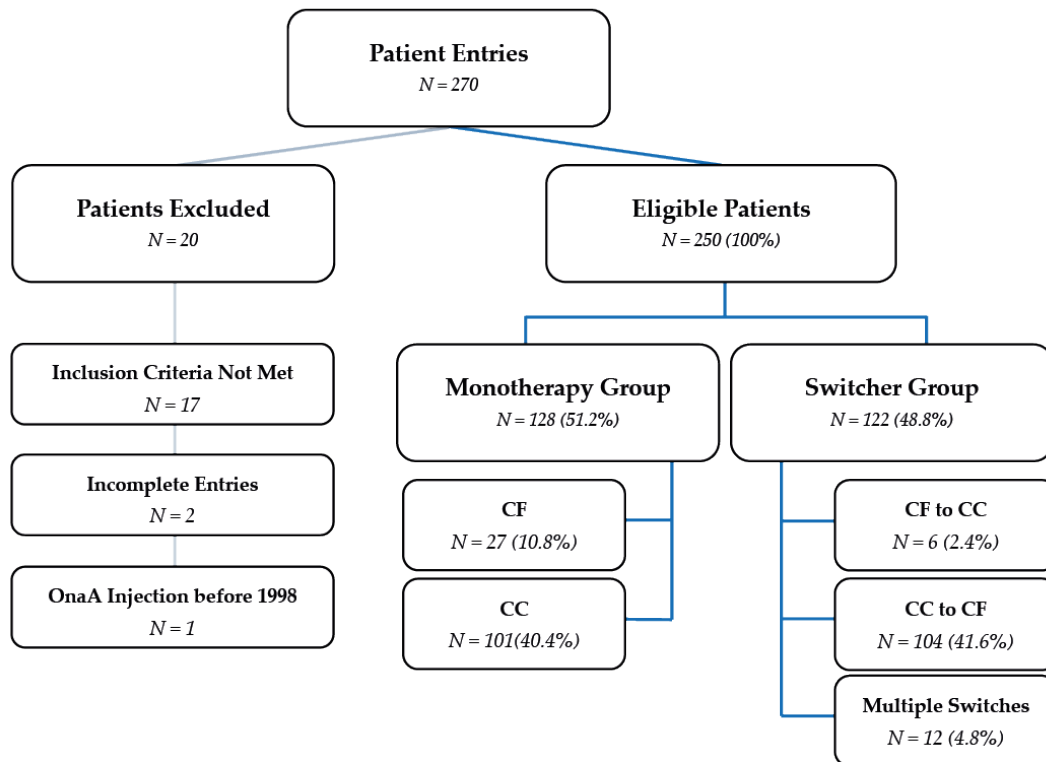


Figure 1. Patient Inclusion. Abbreviations: CF, complex-free; CC, complex-containing; OnaA, onabotulinumtoxinA.

2.3. Baseline Demographics

Table 1 summarizes the demographics and clinical characteristics of the patients in the monotherapy group at first injection/baseline. Patients in the switcher group are found in Table 2. The majority of the 128 patients in the monotherapy group were female (65.5%) and between 40 and 49 years old. The vast majority of patients was diagnosed with idiopathic CD (96.5%).

The most common concomitant diseases reported were “Psychiatric Disorders”, followed by “Vascular Disorders” and “Musculoskeletal and Connective Tissue Disorders”. The most frequent concomitant medications were “Medication used for Treatment of Focal Dystonia” (other than BoNT/A) and “Antidepressant Medication of any Route”.

Table 1. Baseline demographics and clinical characteristics of the monotherapy group.

	Total		CF		CC	
	<i>n</i>	% of Non-missing	<i>n</i>	% of Non-missing	<i>n</i>	% of Non-missing
Total Number of Patients	128		27		101	
<i>Demographic Characteristics</i>						
Sex	128	100%	27	100%	101	100%
Missing	0		0		0	
Male	44	34.4%	11	40.7%	33	32.8%
Female	84	65.6%	16	59.3%	68	67.3%
Intersex	0	0.0%	0	0.0%	0	0.0%
Age at First Injection Visit (Years)	128	100%	27	100%	101	100%
Missing	0		0		0	
18–29	5	3.9%	1	3.7%	4	4.0%
30–39	26	20.3%	5	18.5%	21	20.8%
40–49	41	32.0%	8	29.7%	33	32.7%
50–59	32	25.0%	8	29.6%	24	23.8%
60–64	24	18.8%	5	18.5%	19	18.8%
<i>Clinical Characteristics</i>						
Etiology of CD	114	100%	27	100%	87	100%
Missing	14		0		143	
Idiopathic	110	96.5%	26	96.3%	84	96.6%
Inheritance	2	1.8%	1	3.7%	1	1.1%
Acquired (e.g., Brain Injury)	2	1.8%	0	0.0%	2	2.3%
Concomitant Diseases	111	100%	26	100%	85	100%
Missing	17		1		16	
No	83	74.8%	20	76.9%	63	74.1%
Yes	28	25.2%	6	23.1%	22	25.9%
Endocrine Disorders	4	3.6%	2	7.7%	2	2.4%
Investigations	1	0.9%	1	3.8%	0	0.0%
Metabolism and Nutritional Disorders	3	2.7%	0	0.0%	3	3.5%
Musculoskeletal and Connective Tissue Disorders	6	5.4%	1	3.8%	5	5.9%
Nervous System Disorders	1	0.9%	0	0.0%	1	1.2%
Psychiatric Disorders	13	11.7%	1	3.8%	12	14.1%
Vascular Disorders	8	7.2%	1	3.8%	7	8.2%
Other Relevant Diseases	2	1.8%	2	7.7%	0	0.0%
Concomitant Medication	111	100%	26	100%	85	100%
Missing	17		1		16	
No	80	72.1%	22	84.6%	58	68.2%
Yes	31	27.9%	4	15.4%	27	31.8%
Non-Steroidal Anti-Inflammatory Drugs	5	4.5%	0	0.0%	5	5.9%
Antidepressant Medication of any Route	11	9.9%	2	7.7%	9	10.6%
Opioid Analgesics	1	0.9%	0	0.0%	1	1.2%
Medication used for Treatment of Focal Dystonia	14	12.6%	1	3.8%	13	15.3%
Other Oral Medication Potentially Interfering with BoNT/A Treatment	8	7.2%	1	3.8%	7	8.2%
Medication and Treatment Known to Interfere with BoNT/A Treatment	6	5.4%	0	0.0%	6	7.1%

Abbreviations: CF, complex-free; CC, complex-containing; CD, cervical dystonia; BoNT/A, botulinum neurotoxin type A.

Table 2. Baseline demographics and clinical characteristics of the switcher group.

	Total		CF to CC		CC to CF	
	<i>n</i>	% of Non-Missing	<i>n</i>	% of Non-Missing	<i>n</i>	% of Non-Missing
Total Number of Patients	122		6		104	
<i>Demographic Characteristics</i>						
Sex	122	100%	6	100%	104	100%
Missing	0		0		0	
Male	37	30.3%	2	33.3%	31	29.8%
Female	85	69.7%	4	66.7%	73	70.2%
Intersex	0	0.0%	0	0.0%	0	0.0%
Age at First Injection Visit (Years)	122	100%	6	100%	104	100%
Missing	0		0		0	
18–29	13	10.7%	0	0.0%	13	12.5%
30–39	17	13.9%	1	16.7%	14	13.5%
40–49	29	23.8%	2	33.3%	21	20.2%
50–59	40	32.8%	3	50.0%	36	34.6%
60–64	23	18.9%	0	0.0%	20	19.2%
<i>Clinical Characteristics</i>						
Etiology of CD	116	100%	6	100%	98	100%
Missing	6		0		6	
Idiopathic	115	99.1%	6	100%	97	99.0%
Inheritance	0	0.0%	0	0.0%	0	0.0%
Acquired (e.g., Brain Injury)	1	0.9%	0	0.0%	1	1.0%
Concomitant Diseases	118	100%	6	100%	100	100%
Missing	4		0		4	
No	103	87.3%	5	83.3%	88	88.0%
Yes	15	12.7%	1	16.7%	12	12.0%
Cardiac Disorders	1	0.8%	0	0.0%	1	1.0%
Gastrointestinal disorders	1	0.8%	0	0.0%	1	1.0%
Metabolism and Nutritional Disorders	3	2.5%	0	0.0%	2	2.0%
Musculoskeletal and Connective Tissue Disorders	1	0.8%	0	0.0%	1	1.0%
Psychiatric Disorders	7	5.9%	1	16.7%	5	5.0%
Vascular Disorders	7	5.9%	0	0.0%	5	5.0%
Concomitant Medication	117	100%	6	100%	99	100%
Missing	5		0		5	
No	101	86.3%	5	83.3%	86	86.9%
Yes	16	13.7%	1	16.7%	13	13.1%
Non-Steroidal Anti-Inflammatory Drugs	2	1.7%	0	0.0%	2	2.0%
Antidepressant Medication of any Route	8	6.8%	1	16.7%	6	6.1%
Opioid Analgesics	1	0.9%	0	0.0%	1	1.0%
Medication used for Treatment of Focal Dystonia	5	4.3%	0	0.0%	4	4.0%
Other Oral Medication Potentially Interfering with BoNT/A Treatment	5	4.3%	0	0.0%	5	5.1%
Medication and Treatment Known to Interfere with BoNT/A Treatment	1	0.9%	0	0.0%	1	1.0%

Abbreviations: CF, complex-free; CC, complex-containing; CD, cervical dystonia; BoNT/A, botulinum neurotoxin type A.

2.4. Symptom Onset and CD Diagnosis

The following two parameters were reported assessing the disease duration: the onset of CD symptoms and the time since the diagnosis of CD. Both values described for the monotherapy group were calculated from the first injection visit (baseline) as a reference (Table 3 for the monotherapy group and Table 4 for the switcher group). The time between the onset of symptoms and the first BoNT/A injection was 4.2 years in the total monotherapy group, 2.8 years for the CF group, and 4.5 years for the CC group. The time since diagnosis was 1.3, 0.5, and 1.4 years for these groups, respectively. On average, patients remained undiagnosed for 2.9 years.

Table 3. Time to first injection (baseline) from the onset of symptoms and diagnosis in the monotherapy group.

Time Since CD Symptoms Onset (Years)	Total (n = 127)	CF (n = 27)	CC (n = 100)
Mean (SD)	4.2 (5.1)	2.8 (3.7)	4.5 (5.3)
Median (IQR)	2.0 (1; 6)	1.0 (1; 4)	3.0 (1; 7)
Min, Max	0, 33	0, 17	0, 33
Time Since CD Diagnosis (Years)	Total (n = 128)	CF (n = 27)	CC (n = 101)
Mean (SD)	1.3 (2.4)	0.5 (1.1)	1.4 (2.7)
Median (IQR)	0.3 (0; 1.3)	0.2 (0; 0.4)	0.3 (0; 1.5)
Min, Max	0, 14.2	0, 4.2	0, 14.2

Abbreviations: CD, cervical dystonia; SD, standard deviation; IQR, interquartile range; Min, minimum; Max, maximum. A non-parametric Mann–Whitney U test was performed and no statistical difference was found between groups ($p > 0.5$).

Table 4. Time to first injection (baseline) from the onset of symptoms and diagnosis in the switcher group.

Time Since CD Symptoms Onset (Years)	Total (n = 122)	CF to CC (n = 6)	CC to CF (n = 104)
Mean (SD)	4.5 (5.0)	2.7 (2.3)	4.6 (5.3)
Median (IQR)	2.0 (1; 7)	2.0 (2; 3)	2.0 (1; 7)
Min, Max	0, 23	0, 7	0, 23
Time Since CD Diagnosis (Years)	Total (n = 122)	CF to CC (n = 6)	CC to CF (n = 104)
Mean (SD)	1.4 (2.9)	0.3 (0.4)	1.4 (3.0)
Median (IQR)	0.3 (0.1; 1.2)	0.1 (0; 0.3)	0.3 (0.1; 1.2)
Min, Max	−0.1, 17.3	0, 1.0	−0.1, 17.3

Abbreviations: CD, cervical dystonia; SD, standard deviation; IQR, interquartile range; Min, minimum; Max, maximum. A non-parametric Mann–Whitney U test was performed and no statistical difference was found.

3. Discussion

3.1. DEff and Its Implications

The dose–effect parameter “DEff” introduced in the RELY-CD study offers a novel framework to assess long-term treatment outcomes in CD. By combining dose adjustments and efficacy changes into a single metric, DEff provides a theoretical tool for identifying patterns of clinical resistance.

The analysis of the RELY-CD results will show the DEff’s suitability to identify atypical changes in the dose–effect correlation. Future prospective studies will be required to confirm the clinical meaningfulness of the selected threshold of 1.2. The DEff represents an important step toward a standardized approach to evaluating changes in long-term treatment outcomes in CD.

3.2. Demographics and Clinical Characteristics

The study population predominantly consisted of female patients (65.5%) and the largest mean age group was 40–49 years, which is consistent with previously reported epidemiology of CD [30–32]. The majority of patients were diagnosed with idiopathic CD (96.5%), a finding that aligns with previous studies indicating that idiopathic etiology is the most common form of CD [32,33]. The presence of concomitant psychiatric disorders (11.7%) is noteworthy, as this comorbidity is often observed in dystonia and it can influence the overall management and quality of life in CD patients [34].

3.3. Time to Diagnosis and Treatment

The time between the onset of CD symptoms and the first BoNT/A injection was, on average, 4.2 years for the total monotherapy group. This indicates a significant delay between symptom onset and the initiation of treatment, which can impact the overall disease management and patient quality of life. The time since diagnosis was, on average, 1.3 years, suggesting that patients often remain undiagnosed for approximately 2.9 years. These findings are in alignment with previously reported studies on symptom recognition and diagnosis of CD [35,36]. This delay in diagnosis highlights the need for increased awareness and the early detection of CD to improve treatment outcomes.

3.4. Dose Conversion

To ensure an accurate conversion between incoA (or onaA) and aboA, the respective conversion ratio was entered for each patient individually by the investigators. The mean (median) conversion ratio of incoA/aboA of 1:3 (1:3.3) is in alignment with previously published ratios [37,38].

3.5. Treatment Response

The DEff parameter, which accounts for dose adjustments and clinical outcomes over time, provides a novel measure of the long-term treatment outcome. The DEff can be applied to any efficacy scale commonly used in clinical practice. This allows for the comparison of long-term clinical outcomes in a heterogeneous real-world setting across different centers and countries.

However, the limitations of this novel parameter must be acknowledged. A correlation of treatment outcomes has not been established for all clinical scales permitted in this study. Scales differ in sensitivity, specificity, as well as assessment focus. Some scales include the assessment of dystonic tremors, while others focus on the overall treatment success. These effects are reduced but not completely removed by the consistent use of only one scale for each patient to calculate the DEff.

3.6. Limitations of Statistical Analysis

The main study limitation is the heterogeneity of efficacy scales used for the assessment of the treatment effect due to different clinical practices in a real-world setting [27]. While a correlation between several patient-reported and investigator-observed outcomes had been established [39], differences in perception are possible. An additional factor was the time of assessment, which could be based on the current state at the clinical visit or patient memory.

The COVID-19 pandemic had an impact on various parameters and data collected between 2020 and 2022. Due to lockdown measures, patients might have been injected less frequently than planned and needed.

To minimize the potential error introduced by permitting different efficacy scales, each patient was compared to themselves to form the respective coefficient. A change within the same patients using the same scale ensured the best possible consistency. For the same reason, a change in scale within one patient was not permitted for the analysis of the primary objective.

As this study was a retrospective chart abstraction, there was a possibility of data missing from records.

4. Conclusions

The RELY-CD study protocol provides a comprehensive framework for assessing the long-term clinical response to BoNT/A in patients with CD. This study introduces the novel dose–effect parameter “DEff”, which aims to quantify the relationship between dose adjustments and clinical outcomes over an extended period. The baseline demographics and clinical characteristics of the study population align with the existing literature on CD, providing a solid foundation for future analyses. The study results aim to provide a comprehensive overview of long-term real-world treatment of CD with BoNT/A, including the characterization of muscle patterns and dose–effect development over up to 10 years of treatment. Further studies are needed to investigate the correlation of the DEff with the development of neutralizing antibodies.

5. Methods

5.1. Study Design

RELY-CD is a multicenter, retrospective, real-world observational study designed to evaluate the long-term clinical efficacy and safety of BoNT/A formulations in patients with CD. The study focuses on the dose–effect parameter DEff, a novel metric correlating dose adjustments with clinical outcomes over a follow-up period of up to 10 years. Data were collected from medical records at clinical centers in Germany, Poland, and Spain, representing real-world treatment settings.

5.2. Patient Population

For the primary outcome analysis, patients were included who were treated either only with complex-containing (CC) formulations (onaA, Botox®, AbbVie Ltd., Dublin, Ireland, and aboA, Dysport®, Ipsen Ltd., Paris, France) or the complex-free (CF) formulation (incoA, Xeomin®, Merz Pharmaceuticals GmbH, Frankfurt a.M., Germany). Switches between CC formulations were permitted. The two patient groups are referred to as CC and CF monotherapy. Patients were eligible for inclusion in the primary outcome analysis if they met the following criteria:

- Clinical diagnosis of cervical dystonia (according to the definition of dystonia and focal isolated dystonia described in Albanese et al.) [1].
- Adults (m/f/d) 18–64 years of age at start of the BoNT/A treatment.
- Treatment with BoNT/A for at least 7 consecutive years.
- Complete history of BoNT/A formulations.
- Patients treated with only either complex-containing or complex-free BoNT/A formulations.
- Complete documentation of BoNT/A dose per specified muscle. BoNT/A formulation and same efficacy outcome for ≥ 2 visits in 2nd and 7th treatment years.
- Patient had no known drug addiction or mental illness that was judged to interfere with BoNT/A treatment according to the treating physician.
- Patient was never treated with a botulinum toxin type B formulation.

- Patient did not suffer from additional chronic diseases which may interfere with BoNT/A treatment (e.g., multiple sclerosis or amyotrophic lateral sclerosis).
- Patient did not receive a different BoNT/A formulation for a different indication (in the therapeutic or aesthetic field).
- Patient's written informed consent if required by local and/or national law.

For secondary analyses, additional inclusion criteria were applied. Switches between CC and CF, or vice versa, were permitted, as well as the inclusion of treatment information in treatment years 5 and 10.

5.3. Data Collection

Pseudonymized information from medical charts was entered into the SSL-encrypted INES electronic case report form (eCRF) system (IQVIA Technology Solutions, NJ, USA). The system is based on the following technologies: NET 4.8; Microsoft SQL server 2019; IIS 10.0; SSL validated by Entrust Certification Authority—L1K; WEB server O.S. Windows 2016. Data were collected for all injection visits in the 2nd and 7th treatment years. Optionally, data could be entered for treatment years 5 and 10. A treatment year in this study could span up to 15 months to capture visits affecting the years investigated. In addition, data were collected from the first injection visit on record. To ensure correct data entry and data quality, the following measures were taken: automatic prompts to confirm unusual or illogical data entries (such as unexpectedly high dosages for respective muscles and contradicting dates of visits), clinical and biostatistical review of data to identify outliers, and query management and resolution by CRO regarding all identified potential data entry errors. An overview of the collected parameters is shown in Table 5.

Table 5. Study variables and outcome parameters.

Parameter	Description
Demographics	• Etiology of CD • BoNT/A history • Age group (18–29, 30–39, 40–49, 50–59, 60–64) • Sex (m/f/i) • Concomitant diseases • Time of onset of CD • Time of diagnosis of CD
Efficacy Outcomes (in order of priority for DEff calculation)	1. TWSTRS total score 2. TWSTRS severity subscore 3. Tsui score 4. 7-point CGIC 5. 7-point PGIC 6. 7-, 8- or 10-point VAS (excluding “0”) 7. 8-, 9- or 11-point VAS (including “0”) 8. 8- or 10-point Likert PEGR (excluding “0”) 9. 9- or 11-point Likert PEGR (including “0”) 10. 7-, 8-, or 10-point Likert scale or NRS (excluding “0”) 11. 8-, 9-, or 11-point Likert scale or NRS (including “0”) 12. 100-point scale treated as 10-point scale (divided by 10)
Quality of Life	• SF-36 • EQ-5D • CDQ-24

Table 5. Cont.

Parameter	Description
Clinical Parameters	• Total body dose of BoNT/A per injection visit • BoNT/A dose of respective individual treated muscles • BoNT/A conversion ratio used (switches from or to aboA) • Adverse events • BoNT/A formulation used • Injection guidance technique (EMG, US, anatomic landmarks, and palpation) • Injection date (calendar week and year, or month and year) • Concomitant medication • Clinician's rationale for immunogenicity-related signs • Duration of effect (onset/complete waning of effect) • Sick leaves • Additional phone calls

Abbreviations: CD, cervical dystonia; m/f/i, male/female/intersex; TWSTRS, Toronto Western Spasmodic Torticollis Rating Scale; CGIC, Clinician's Global Impression of Change; PGIC, Patient's Global Impression of Change; VAS, Visual Analog Scale; PEGR, Patient Evaluation of Global Response; NRS, Numeric Rating Scale; SF-36, Short-Form 36; EQ-5D, EuroQol 5 Dimensions; CDQ-24, Craniocervical Dystonia Questionnaire 24; BoNT/A, botulinum neurotoxin type A; aboA, abobotulinumtoxinA; EMG, electromyography; US, ultrasound.

5.4. Study Outcomes

5.4.1. Primary Outcome

The primary outcome was the percentage of patients with a clinically meaningful worsening in dose–effect (DEff) at year 7 compared to reference year 2 between complex-free and complex-containing BoNT/A monotherapy. Year 2 was chosen as reference year to account for higher variability in the 1st treatment year due to dose adjustments, muscle selection optimization, and patient expectation management. Year 7 was chosen based on observed worsening effects in antibody-positive patients from 5 years onwards and the decrease in probability to remain antibody-negative within this time frame [4,40]. A clinically meaningful worsening was considered a DEff of >1.2. This cutoff was based on clinical assessment and experience.

The DEff was founded in the expected dose/effect correlation observed with BoNT/A treatment, i.e., the effect strength increases with the increased dose [3,41]. The following factors were defined with the potential to influence dose and/or effect changes over time and, therefore, the DEff:

- Disease progression;
- Developing drug resistance;
- Complete drug resistance;
- Physical changes (e.g., weight gain/loss);
- Psychological trigger (e.g., stress and depression).

5.4.2. Calculation of the DEff

The DEff is the product of dose and effect change (Dose and Effect Coefficients) of a treatment year compared to reference year 2. The dose coefficient does not assess the total dose change over time, but rather the sum of changes in doses of individual muscles. This is to take into account changes in treatment patterns unrelated to the development of clinical resistance. Muscles that were injected in only one comparator year, or less than twice in a comparator year, were set to 1 by definition. They do not impact the DEff. The

Dose Coefficient Q_D is the geometric mean of the quotients of muscle doses of treatment year 7 and reference year 2, as follows:

$$Q_D = \sqrt[m]{\prod_{i=1}^m \left(\frac{Dose(year\ 7)_i}{Dose(year\ 2)_i} \right)} \quad i = 1 \dots m \text{ treated muscles}$$

The Effect Coefficient Q_E is the quotient of mean effect observed in treatment year 7 compared to reference year 2, as follows:

$$Q_E = \text{Efficacy (year 7)} / \text{Efficacy (year 2)}$$

To account for differences in permitted scales, all of the scales were converted to a unified 100-point scale before the calculation (0 is best; 100 is no effect). Scales within single patients did not change between years. This way, >1 represents a worsening for both coefficients.

DEff is the product of Q_D and Q_E , as follows:

$$DEff = Q_D * Q_E$$

The product was chosen rather than the mean for several reasons:

1. *Multiplicative Relationship:*

- Interaction Between Dose and Efficacy: The DEff aims to capture the combined effect of changes in both the dose and efficacy. By multiplying Q_D and Q_E , the DEff reflects how changes in the dose and efficacy interact with each other. If either the dose or efficacy changes significantly, the product will highlight this interaction more effectively than a mean would.
- Sensitivity to Changes: Multiplication is more sensitive to changes in either parameter. For example, if the dose increases significantly but the efficacy decreases, the product will show a more pronounced effect, indicating a potential issue with treatment resistance.

2. *Geometric Mean Concept:*

- Proportional Changes: The use of the product aligns with the concept of the geometric mean, which is suitable for proportional changes. The geometric mean is often used in situations where values are multiplicative rather than additive.
- Normalization: Multiplying Q_D and Q_E normalizes the DEff, making it easier to compare across different patients and treatment regimens. It provides a single, unified measure that captures the overall treatment effect.

3. *Clinical Relevance:*

- Thresholds for Clinical Significance: The product of Q_D and Q_E allows for the establishment of clinically meaningful thresholds.
- Highlighting Extremes: By using the product, the DEff can highlight extreme cases where either the dose or efficacy changes drastically. This is important for identifying patients who may be developing resistance or experiencing significant changes in treatment response.

5.4.3. Visualization of the DEff

Figure 2 shows a visual representation of the DEff interpretation. The symbols represent putative patients responding differently to dose adjustments. The following scenarios of changes in the dose–effect are illustrated:

Scenario 1 (circle): The patient has Dose and Effect Coefficients < 1 , which means that the treatment effect increased and the required dose decreased over time. The DEff is < 1.0 and the patient is in the green area (= improvement of dose–effect).

Scenario 2 (solid square): The patient has an Effect Coefficient < 1 and Dose Coefficient > 1 . The effect has improved correlating to the dose increase. Therefore, the patient is also still in the green area.

Scenario 3 (hollow square): The patient has had no dose change and minor effect deterioration, and is therefore in the white area. The deterioration is not clinically meaningful.

Scenario 4 (arrow heads): These patients deteriorated in effect and required a dose increase. One patient (head up) had a stronger dose increase, the other patient (head down) had a stronger effect decrease. Therefore, both have a similar and clinically meaningful worsening of the DEff (> 1.2).

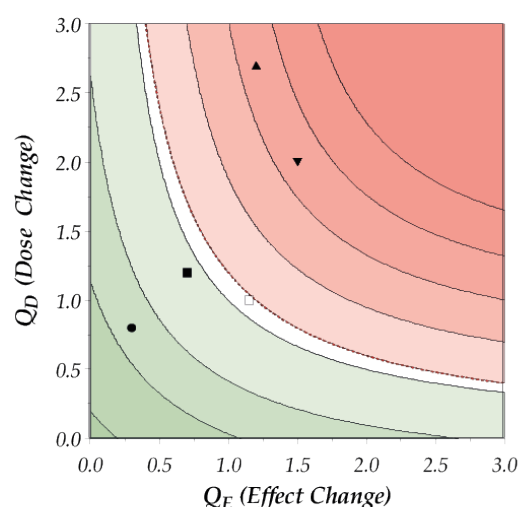


Figure 2. Visualization and Example Values for DEff Interpretation. Each symbol represents the Dose Change/Coefficient (Q_D) and Effect Change/Coefficient (Q_E) of a putative patient between two timepoints. Values > 1 show a dose increase and effect decrease over time. The green area under the black curve includes all values with a DEff < 1.0 ($< Q_D = 1/Q_E$), i.e., an improvement of dose–effect. The red area above the dotted line includes all values with a DEff > 1.2 ($> Q_D = 1.2/Q_E$), i.e., a worsening of the dose–effect. The white area between the lines represents an increased but not clinically meaningful DEff. Examples: Patient A (circle) had a dose decrease and efficacy increase over time, i.e., $DEff = Q_D \cdot Q_E = 0.8 \cdot 0.3 = 0.24$. Patient B (arrow head up) had a dose increase and efficacy decrease over time, i.e., $DEff = Q_D \cdot Q_E = 1.5 \cdot 2.0 = 3.0$. Abbreviations: Q_D , Dose Coefficient; Q_E , Effect Coefficient; DEff, dose–effect parameter.

5.4.4. Secondary Endpoints

Secondary endpoints are the difference in the mean DEff between CF and CC monotherapy populations in the years 5, 7, and 10 compared to reference year 2, and the percentage of patients with a DEff > 1.2 in years 5 and 10 compared to reference year 2. For all secondary DEff analyses, data of 1 visit or more per optional year are permitted.

A further secondary endpoint is the clinically meaningful change in the patient-reported effect from baseline (first injection visit on record) or previous injection visit for change scales. Physician-reported scales do not take peak effect into account and were therefore excluded from this endpoint. The minimal clinically meaningful change for each

scale is either based on previously reported values or clinical experience, and summarized in Table 6.

Table 6. Clinically meaningful change in patient-reported outcomes.

Scale	Threshold for Clinical Meaningfulness
7-point GICS (CGIC, PGIC)	≥ 5 on GICS (+1, at least “minimally improved”) or 1-point improvement on VAS/Likert/NRS [39].
7-point VAS	
7-point Likert	
7-point NRS	
8-point PEGR	$\geq +2$ compared to baseline [42].
10-point PEGR	
8–10-point VAS	
8–10-point Likert	
8–10-point NRS	

Abbreviations: GICS, Global Impression of Change Scale; VAS, Visual Analog Scale; NRS, Numeric Rating Scale; PEGR, Patient Evaluation of Global Response.

5.4.5. Other Endpoints and Safety

Other endpoints include the change in duration or waning of effect, the change in total dose and dose per muscle over time, and the incidence of frequent adverse events (AEs) overall and in patients with altered DEff.

Documented AEs are coded using MedDRA version 26. Frequent AEs were defined as AEs reported in more than 1% in a previous open-label extension trial in CD [42]. The investigator decided whether an AE is treatment-related. The incidence of frequent AEs per year (2, 5, 7, and 10) was compared descriptively overall and between patients with a dose–effect > 1.2 and patients with a dose–effect ≤ 1.2 .

Clinician’s Rationale for Observed Changes Potentially Related to Immunogenicity

Investigators were provided with a list of clinical changes potentially related to immunogenicity. If any changes were observed (multiple selections possible), investigators could choose from a second list the documented cause/rationale of/for the observed change. The selection of clinical changes and respective causes are listed in Table 7.

Table 7. Clinical changes potentially related to immunogenicity.

Clinical Change	Cause/Rationale
Dose Increase	Disease Progression
Effect Duration Decrease	Developing Drug Resistance
Decrease in Efficacy	Complete Drug Resistance
Lack of Efficacy	Physical Changes (e.g., weight gain/loss)
Patient-Reported Dissatisfaction	Psychological Trigger (e.g., stress, depression)
Other (Free text)	Other (Free text)

5.4.6. Quality of Life

Quality of Life (QoL) questionnaires are summarized descriptively.

5.5. Statistics

Descriptive analyses were performed. For all analyses, SAS version 9.4 was used. Non-parametric Mann–Whitney U tests were performed in GraphPad Prism vers. 10.4.1 (GraphPad Software, LLC, Boston, MA, USA). Figures were made in GraphPad Prism vers. 10.4.1 and Adobe Illustrator v. 29 (Adobe Systems Software Ireland Limited, Dublin, Ireland). Continuous variables are summarized as the mean and standard deviation (SD), median, interquartile range (IQR), minimum, and maximum. Categorical variables are

presented as absolute (number) and relative (%) frequencies. For categorical variables, each documented category was considered only once per patient in a treatment year and not multiple times, as most treatment years included at least two injection visits.

Missing data were not imputed. Descriptive analyses were performed using the available data. The absolute number and proportion of patients with missing data were reported for each measured variable in the study.

Sample Size

Based on the mean incidence of (partial) secondary treatment failure reported previously [4,5], the prevalence in year 7 was estimated for the CC and CF monotherapy groups. Since no antibody-induced treatment failure was reported in CF patients, a prevalence of 1% was chosen to account for other causes of treatment failure.

A Fisher's exact test with a 5% significance level and 90% power was used, assuming a proportion of 0.01 in the CF group and 0.063 in the CC group. The sample size per group was 262, leading to a total of 524 patients.

Analysis Sets

The following analysis sets were evaluated:

- Full Analysis Set (FAS)
 - All patients enrolled who met the selection criteria.
- Monotherapy Analysis Set = Monotherapy Group
 - CC monotherapy group (including switches between CC products);
 - CF monotherapy group.
- Switcher Analysis Set = Switcher Group
 - CF to CC group;
 - CC to CF group;
 - All switchers, including multiple switches between CC and CF.

Author Contributions: Conceptualization, B.W. and P.A.; methodology, B.W. and G.C.; validation, B.W. and G.C.; formal analysis, G.C.; investigation, B.W., H.S., and P.A.; data curation, B.W. and G.C.; writing—original draft preparation, B.W.; writing—review and editing, B.W., G.C., H.S., and P.A.; visualization, B.W.; supervision, H.S. and P.A.; project administration, B.W.; funding acquisition, B.W.; data accumulation and entry, J.-I.L., T.K., R.J., M.B., S.O., M.T., P.S., S.S.V., E.L.-V., P.M., S.J., E.O.-L., P.S.A., E.P., and G.S. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Medical Faculty of the University of Düsseldorf (protocol code 2022-2205, approved 30 January 2023). In Spain, the study was approved by the Reference Ethics Committee of the Hospital Universitario Clínico San Carlos (protocol code 23/121-O_M_NoSP, approved 8 March 2023). In Poland, the study was approved by the Ethics Committee of the Medical University of Lublin (protocol code 60/2023/KB/IX, approved 25 April 2023).

Informed Consent Statement: Informed consent was obtained in Germany and Poland from all subjects involved in the study. In Spain, patient consent was waived due to the exemption regarding pseudonymized retrospective clinical studies described in Royal Decree 957/2020 of 3rd November.

Data Availability Statement: The study is registered on clinicaltrials.gov under the ID NCT05884528.

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Conflicts of Interest: B.W. has a dual role as PhD student at Heinrich Heine University Düsseldorf and as an employee of Merz Therapeutics GmbH. G.C. is an employee of Merz Therapeutics GmbH. Merz Therapeutics GmbH is a daughter company of Merz Pharmaceuticals GmbH, market authorization holder of XEOMIN[®], which is one of the three products under investigation. S.S.V. has received financial support (including research funding and speaker honoraria) from Zambon, Abbvie, Bial, Esteve and expects to receive financial support from Merz. J.-I.L. is Review Editor for Frontiers in Neurology and has received honoraria for speaking/consultation from Boehringer Ingelheim, Daiichi Sankyo, Allergan, Abbvie, Ipsen, Novartis, Teva, Lilly, Pfizer, Perfood, Merz as well as travel grants from Allergan, Abbvie, Ipsen, Merz, Bayer, Novartis, and grants from Merz, all outside the submitted work. T.K. has received travel grants and/or honoraria for lectures from Merck, AbbVie, Ipsen, and Merz, all outside the submitted work. P.M. has received financial support from the Spanish Ministry of Science and Innovation (RTC2019-007150-1), the Instituto de Salud Carlos III-Fondo Europeo de Desarrollo Regional (ISCIII-FEDER) (PI16/01575, PI18/01898, PI19/01576, PI20/00613, PI21/01875, PI22/01704, PI23/00512), the Consejería de Economía, Innovación, Ciencia y Empleo de la Junta de Andalucía (CVI-02526, CTS-7685), the Consejería de Salud y Bienestar Social de la Junta de Andalucía (PI-0471-2013, PE-0210-2018, PI-0459-2018, PE-0186-2019), the Consejería de Transformación Económica, Industria, Conocimiento y Universidades de la Junta de Andalucía (PY20_00896). He has also received support for attending meetings and/or travel or honorarium for lecturing from Abbott, Allergan, Abbvie, Bial, Britannia, Italfarmaco, Merz, UCB, Teva and Zambon. H.S. has received a speaker honorarium from Merz Therapeutics unrelated to this project. G.S. has received funds from Ipsen, AbbVie, and Merz. P.A. received compensation for serving on Scientific Advisory Boards for Abbvie/Allergan, Biogen, Celgene, Janssen Cilag, Ipsen, Lilly, Merck, Merz, Novartis, Roche, Teva; he received speaker honoraria and travel support from Abbvie/Allergan, Biogen, Celgene, Janssen Cilag, Ipsen, Lilly, Merck, Merz, Novartis, Roche, Teva; he received research support from German Research Association (DFG), EFRE-NRW, Abbvie/Allergan, Biogen, Celgene, Janssen Cilag, Ipsen, Merck, Merz, Novartis, and Roche. M.B., S.O., M.T., P.S., E.L.-V., S.J., E.P., R.J., and P.S.A. declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

MHDA	mouse hemidiaphragm assay
MPN	mouse phrenic nerve
NRS	Numeric Rating Scale
onaA	onabotulinumtoxinA
PEGR	Patient Evaluation of Global Response
PGIC	Patient's Global Impression of Change
QoL	quality of life
RELY-CD	Real-World Evidence of Longevity of BoNT/A in Cervical Dystonia
SF-36	Short-Form 36
TWSTRS	Toronto Western Spasmodic Torticollis Rating Scale
US	ultrasound
VAS	Visual Analog Scale
aboA	abobotulinumtoxinA
AEs	adverse events
BoNT/A	botulinum neurotoxin type A
CD	cervical dystonia
CDQ-24	Craniocervical Dystonia Questionnaire 24
CGIC	Clinician's Global Impression of Change
DEff	dose-effect parameter
eCRF	electronic case report form
EMA	European Medical Agency
EMG	electromyography
EQ-5D	EuroQol 5 Dimensions

FAS	full analysis set
incoA	incobotulinumtoxinA
kDa	kilodalton
m/f/d	male/female/diverse

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12.3 LONG-TERM TREATMENT RESPONSE OF BOTULINUM TOXIN TYPE A AND DOSE-EFFECT PATTERNS IN CERVICAL DYSTONIA

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Authors' Roles

Conceptualization, B.W. and P.A.; methodology, B.W. and G.C.; validation, B.W. and G.C.; formal analysis, G.C.; investigation, B.W., H.S., and P.A.; data curation, B.W. and G.C.; writing - original draft preparation, B.W.; writing - review and editing, B.W., G.C., H.S., and P.A.; visualization, B.W.; supervision, H.S. and P.A.; project administration, B.W.; funding acquisition, B.W.; data accumulation and entry, J.-I.L., T.K., R.J., M.B., S.O., P.S., S.S.V., E.L.-V., P.M., S.J., E.O.-L., P.S.A., E.P., and G.S. All authors have read and agreed to the published version of the manuscript.

Financial Disclosures (last 12 months)

B.W. has a dual role as PhD student at Heinrich Heine University Düsseldorf and as an employee of Merz Therapeutics GmbH. G.C. is an employee of Merz Therapeutics GmbH. Merz Therapeutics GmbH is a daughter company of Merz Pharmaceuticals GmbH, market authorization holder of XEOMIN®, which is one of the three products under investigation. S.S.V. has received financial support (including research funding and speaker honoraria) from Zambon, AbbVie, Bial, and Esteve and expects to receive financial support from Merz. J.-I.L. is review editor for *Frontiers in Neurology* and has received honoraria for speaking/consultation from Boehringer Ingelheim, Daiichi Sankyo, Allergan, AbbVie, Ipsen, Novartis, Teva, Lilly, Pfizer, Perfood, and Merz; travel grants from Allergan, AbbVie, Ipsen, Merz, Bayer, and Novartis; and grants from Merz, all outside the submitted work. T.K. has received travel grants and/or honoraria for lectures from Merck, AbbVie, Ipsen, and Merz, all outside the submitted work. P.M. has received financial support from the Spanish Ministry of Science and Innovation (RTC2019-007150-1), the Instituto de Salud Carlos III-Fondo Europeo de Desarrollo Regional (ISCIII-FEDER; PI16/01575, PI18/01898, PI19/01576, PI20/00613, PI21/01875, PI22/01704, PI23/00512), the Consejería de Economía, Innovación, Ciencia y Empleo de la Junta de Andalucía (CVI-02526, CTS-7685), the Consejería de Salud y Bienestar Social de la Junta de Andalucía (PI-0471-2013, PE-0210-2018, PI-0459-2018, PE-0186-2019), and the Consejería de Transformación Económica, Industria, Conocimiento y Universidades de la Junta de Andalucía (PY20_00896). He has also received support for attending meetings and/or travel or honorarium for lecturing from Abbott, Allergan, AbbVie, Bial, Britannia, Italfarmaco, Merz, UCB, Teva, and Zambon. S.J. has received grants from Instituto de Salud Carlos III (PI18/01898) and PI21/01901) and Consejería de Salud (PI-0459-2018). She has also received Honoraria for AbbVie, Abbott, Allergan, Bial, Merz, UCB, and Zambon, and support for meetings from AbbVie, Zambon, Bial and Merz. H.S. has received a speaker honorarium from Merz Therapeutics unrelated to this project. E.O.-L. was supported by the “Río Hortega” program [CM23/00288] from the Instituto de Salud Carlos III (ISCIII-FEDER). G.S. has received funds from Ipsen, AbbVie, and Merz. P.A. has received compensation for serving on scientific advisory boards for AbbVie/Allergan, Biogen, Celgene, Janssen Cilag, Ipsen, Lilly, Merck, Merz, Novartis, Roche, and Teva; speaker honoraria and travel support from AbbVie/Allergan, Biogen, Celgene, Janssen Cilag, Ipsen, Lilly, Merck, Merz, Novartis, Roche, and Teva; and research support from the German Research Association (DFG), EFRE-NRW, AbbVie/Allergan, Biogen, Celgene, Janssen Cilag, Ipsen, Merck, Merz, Novartis, and Roche. P.S., E.L.-V., E.P., R.J., and P.S.A. declare no conflicts of interest.

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Abstract

Background: Immunogenicity with repeated botulinum neurotoxin type A (BoNT/A) injections can limit long-term management of cervical dystonia (CD). Complex-free (CF) incobotulinumtoxinA (incoA) seems to demonstrate lower secondary failure rates than complex-containing (CC) formulations, but comprehensive real-world multicenter comparisons remain limited.

Objectives: To characterize long-term dose-effect patterns across three BoNT/A formulations using a novel dose-effect parameter (DEff) that integrates dose change with clinical effect over 7 years of continuous therapy.

Methods: International multicenter retrospective chart-abstraction including 250 patients with CD treated with BoNT/A for ≥ 7 years. Three formulations were compared: incobotulinumtoxinA (incoA) [CF, $n=27$], onabotulinumtoxinA (onaA) and abobotulinumtoxinA (aboA) [CC, $n=101$], and patients who switched between CC and CF [$n=122$]. A 20% increase in DEff was predefined as clinically meaningful worsening. Primary efficacy measures included TWSTRS total score and Tsui score.

Results: At year 7, clinically meaningful worsening ($\text{DEff} \geq 1.2$) occurred in 11.1% of CF monotherapy patients, 24.2% in CC monotherapy, and 24.8% of switchers. Median total doses were 200 incoA U, 200 onaA U, and 750 aboA U. TWSTRS scores worsened significantly in the CC ($p=0.039$) and CC-to-CF groups ($p<0.001$); the small CF group ($n=6$) did not reach statistical power. Tsui scores remained numerically more stable. Patients switching to incoA demonstrated post-switch improvement in Tsui scores.

Conclusions: Long-term BoNT/A treatment in CD remains broadly effective across formulations, with a more stable DEff profile in CF monotherapy. The DEff parameter provides a practical real-world tool for monitoring treatment response and optimizing long-term outcomes in CD management.

Introduction

Cervical dystonia (CD) is the most common form of adult-onset focal dystonia and is characterized by involuntary contractions of cervical muscles leading to abnormal head postures, pain, and functional impairment.¹⁻³ BoNT/A is the first-line treatment for CD and provides consistent symptom relief when administered at regular intervals.⁴ BoNT/A acts by cleaving the SNARE protein SNAP-25, thereby blocking acetylcholine release at the neuromuscular junction and inducing reversible chemodenervation.^{5,6} Because CD requires lifelong, repeated injections, long-term therapeutic response is influenced not only by dosing strategies and muscle selection but also by the possibility of immunogenicity with repeated exposure to a foreign protein.⁷⁻⁹

Immunogenicity against BoNT/A has been recognized for more than two decades as a clinically relevant limitation of therapy, particularly in long-term high-dose treatment.¹⁰⁻¹⁵ Neutralizing antibodies (NABs) can reduce biological activity of the neurotoxin, leading to reduced duration of effect, a need for increasing doses, partial secondary treatment failure (STF), or, more rarely, complete secondary failure.^{11,13,16,17} Although only approximately half of clinically defined secondary nonresponders demonstrate NABs on sensitive assays, patients with detectable NABs show significantly worse clinical severity and require higher doses than do antibody-negative patients.^{8,11} Long-term observational studies have confirmed that cumulative dose and dose per injection cycle are the strongest predictors of immunogenicity, together with formulation differences and switching between products.^{8,13,14}

Formulation-specific immunogenic risks have been documented in long-term CD treatment. Clinical cohorts consistently show lower rates of NABs and secondary failure with incoA than with complex-containing (CC) formulations onaA and aboA.^{8,13,14,18-21} These differences align with variations in foreign protein content, as complex-free (CF) incoA lacks the additional clostridial proteins present in onaA and aboA that may augment immune responses.^{7,22-25}

However, no large multicenter study has systematically characterized long-term dose-response patterns across all three formulations under routine care conditions while simultaneously accounting for real-world injection practice. Existing real-world antibody studies rely on single-center cohorts and specialized laboratory assays not routinely available.^{8,13,17,24,26} Real-world assessments have also highlighted substantial heterogeneity in the documentation of clinical scales, dose adjustments, and

suspected resistance signs across centers, underscoring the need for standardized, multicenter evaluation in heterogeneous environments.^{27,28}

The RELY-CD study was designed to address this gap. Building upon extensive feasibility work identifying routinely collected parameters, dose per muscle, and at least one established efficacy measure, the study introduced the novel dose-effect parameter (DEff) that integrates both change in dose and change in clinical effect over time.^{27,28} This enables identification of partial treatment failure in real-world practice without requiring specialized antibody.²⁹ DEff reflects the central clinical phenomenon of concern: a worsening treatment effect despite dose escalation. This aligns with the clinical definition of partial STF proposed by Hefter et al.³⁰ and addresses limitations of previous long-term studies that either lacked dose-normalized outcomes or relied solely on patient-reported worsening.

The present manuscript reports the main long-term outcomes of RELY-CD, with analyses focusing on treatment years 2 and 7. Specifically, we evaluated the prevalence and distribution of dose-effect worsening (DEff >1.2), compared long-term patterns between CF and CC BoNT/A formulations, characterized switching trajectories, and assessed longitudinal clinical outcomes using the Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) and Tsui scores. By combining detailed injection documentation, multicenter real-world data, and a standardized dose-effect framework, RELY-CD provides a comprehensive evaluation of long-term clinical response longevity and resistance phenomena in the routine treatment of CD.

Methods

Study Design

This was an international, multicenter, retrospective chart-abstraction study designed to assess long-term clinical response to BoNT/A in patients with CD. Clinical data from routine care were extracted from medical records at participating centers and entered into a standardized electronic case report form. No study-specific interventions were performed.

The overall study design and baseline characteristics were reported previously.²⁹ The current manuscript focuses on prespecified long-term analyses of the dose-effect relationship and clinical outcome trajectories.

Three licensed BoNT/A formulations were included: incobotulinumtoxinA (incoA; Xeomin®, Merz Therapeutics GmbH, Frankfurt am Main, Germany), onabotulinumtoxinA (onaA; Botox®, Allergan/AbbVie, Irvine, CA, USA), and abobotulinumtoxinA (aboA; Dysport®, Ipsen Pharma, Boulogne-Billancourt, France). For group-level analyses, incoA was classified as CF and onaA and aboA as CC formulations.

Ethical and Regulatory Compliance

The study complied with the Declaration of Helsinki, ICH-GCP, and applicable data-protection laws. Ethics approval/notification was obtained in all countries as required.²⁹ Data were pseudonymized in the eCRF; no direct identifiers were collected. Written informed consent was obtained where mandated. ClinicalTrials.gov: NCT05884528.

Eligibility and Cohorts

Per protocol (details in ²⁹), records of adult patients with a clinical diagnosis of CD and ≥7 years of BoNT/A treatment at the same center were eligible. Patients were assigned to:

- CF monotherapy: incoA exclusively;
- CC monotherapy: onaA and/or aboA exclusively (within-class switches did not constitute a switch event);
- Switchers: CF→CC, CC→CF, or more complex sequences (included in overall switcher analyses).

Dose normalization used site-reported conversion ratios when available; otherwise, the median reported ratios were applied: incoA:onaA = 1:1 and incoA:aboA = 1:3.²⁹

Data Collection

Data were abstracted into a secure eCRF (INES; IQVIA Technology Solutions, NJ, USA). For this analysis we captured:

- Injection data: muscles injected, dose per muscle (converted to incoA-equivalent units), total session dose, and formulation;
- Efficacy: TWSTRS total and Tsui scores, transformed to the protocol's 0-100 metric;
- Potential immunogenicity-related documentation: physician-reported dose increases, reduced/shortened efficacy, lack of efficacy, and patient dissatisfaction, with clinician interpretation;
- Switching variables: calendar year, time since first injection, and product sequence.

Baseline was the first recorded injection. Reinjection visits in years 2 and 7 were required for the primary analysis; years 5 and 10 were optional. To enhance pseudonymization, visit timing used calendar weeks, allowing a "treatment year" of up to ~15 months if visits straddled cut-offs.

Derived Parameters

Dose Coefficient

Dose change was evaluated per muscle to avoid confounding by changes in injection pattern. For each muscle injected in both comparator years, the dose coefficient was defined as shown in Equation 1,

$$Q_D = \left(\prod_{i=1}^m \frac{\text{Dose}_{i,Y}}{\text{Dose}_{i,2}} \right)^{\frac{1}{m}} \quad \text{Equation 1.}$$

where Q_D is dose coefficient, Y denotes the follow-up year (5, 7, or 10) and m the number of muscles included. Muscles injected fewer than twice in a given year, or muscles added or discontinued between years, were set to 1 (no change). Doses were converted to incoA-equivalent units using the center-specific or predefined conversion ratios.

Effect Coefficient

All efficacy scales were converted to the standardized 100-point metric defined in the protocol (0 best, 100 worst score). The effect coefficient was calculated as shown in Equation 2,

$$Q_E = \frac{\text{Mean efficacy score}_Y}{\text{Mean efficacy score}_2} \quad \text{Equation 2.}$$

where Q_E is effect coefficient. Values >1 indicate worsening of clinical effect in year Y relative to year 2.

DEff

The combined DEff was defined as shown in Equation 3.

$$\text{DEff} = Q_D \times Q_E \quad \text{Equation 3.}$$

A DEff value >1.2 was predefined as clinically meaningful worsening, based on outcome thresholds for clinical meaningfulness in literature and clinical experience.^{31,32}

Outcomes

The primary outcome was the proportion of patients receiving CF and CC monotherapy with a DEff >1.2 at treatment year 7 compared with year 2, reflecting clinically meaningful worsening of treatment response.

Exploratory analyses included the distribution of DEff values and dose-effect scatterplots in patients receiving monotherapy at year 7 and corresponding DEff results in the overall switcher population at the same timepoint. Additional analyses evaluated DEff using Tukey box plots for CF, CC, and

CC→CF switchers in treatment years 5, 7, and 10 and described doses administered across all individual visits in patients receiving monotherapy, expressed as percentages of maximum label dose and product-specific dose (incoA, onaA, and aboA). Physician-reported rationales for potential signs of immunogenicity were summarized for treatment year 7. Longitudinal clinical trajectories were assessed through TWSTRS and Tsui score development across treatment years in both monotherapy and switcher groups. Finally, dose-time and Tsui-time relationships were examined using models adjusted for calendar year around the time of product switch. All other protocol-defined outcomes are described in detail in the protocol and baseline demographics publication.²⁹

Statistical Analysis

Primary analyses were performed in SAP (version 2.0, December 2024). Additional statistical analyses and graphics were performed using GraphPad Prism (version 10.6.1; GraphPad Software, San Diego, CA, USA). No imputation of missing data was performed; analyses were based on observed data.

The planned monotherapy sample size ($n \approx 656$; 1:1 CF:CC) was based on detecting differences in the expected prevalence of partial STF between formulations. Using proportions of ~1% for CF and ~6% for CC (derived from Albrecht et al.⁸ and Walter et al.¹³ and estimation of false positives), a Fisher's exact test with 90% power and $\alpha = 0.05$ required ~262 patients per group (~328 per group after accounting for up to 20% missingness). As this target was not met, the primary analysis was descriptive.

For efficacy, non-normal distributions motivated nonparametric procedures. Within-group change across time points used Friedman tests with Wilcoxon post hoc comparisons (baseline vs year 2, baseline vs year 7, year 2 vs year 7). Between-group comparisons used Kruskal-Wallis with Mann-Whitney U tests and Bonferroni adjustment ($\alpha = 0.0167$ for three comparisons). Only patients with data at all three time points were included in these tests.

For CC→CF switchers, dose and score trajectories were modeled using linear mixed-effects models with random patient effects and fixed effects for calendar year and time since first treatment; spline terms allowed flexible modeling around the switch. Simple linear regressions were also fit to pre- and post-switch periods to compare slopes.

Protocol Deviations and Data Handling

Post-lock review identified protocol deviations relevant to DEff analyses. Patients not meeting selection criteria, most commonly due to incomplete documentation of mandatory years, were excluded from DEff but retained in secondary analyses. For optional years 5 and 10, if only one injection visit was available, observed doses from year 2 and the optional year were used directly (not set to 1). Muscles injected only once in one comparator year but not both continued to be set to 1, per standard rules. Minor data corrections aligned entries with validated scoring or protocol conventions (e.g., recoding combined muscles that precluded dose reconstruction; harmonizing muscle names; correcting isolated Tsui inconsistencies).

Results

Study Population and Baseline Characteristics

The baseline characteristics are summarized in **Table 1**. A total of 250 patients were included in the analysis. Of these, 27 received CF monotherapy, 101 received CC monotherapy (started on onaA $n=51$; started on aboA $n=50$), and 122 were in the switcher population. Switchers comprised CC→CF ($n=104$), CF→CC ($n=6$), and multiple-switch patients ($n=12$).

Across all groups, the majority were female (CF 59.3%; CC 67.3%; switchers 69.7%). Idiopathic CD predominated ($\geq 96\%$ in each group). Baseline disease duration differed modestly, with shorter time since diagnosis in CF monotherapy (mean 6.3 months) than with CC monotherapy (17.3 months) and switchers (16.4 months). Time since symptom onset ranged between approximately 3 and 5 years across groups.

Table 1. Baseline characteristics of dose-effect analysis groups.

Baseline characteristic	CF monotherapy (n=27)	CC monotherapy (n=101; onaA n=51, aboA n=50)	Switchers (any) (n=122; CF→CC n=104, CF→CC n=6, multiple n=12)
Female, n (%)	16 (59.3)	68 (67.3)	85 (69.7)
Idiopathic etiology, n (%)	26 (96.3)	84 (96.6 of non-missing)	115 (99.1 of non-missing)
Time since CD diagnosis, mean (SD), months	6.3 (13.0)	17.3 (31.9)	16.4 (34.6)
Time since symptom onset, mean (SD), years	2.8 (3.7)	4.5 (5.3)	4.5 (5.0)
TWSTRS score, mean (SD), n (%)	43.2 (9.8), 6 (22.2)	39.6 (8.8), 29 (28.7)	40.8 (6.4), 40 (32.8)
Tsui score, mean (SD), n (%)	6.4 (2.3), 14 (51.9)	7.3 (3.4), 52 (51.5)	7.6 (3.9), 23 (18.9)

Values are presented as n (%), mean (SD), or n with available data as indicated. TWSTRS and Tsui baseline scores reflect the number of patients with documented assessments prior to first recorded BoNT/A injection. Percentages for idiopathic etiology are calculated among patients with non-missing data. CF = complex-free monotherapy (incobotulinumtoxinA); CC = complex-containing monotherapy (onabotulinumtoxinA or abobotulinumtoxinA). Switchers (any) include all patients who changed between CF and CC formulations at least once.

Baseline TWSTRS or Tsui scores were available for subsets of patients. Mean TWSTRS values were 43.2 (CF), 39.6 (CC), and 40.8 (switchers). Baseline Tsui scores averaged 6.4 (CF), 7.3 (CC), and 7.6 (switchers).

Baseline measures did not differ significantly between monotherapy groups in either TWSTRS (Kruskal-Wallis $p = 0.84$) or Tsui scores ($p = 0.53$). For demographic variables (age, sex distribution, time since diagnosis/symptom onset), no statistically significant group differences were detected using appropriate non-parametric tests (Kruskal-Wallis for continuous variables; Fisher's exact test for categorical variables). A detailed description of baseline variables can be found in the protocol and baseline demographics publication.²⁹

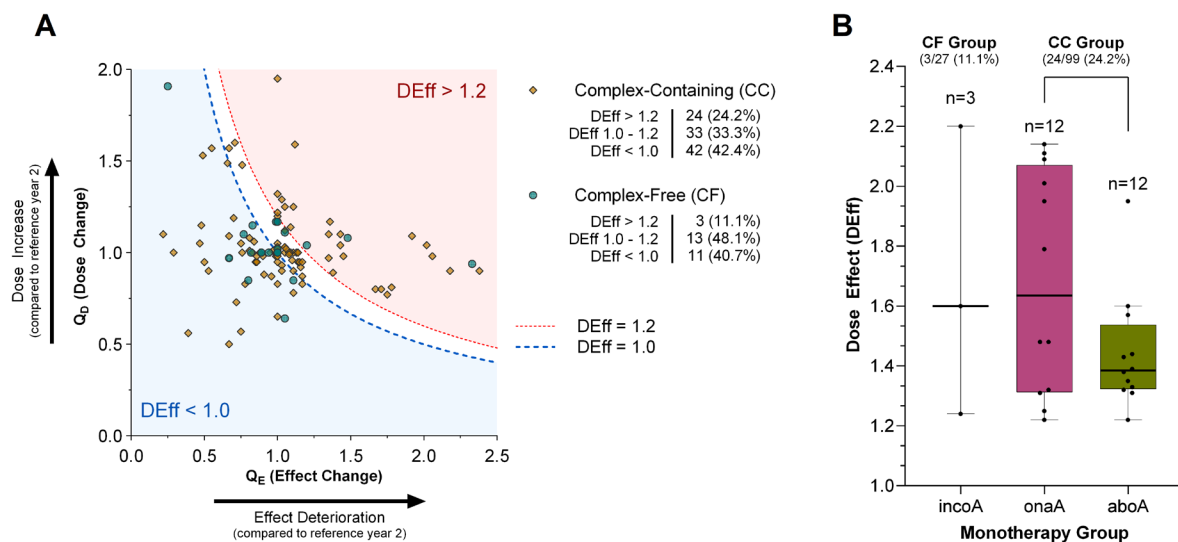


Figure 1. Dose-effect analysis in patients monotherapy patients at treatment year 7.

(A) Scatterplot of individual dose coefficients (Q_D) and effect coefficients (Q_E) for complex-free (CF) and complex-containing (CC) monotherapy patients ($DEff = Q_D \cdot Q_E$). The blue dashed line represents dose-effect parameter ($DEff$) = 1.0 (expected effect response following dose adjustments compared with year 2), and the red dashed line represents $DEff = 1.2$, the predefined threshold for clinically meaningful worsening. (B) Distribution of $DEff > 1.2$ values in monotherapy patients, shown using Tukey box plots.

Long-term dose-effect changes in monotherapy groups after 7 years

In total, 24 (24.2%) patients in the CC group and 3 (11.1%) in the CF group had a DEff >1.2 in the 7th treatment year (change from reference year 2) (**Figure 1A**). Most patients with DEff >1.2 showed effect deterioration at little change in dose per muscle. **Figure 1B** shows the distribution of DEff >1.2 patients on the product level. Half of the CC patients were in each of the onaA and aboA patient subgroups. Median and spread are numerically higher in the onaA subgroup.

Almost half of the CF group and one-third of the CC group (48.1% and 33.3%) had a DEff between 1 and 1.2, indicating little to no change in the dose-effect correlation; 42.4% in the CC group and 40.7% in the CF group had a reduced DEff of <1.2, signaling an improved dose-effect profile (i.e., increased effect at stable dose per muscle, stable effect at decreased dose, or both).

Dose-effect in all analysis groups at years 5, 7, and 10

Figure 2 shows the DEff distribution of the monotherapy and switcher groups. Patients with DEff >1.2 were found in the CC monotherapy and switchers (any) groups. Since not all patient data were entered in year 5, no direct comparison to year 7 was possible. The switchers at year 7 showed a distribution profile and proportion of patients with DEff >1.2, 1.0-1.2, and <1.0 similar to the CC monotherapy group (**Figure 2** and **Suppl. Figure 1**). The median DEff remained stable in all groups in year 10 (**Figure 2**).

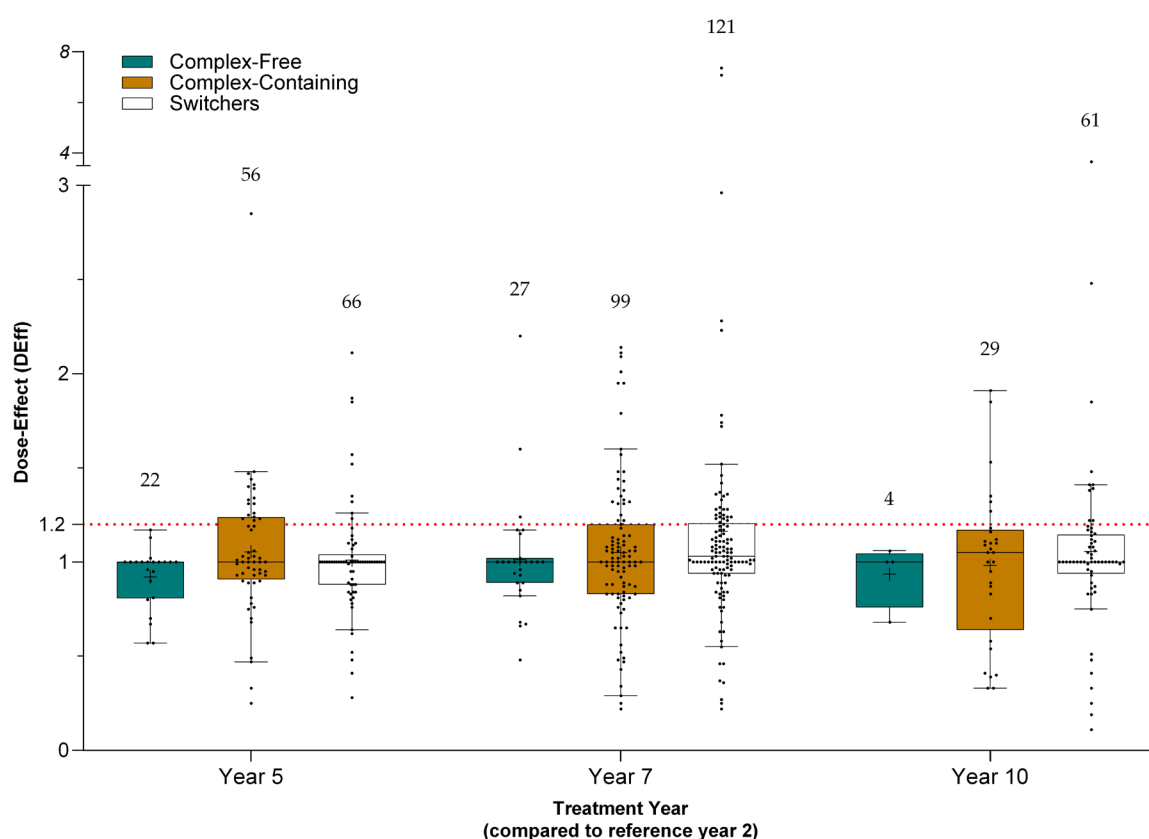


Figure 2. Dose-effect analysis in complex-free (CF), complex-containing (CC), and switcher groups for years 5, 7, and 10 (compared with year 2).

Tukey box plots show the distribution of dose-effect parameter (DEff) values in CF, CC, and switcher groups. Boxes represent the interquartile range with the median line and mean cross; whiskers extend to 1.5× the interquartile range; individual patient values are plotted over each box. Numbers represent individual patients. Red dashed line represents DEff = 1.2.

Average BoNT/A dose across all treatments

The total body dose used was quantified for all monotherapy populations (incoA, onaA, aboA) as percentage of maximum dose per label and as product-specific units (**Figure 3**). The total body doses (% of maximum label) as mean/median for incoA, onaA, and aboA were 60.8/66.7, 58.9/66.6, and

78.9/75.0. There was no statistically significant difference between incoA and onaA. The mean/median total body dose used for aboA was significantly higher than for the other products ($p < 0.001$).

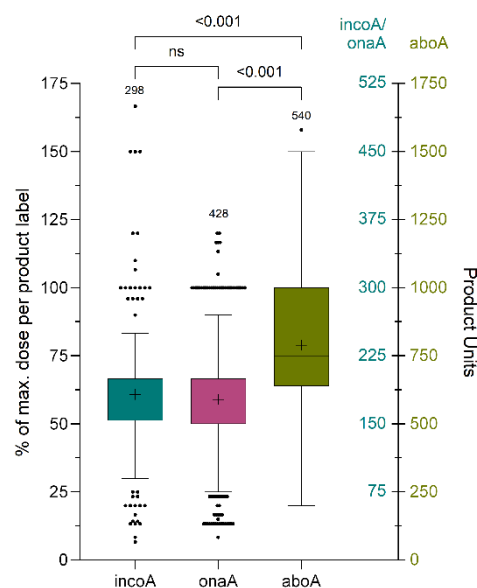


Figure 3. Doses used in monotherapy groups by product.

Tukey box plots show the distribution of dose usage for incobotulinumtoxinA (incoA), onabotulinumtoxinA (onaA), and abobotulinumtoxinA (aboA) expressed as a percentage of the respective product's maximum label dose (left y-axis) and product units (right y-axis). Boxes represent the interquartile range with the median line and mean cross; whiskers extend to $1.5 \times$ the interquartile range; group comparisons were performed using Mann-Whitney U tests because of the non-normal distributions of dose percentages (p -values shown above brackets).

Change in treatment effect over time

Treatment effects using the TWSTRS or Tsui scales were taken for all patients at reinjection. No peak effect changes were recorded using these scales. **Figure 4** shows the development of the Tsui (4A) and TWSTRS total (4B) scores at baseline, year 2, and year 7 (baseline first visit on record, all visits for years 2 and 7). The Tsui score shows a numerical improvement from baseline in years 2 and 7 for

CF and CC and an initial improvement in year 2 but deterioration in year 7 in the CC→CF group. None of the groups were powered to detect statistical differences. TWSTRS total scores increased progressively over time in all groups, with mean increases from baseline to year 7 of 2.0 points in CF, 4.1 points in CC, and 5.7 points in CC-to-CF. The increase was statistically significant in the CC ($p \leq 0.039$) and CC-to-CF groups ($p < 0.001$) but not in the smaller CF group ($n=6$). A correlation analysis between TWSTRS total and Tsui scores in patients with both assessments revealed a moderate correlation ($r=0.58$, $R^2=0.34$, $p \leq 0.0001$, **Suppl. Figure 2**).

Effect of switching on treatment effect

The impact on treatment effect of switching BoNT/A formulation was assessed using a linear mixed-effects model, adjusting for treatment year as covariate. As the sample size was low in the other switcher groups, only CC→CF was analyzed. The analysis included 16 patients evaluated with the Tsui score and 36 patients with the TWSTRS total score. Both fitted curves showed improvement up to 6–4 years before switch before deterioration (**Figure 5**). Peak of worsening was projected at 1 year after switch in the Tsui group and 4 years after switch in the TWSTRS group. The Tsui group reached the pre-switch improvement level 4 years after switch and continued to improve. The TWSTRS group did not reach pre-switch improvement levels within the observed timeframe but continued the trend after the peak at 4 years.

Clinician's observation of immunogenicity-related signs

Clinical records were analyzed for treatment response changes potentially related to immunogenicity or other clinical observations. Treatment response changes included dose increase, effect duration or efficacy decrease, lack of efficacy, and patient-reported dissatisfaction. The corresponding clinical observations of suspected reasons were also collected. This included disease progression, developing or complete drug resistance, physical changes, or psychological triggers. The results in year 7 are summarized in **Table 2**.

Overall, four clinical observations were reported in the CF group, 63 in the CC group, and 93 in the CC→CF group (all group differences significant; Pearson's chi-squared test for overall comparison: $p < 0.001$, Fisher's exact test for pairwise comparisons: CF vs. CC $p < 0.001$, CF vs. CC→CF $p < 0.001$, CC vs. CC→CF $p=0.012$). The most frequently documented suspected cause was disease progression ($n=132$), followed by psychological triggers ($n=15$), and developing drug resistance ($n=12$). The number of unique patients with at least one treatment response change were 3 (11.1%) in the CF, 23 (22.8%) in the CC, and 39 (37.5%) in the CC→CF groups, respectively (Pearson's chi-squared test for group comparison: $p=0.015$).

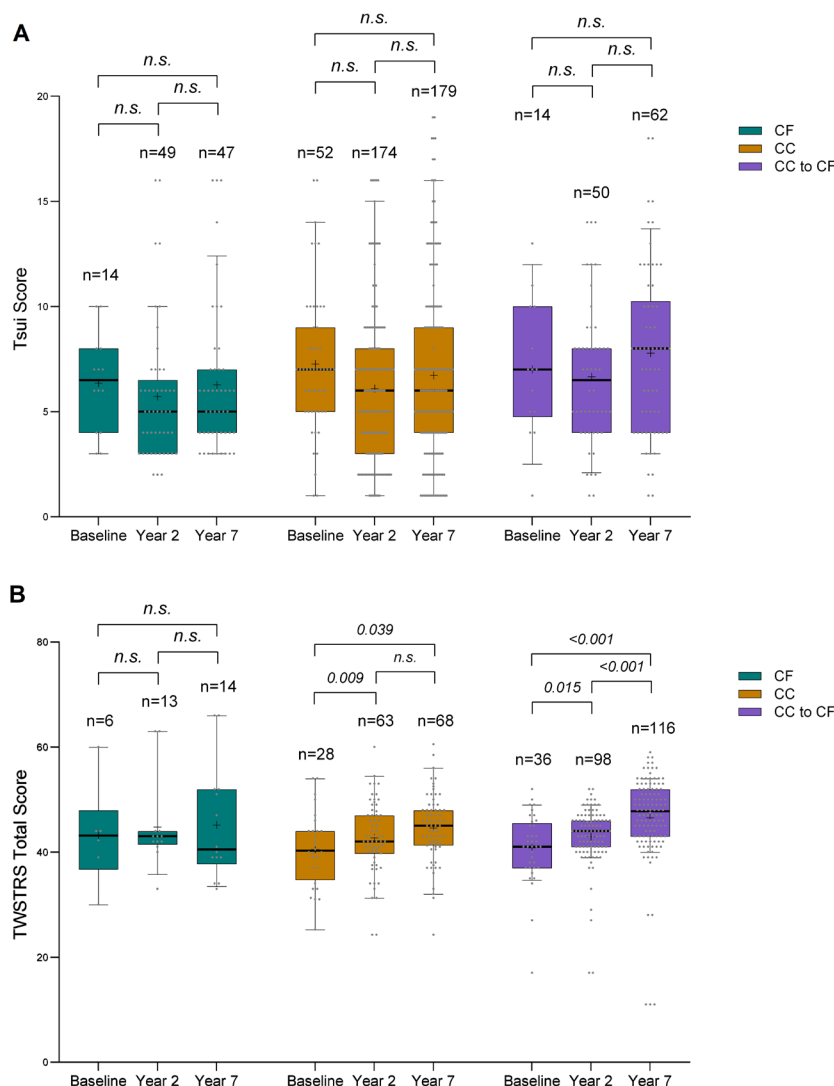


Figure 4. Tsui and Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) score development at baseline, year 2, and year 7.

Tukey box plots show the development of (A) Tsui and (B) TWSTRS scores in complex-free (CF), complex-containing (CC), and CC→CF switcher groups at baseline and treatment years 2 and 7. Boxes represent the interquartile range with the median line and mean cross; whiskers extend to 1.5× the interquartile range; individual patient values are plotted over each box. Statistical comparisons used the Friedman test with Wilcoxon signed-rank post hoc tests because of the non-normal score distributions and differing sample sizes across timepoints (p-values above brackets; n.s. = not significant). N equals all scores recorded in the respective group and year.

Discussion

A Combined Perspective on Dose and Effect

Long-term observational studies have established the durability of BoNT/A in CD. Petracca et al.³³ reported a 25-year single-center experience including all modern formulations, describing dose evolution, benefit duration, and safety despite small numbers inherent to acquired CD. Jochim et al.³⁴ analyzed up to 27 years of treatment with aboA and onaA, detailing dose development, intervals, safety, patient satisfaction, and injection pattern changes (conceptually similar to the detailed muscle-level dose change in RELY-CD). Likewise, Yahalom et al.³⁵ provided additional longitudinal data in idiopathic CD. These single-center cohorts often relied on patient-reported ratings, but mostly lack comprehensive incoA data and a standardized framework linking dose changes to clinical effect.

RELY-CD extends this literature by assembling a multicenter, multinational cohort with dose-per-muscle comparisons, routine Tsui/TWSTRS ratings, and an integrated DEff, enabling granular assessment of long-term trajectories. It also provides one of the most extensive routine-care datasets for incoA in a long-term, real-world setting. Prior immunogenicity-focused studies (e.g., Albrecht et al.⁸, Walter et al.¹³) concentrated on neutralizing antibodies (NAbs) and secondary non-response in expert centers; RELY-CD instead evaluates long-term patterns under routine care, allowing potential signals of waning to emerge outside specialized settings.

Table 2. Treatment Response Changes and Clinical Observations by Treatment Group in Year 7

Observed in any category (% relative to total number of assessment opportunities)	Treatment Year 7 [n(%)]														
	CF						CC						CC to CF		
	4 (3.0)						63 (12.2)						93 (17.8)		
	Dose Increase			Effect Duration Decrease			Decrease in Efficacy			Lack of Efficacy			Patient-Reported Dissatisfaction		
	CF	CC	CC to CF	CF	CC	CC to CF	CF	CC	CC to CF	CF	CC	CC to CF	CF	CC	CC to CF
Not observed	26 (96.3)	84 (83.2)	77 (74.0)	26 (96.3)	85 (84.2)	74 (71.2)	26 (96.3)	88 (87.1)	75 (72.1)	27 (100.0)	100 (99.0)	103 (99.0)	26 (96.3)	98 (97.0)	100 (96.2)
Observed (any)	1 (3.7)	20 (19.8)	28 (27.0)	1 (3.7)	17 (16.9)	30 (28.0)	1 (3.7)	17 (16.9)	30 (28.8)	0 (0.0)	3 (3.0)	1 (1.0)	1 (3.7)	6 (6.0)	4 (3.9)
Disease progression	1 (3.7)	17 (16.8)	27 (26.0)	0 (0.0)	12 (11.9)	27 (26.0)	0 (0.0)	13 (12.9)	28 (26.9)	0 (0.0)	1 (1.0)	1 (1.0)	0 (0.0)	2 (2.0)	3 (2.9)
Developing drug resistance	0 (0.0)	3 (3.0)	1 (1.0)	0 (0.0)	1 (1.0)	1 (1.0)	0 (0.0)	3 (3.0)	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)	0 (0.0)	2 (2.0)	0 (0.0)
Complete drug resistance	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Physical changes	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Psychological triggers	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.7)	4 (4.0)	2 (1.9)	0 (0.0)	1 (1.0)	2 (1.9)	0 (0.0)	1 (1.0)	0 (0.0)	1 (3.7)	2 (2.0)	1 (1.0)
Other	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Data are presented as n (%). Statistical comparisons between treatment groups were performed using Pearson's chi-square test or Fisher's exact test as appropriate, followed by pairwise comparisons using Fisher's exact test. **Observed in any category:** all group differences significant; Pearson's Chi-square test for overall comparison: $p < 0.001$, Fisher's exact test for pairwise comparisons: CF vs. CC $p < 0.001$, CF vs. CC→CF $p < 0.001$, CC vs. CC→CF $p = 0.012$. **Dose increase:** overall χ^2 test $p = 0.028$, with significant difference between CF vs CC to CF ($p = 0.008$), but not CF vs CC ($p = 0.074$) or CC vs CC to CF ($p = 0.250$). **Effect duration decrease:** overall χ^2 test $p = 0.007$, with significant differences between CF vs CC to CF ($p = 0.005$) and CC vs CC to CF ($p = 0.046$), but not CF vs CC ($p = 0.119$). **Decrease in efficacy:** overall χ^2 test $p = 0.006$, with significant differences between CF vs CC to CF ($p = 0.005$) and CC vs CC to CF ($p = 0.046$), but not CF vs CC ($p = 0.120$). **Lack of efficacy:** overall Fisher's exact test $p = 0.373$, with no significant pairwise differences (CF vs CC $p = 1.000$, CF vs CC to CF $p = 1.000$, CC vs CC to CF $p = 0.369$). **Patient-reported dissatisfaction:** overall Fisher's exact test $p = 0.905$, with no significant pairwise differences (CF vs CC $p = 1.000$, CF vs CC to CF $p = 1.000$, CC vs CC to CF $p = 0.748$). Multiple reports per patient were possible.

DEff as a Marker of Treatment Dynamics in Routine Care

In monotherapy groups, DEff characterized long-term stability and signs of waning under real-world conditions. Because the planned monotherapy sample size was not reached, results are descriptive, emphasizing the prespecified year-7 comparison versus year 2. Most patients maintained stable or improved DEff, supporting durable benefit across formulations. The clustering of values within DEff 1.0-1.2 supports a >20% threshold as a practical marker of meaningful deviation, recognizing it was not derived from formal sensitivity/specificity testing.

Proportions with DEff > 1.2 exceeded rates of STF or NABs summarized by Walter et al.¹⁴ This was expected because DEff captures all causes of clinical worsening (e.g., disease progression, pattern change, psychological and technical factors), not immunogenicity alone. Thus, DEff is a broader indicator of real-world variability rather than a surrogate for immunogenic STF. At year 7, patients receiving incoA monotherapy had the lowest proportion of DEff >1.2 (11.1%), whereas those receiving CC monotherapy and switcher patients (92.6% of whom were initiated on onaA or aboA) demonstrated a numerically higher proportion of clinically relevant worsening (24.2% and 24.8%, respectively). This aligns with prior evidence of lower immunogenic risk with incoA than with onaA or aboA in CD, although RELY-CD did not include antibody testing.^{7,14,19-21}

Selection effects also merit consideration. RELY-CD included only patients with ≥7 years of continuous treatment at the same centers; individuals discontinuing earlier, often due to reduced benefit, were not captured. Consequently, year-7 DEff > 1.2 proportions likely underestimate the true prevalence of long-term worsening in practice, a bias recognized in CD cohorts where persistent responders predominate over time.^{36,37}

Total Body Dose Requirements Across Formulations

In monotherapy, median product-specific doses were 200 U for incoA, 200 U for onaA, and 750 U for aboA, consistent with real-world patterns. The dose equivalence of incoA and onaA has been repeatedly shown; aboA totals fall within commonly reported ranges.^{33-35,38-40} Literature-based conversion factors of ~1:2.5-1:4 support the cohort's center-reported ratios (mean/median 1:3.3/1:3.0), with observed medians corresponding to ~1:3.75, an observation that warrants confirmation in larger controlled datasets.^{8,34,39,41}

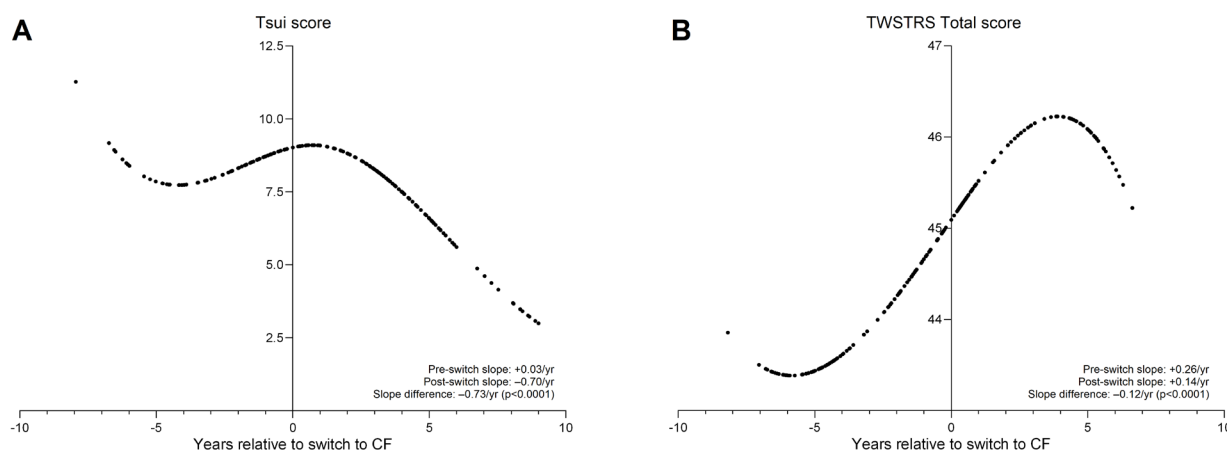


Figure 5. Tsui and Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) trajectories before and after switching from complex-containing (CC) to complex-free (CF) formulations.

Spline functions were used for flexible modeling of the efficacy score dynamics around the time of switch. Both functions showed a pattern of improvement followed by decline of effect before the switch. After switching, Tsui scores ($n(\text{patients})=16$) improved within the first year, whereas TWSTRS ($n(\text{patients})=36$) showed later improvement beginning around year four. To quantify these changes, linear slopes were compared before and after the switch: TWSTRS slopes decreased from +0.26 to +0.14 points/year ($\Delta = -0.12$, $p < 0.0001$), and Tsui slopes shifted from flat (+0.03 points/year) to improving (-0.70 points/year; $\Delta = -0.73$, $p < 0.0001$). These results indicate significantly more favorable long-term trajectories following the switch.

Clinical Trajectories and Modifiers of Response

Across groups, Tsui and TWSTRS trajectories indicated largely maintained long term treatment response with only modest changes. Direct scale comparison is limited by differing constructs, although intra patient correlation (Suppl. Fig. 2) supports a consistent relationship. CF and CC groups improved in Tsui at year 2 and remained generally stable; CC→CF switchers showed later subtle worsening, while TWSTRS exhibited gradual worsening in CC and switchers, with CF inconclusive due to small numbers. Overall, these patterns are consistent with durable long term benefit.

Model based analyses around switching from complex containing to complex free formulations suggested attenuation of pre switch decline, with earlier improvement signals in Tsui and delayed improvement in TWSTRS, compatible with prior reports of stabilizing benefit after transitioning to incoA.^{42,43} Given small switcher samples and absence of antibody testing, these modelled trajectories should be interpreted cautiously.

Clinician reported observations of treatment response change were more frequent in CC monotherapy and switchers than in CF monotherapy, most often attributed to disease progression. Baseline characteristics do not suggest differences that would fully explain this pattern, implying that non progression contributors (e.g., gradual decline in treatment responsiveness) may also play a role but be difficult to disentangle from progression in routine practice; an issue highlighted in prior long-term work.^{14,35,44}

Conclusion

In this multicenter, real-world cohort, long-term BoNT/A treatment in CD remained broadly stable across formulations, with most patients showing unchanged or improved dose-effect relationships over time. Differences in DEff distributions between groups were consistent with previously reported product-specific patterns, but no mechanistic conclusions can be drawn in the absence of antibody testing. The DEff parameter itself requires prospective validation to confirm its clinical meaningfulness; however, it represents a practical tool for summarizing treatment changes in routine care and can be applied across different rating scales and potentially other therapeutic indications. Overall, RELY-CD extends long-term observational evidence by integrating dose and effect in a unified framework and demonstrating the feasibility of such an approach in heterogeneous, everyday clinical practice.

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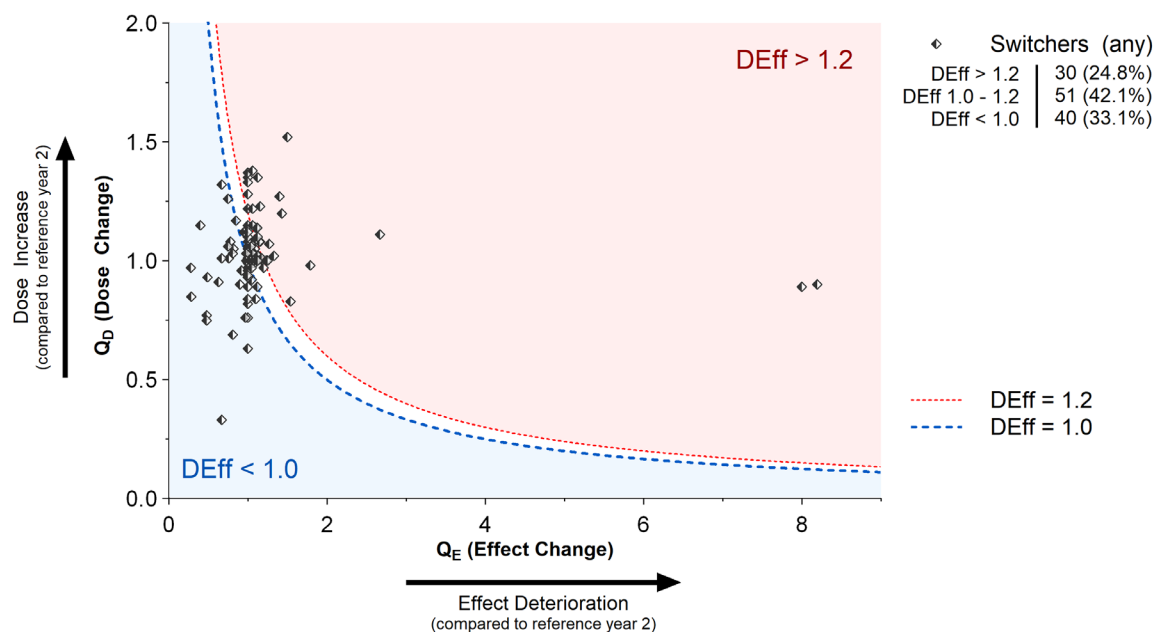
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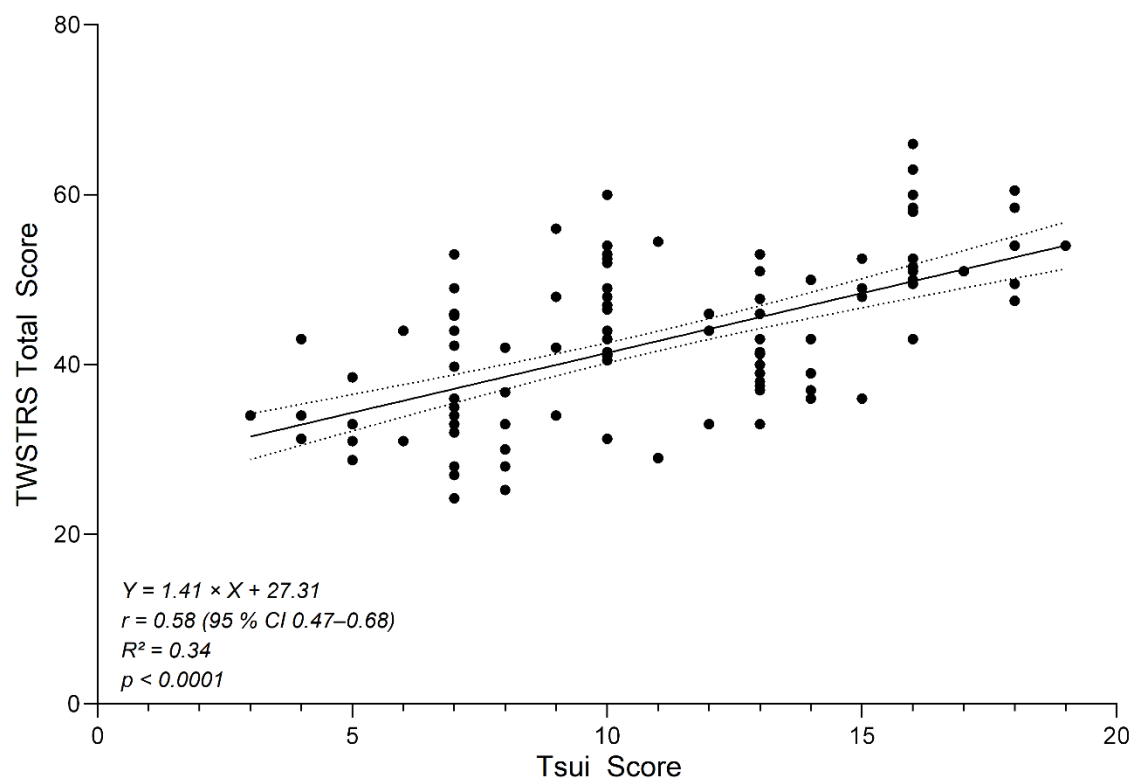
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Supplementary Figures



Suppl. Figure 1. Dose-effect analysis in switcher patients at treatment year 7.

Scatterplot of individual dose coefficients (Q_D) and effect coefficients (Q_E) for switcher (any) patients (dose-effect parameter $[DEff] = Q_D \times Q_E$). The blue dashed line represents $DEff = 1.0$ (expected effect response following dose adjustments compared with year 2), and the red dashed line represents $DEff = 1.2$, the predefined threshold for clinically meaningful worsening.



Suppl. Figure 2. Intra-patient correlation analysis of Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) and Tsui scores.

The correlation analysis was performed in patients with both TWSTRS and Tsui scores available for the same visit. The analysis revealed a moderate correlation between scores.

12.4 LONG-TERM DOSE DEVELOPMENT AND TREATMENT PATTERNS WITH BOTULINUM TOXIN TYPE A IN CERVICAL DYSTONIA

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B.W. contributed to the research project through conception, organization, and execution; to the statistical analysis through design and execution; and to manuscript preparation through writing of the first draft.

Manuscript submitted to “Movement Disorders Clinical Practice”

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1. Research Project: A. Conception, B. Organization, C. Execution
2. Statistical Analysis: A. Design, B. Execution, C. Review and Validation
3. Manuscript Preparation: A. Writing of the first draft, B. Review and Critique

BW: 1A, 1B, 1C, 2A, 2B, 3A

GC: 2C, 3B

HS: 1A, 3B

PA: 1A, 1C 1B, 3B

Abstract

Background

Long-term treatment of cervical dystonia with botulinum neurotoxin type A requires continuous adaptation of dose and injection patterns. The relationship between muscle-specific dose development and patient- and clinician-reported outcomes remains insufficiently characterized.

Methods

This post-hoc analysis is based on the international, multicenter, retrospective Real-World Evidence of Longevity of Botulinum Toxin Type A in Cervical Dystonia (RELY-CD) study. Patients receiving abobotulinumtoxinA, incobotulinumtoxinA, onabotulinumtoxinA, or switching from complex-containing (abo-/onabotulinumtoxinA) to complex-free (incobotulinumtoxinA) formulations were evaluated at baseline and Years 2, 5, 7, and 10. Doses were converted to incobotulinumtoxinA-equivalent units. Injection frequencies and dose trajectories were analyzed descriptively and using linear mixed-effects models adjusted for calendar year. Patient and Clinician Global Impression of Change were assessed.

Results

Injection targets remained stable, with sternocleidomastoid (88.8%) and splenius capitis (82.4%) most frequently injected. Mean doses were highest in larger muscles, including splenius capitis (75.5 units) and sternocleidomastoid (61.7 units). Differences between formulations were most pronounced in these muscles, for example in the splenius capitis at Year 2 (abobotulinumtoxinA 111.7 units vs incobotulinumtoxinA 67.5 units), whereas doses were similar in the levator scapulae (~33 units). Calendar-year adjustment altered trajectory shapes but showed consistent effects across formulations. Global impression scores remained positive, with >90% achieving clinically meaningful improvement.

Conclusions

Over up to 10 years, treatment is characterized by dynamic, muscle-specific dose adaptation. Despite variability in dosing, sustained global improvement indicates effective clinical management. These findings highlight the importance of evaluating dose and efficacy jointly and support integrated measures such as the dose-effect parameter (DEff).

Introduction

Botulinum toxin type A (BoNT-A) is the established first-line treatment for cervical dystonia (CD), but long-term management is dynamic rather than static. Over years of repeated treatment, clinicians adjust dose, muscle selection, and injection strategy over time in response to symptom pattern, tolerability, and perceived treatment effect. Although the long-term efficacy and safety of BoNT-A in CD are well documented, these studies have focused on global clinical outcomes, total body doses, or treatment intervals [1], [2], [3], [4]. The development of dose patterns at the level of individual muscles has received less systematic attention [5], [6].

Differences between BoNT-A formulations further complicate the interpretation of long-term dosing. Dose equivalence between incoA and onaA is generally accepted as approximately 1:1, whereas

reported conversion ratios between aboA and the other formulations vary more widely in clinical practice [7], [8]. Furthermore, long-term observational cohorts have shown that dose escalation does not necessarily correspond to improved clinical benefit, as dose changes may also reflect disease progression, evolving injection strategies, or changes in treatment response over time [5], [9].

While several studies have described long-term treatment outcomes in CD, the relationship between evolving injection patterns, dose adaptation, and patient- and clinician-reported global improvement has not been systematically examined. This is clinically relevant because routine treatment decisions are typically guided by iterative adjustments of muscle selection and dosing in response to perceived treatment effects at reinjection visits, rather than by standardized rating scales alone.

The present analysis therefore focuses on long-term evolution of dose and treatment patterns in CD, with particular emphasis on frequently injected muscles and on Patient Global Impression of Change (PGIC) and Clinician Global Impression of Change (CGIC). The aim was to describe how muscle selection and dosing evolve over time across BoNT-A formulations, and whether these longitudinal adaptations are accompanied by sustained positive global impressions of treatment effect.

Methods

Study design and population

This analysis is based on the RELY-CD study, an international, multicenter, retrospective chart-abstraction study of patients with CD receiving long-term treatment with BoNT-A. The overall study design and baseline characteristics have been described previously (Waeschle *et al.*, 2025). Patients treated with CF or CC formulations, as well as patients who switched from CC to CF during follow-up (CC to CF), were included. Analyses were performed at baseline and at follow-up visits in Years 2, 5, 7, and 10.

Treatment and dose standardization

Patients received routine clinical treatment according to local practice. Different BoNT-A formulations (aboA, incoA, and onaA) were used. To allow comparison across formulations, all doses were converted to incoA-equivalent units using center-specific conversion ratios as reported by participating sites. For aboA, the mean (median) reported conversion ratio across sites was 1:3.3 (1:3) [10]; if no center-specific ratio was available, the median ratio of all centers (1:3) was applied. The incoA:onaA conversion ratio was 1:1.

Table 1. Long-term dose development per individual muscle and formulation

Muscle	Year	aboA		incoA		onaA		CC to CF	
		n	Units, mean (SD)	n	Units, mean (SD)	n	Units, mean (SD)	n	Units, mean (SD)
Levator scapulae	0	5	36.7 (7.5)	10	32.0 (9.2)	15	27.0 (8.0)	43	29.5 (12.4)
	2	37	32.9 (12.7)	38	33.0 (9.1)	53	30.5 (8.3)	142	37.1 (13.8)
	5	27	37.0 (10.1)	32	32.2 (6.8)	31	38.1 (21.6)	22	36.1 (12.9)
	7	61	33.2 (10.6)	38	34.3 (6.2)	57	37.0 (17.0)	205	42.8 (17.3)
	10	10	36.0 (3.4)	0	-	2	37.5 (17.7)	70	49.4 (11.4)
Obliquus capitis inferior	0	0	-	0	-	0	-	3	25.0 (0.0)
	2	4	50.0 (13.6)	4	57.5 (5.0)	1	40.0	7	32.6 (10.7)
	5	24	42.1 (13.1)	9	26.7 (5.0)	2	35.0 (7.1)	9	40.4 (18.1)
	7	26	42.9 (12.6)	25	34.4 (12.5)	5	33.0 (9.7)	26	30.6 (10.0)
	10	0	-	0	-	2	37.5 (17.7)	48	28.4 (8.5)
Scalene muscles	0	8	36.5 (19.9)	7	25.0 (7.6)	5	27.0 (13.0)	10	24.2 (16.6)
	2	18	43.1 (17.1)	20	25.3 (8.2)	23	27.6 (11.1)	28	17.5 (4.9)
	5	9	38.0 (16.7)	14	23.9 (4.9)	15	23.7 (3.0)	21	28.7 (14.8)
	7	14	31.5 (9.3)	12	22.9 (4.5)	19	27.1 (12.1)	78	26.4 (13.5)
	10	7	85.7 (6.3)	0	-	4	20.0 (0.0)	14	22.9 (10.1)
Semispinalis capitis	0	6	43.3 (19.1)	1	20.0	6	61.7 (44.9)	20	35.8 (15.9)
	2	18	42.6 (37.4)	21	34.0 (15.3)	21	37.4 (15.0)	83	45.7 (19.0)
	5	17	57.1 (30.4)	10	27.5 (5.9)	7	35.0 (14.4)	4	33.8 (11.1)
	7	32	51.1 (26.6)	23	43.5 (12.2)	31	46.4 (13.4)	129	47.0 (11.6)
	10	0	-	0	-	2	37.5 (17.7)	43	43.4 (21.4)
Semispinalis cervicis	0	0	-	5	20.0 (0.0)	11	20.9 (2.0)	4	41.3 (11.8)
	2	9	40.7 (8.8)	22	25.0 (13.0)	47	24.7 (9.4)	15	45.3 (16.9)
	5	11	50.0 (10.5)	15	20.7 (2.6)	34	22.6 (8.6)	11	25.0 (13.4)
	7	9	33.3 (14.4)	16	23.1 (8.7)	35	24.9 (10.5)	36	36.5 (12.6)
	10	2	29.2 (5.9)	0	-	4	25.0 (0.0)	28	45.5 (16.7)
Splenius capitis	0	54	97.0 (57.0)	22	64.1 (27.2)	30	55.3 (30.8)	91	50.7 (20.6)
	2	154	111.7 (60.6)	73	67.5 (30.0)	79	67.8 (36.9)	258	56.9 (26.7)
	5	84	81.8 (52.1)	68	57.9 (29.7)	33	51.1 (24.9)	106	52.6 (24.1)
	7	162	95.2 (58.1)	79	60.4 (26.0)	93	64.9 (30.3)	306	64.6 (28.7)
	10	32	100.8 (61.1)	13	66.2 (27.3)	21	60.7 (22.7)	101	57.6 (26.2)
Splenius cervicis	0	0	-	2	35.0 (7.1)	6	46.7 (23.8)	10	45.5 (27.2)
	2	2	83.3 (47.1)	12	38.8 (15.1)	22	36.1 (15.3)	14	44.3 (22.9)
	5	0	-	7	32.9 (7.6)	16	28.8 (8.7)	5	30.0 (14.1)
	7	2	75.0 (58.9)	9	31.1 (7.4)	17	45.6 (24.6)	33	44.8 (14.7)
	10	8	27.1 (3.9)	2	75.0 (35.4)	1	50.0	6	51.7 (22.7)
Sternocleidomastoid	0	40	77.3 (42.6)	26	55.1 (29.2)	30	51.2 (30.5)	100	53.4 (18.2)
	2	126	69.8 (36.8)	80	65.2 (29.7)	75	52.5 (25.3)	269	54.7 (21.5)
	5	82	74.9 (47.0)	65	64.7 (25.8)	43	57.4 (30.4)	106	59.4 (19.7)
	7	139	61.0 (37.7)	79	67.0 (26.8)	96	48.3 (24.8)	333	53.2 (17.3)
	10	39	71.7 (34.7)	15	85.7 (20.1)	32	53.9 (32.4)	117	65.3 (20.1)
Trapezius	0	24	70.1 (31.8)	16	43.4 (23.1)	24	47.1 (14.1)	62	49.9 (30.6)
	2	82	56.2 (29.0)	64	43.3 (21.7)	67	49.5 (26.2)	172	48.7 (28.7)
	5	59	55.2 (18.4)	51	40.0 (20.3)	31	36.5 (13.7)	93	48.7 (21.9)
	7	81	53.3 (30.2)	56	44.6 (19.1)	86	46.0 (22.7)	248	43.3 (17.8)
	10	23	41.8 (26.1)	16	30.3 (8.5)	22	51.4 (20.4)	93	59.1 (20.7)
Other	0	4	72.5 (86.2)	2	12.5 (10.6)	2	15.0 (7.1)	6	70.5 (54.1)
	2	9	42.2 (21.5)	20	29.3 (18.3)	9	25.5 (18.8)	31	50.3 (35.7)
	5	6	14.4 (17.4)	8	27.1 (9.9)	9	26.7 (9.0)	1	25.0
	7	31	93.5 (78.5)	23	22.9 (14.6)	29	43.3 (24.4)	94	50.2 (14.0)
	10	0	-	1	25.0	0	-	3	35.0 (8.7)

Doses in incoA-adjusted Units; the conversion ratios as reported by centers were used. "n" represents the number of recorded injections of a muscle (not number of patients or injection points per muscle). Most frequently injected "other" muscles were Longus capitis/colli. Abbreviations: aboA, abobotulinumtoxinA; incoA, incobotulinumtoxinA; onaA, onabotulinumtoxinA; CC, complex-containing; CF, complex-free; n, number; SD, standard deviation.

Muscle analyses

Analyses of injection frequency were restricted to muscles injected in more than 10% of patients at baseline or at mandatory follow-up visits (Years 2 and 7).

Dose trajectories were calculated for muscles $\geq 30\%$ injection frequency at baseline to allow for sufficient data point availability. Linear regression was fitted using exact injection timepoints. These were simplified to treatment years in the graph.

Outcomes

PGIC and CGIC were assessed at reinjection visits using 7-point scales ranging from -3 to +3. Clinically meaningful improvement was defined as “+1” (minimally improved or better) in alignment with previous reports [4].

Statistical analysis

Descriptive statistics were used to summarize injection frequencies, dose distributions, and PGIC and CGIC scores. Mean doses were calculated per muscle, formulation, and timepoint.

Dose trajectories over time were analyzed using linear mixed-effects models with patient as a random effect. Models were adjusted for calendar year of measurement. Results are presented as trajectories of unadjusted means and adjusted estimates.

Between-group comparisons for PGIC and CGIC were performed at each timepoint using pairwise Welch t-tests based on group means, standard deviations, and sample sizes, accounting for unequal variances and group sizes. Multiplicity across pairwise comparisons within each timepoint was controlled using Holm adjustment.

No formal statistical comparisons between formulations were performed for dose analyses because of the observational design and repeated measurements per patient.

Results

Muscle selection and injection frequency

Figure 1A illustrates the combined injection frequencies (all formulation groups) for muscles injected in more than 10% of patients at baseline or mandatory follow-up visits. At baseline, the most frequently injected muscles were the sternocleidomastoid (88.8%) and splenius capitis (82.4%), followed by the trapezius (52.8%) and levator scapulae (32.0%). Lower injection rates were observed for the scalene muscles (14.4%), semispinalis capitis and cervicis (13.2% and 7.6%), splenius cervicis (7.6%), and obliquus capitis inferior (1.6%).

Injection frequencies remained largely stable over time for most muscles. Notable increases were observed for the semispinalis capitis and cervicis between baseline and Year 2 (13.2% to 30.0% and 7.6% to 16.0%, respectively), with values remaining elevated at Year 7. The obliquus capitis inferior showed the strongest relative increase, from 1.6% at baseline to 6.4% at Year 2 and 20.3% at Year 7, and was the only muscle with a marked increase between Years 2 and 7. Gradual increases were also observed for the levator scapulae (32.0% to 47.7%) and trapezius (52.8% to 64.8%), while the sternocleidomastoid and splenius capitis remained consistently high across all timepoints.

Dose distribution across muscles

Figure 1B (see Supplementary Table 1 for overall mean dose and standard deviation) and Table 1 show mean doses per muscle over time. Overall, unadjusted mean doses remained relatively stable across treatment years. Highest total mean doses were observed in frequently injected, large muscles, including splenius capitis (75.5 U), sternocleidomastoid (61.7 U), trapezius (49.2 U), semispinalis capitis (44.6 units), splenius cervicis (39.8 U), and levator scapulae (34.9 U).

The obliquus capitis inferior, a comparatively small deep cervical muscle, showed mean doses similar to or slightly exceeding those of larger muscles such as the levator scapulae (35.7 U vs. 34.9 U).

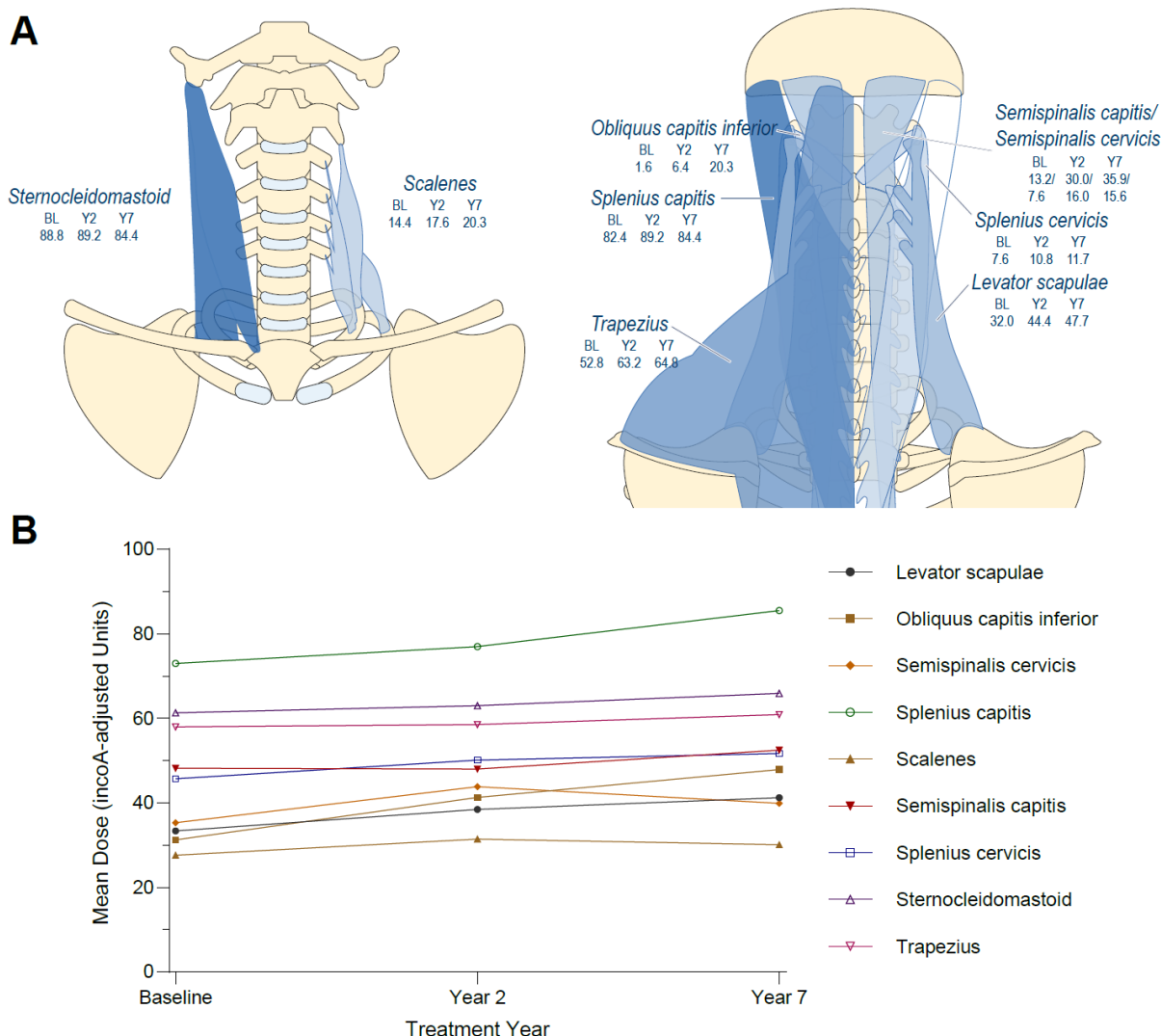


Figure 1. Muscle injection frequency and dose development

A: Schematic representation of most frequently injected muscles (>10 % at BL, Y2, or Y7). Anterior view shown left, posterior view right. Darker tint equals higher frequency (at BL). Frequency shown in percent (%). B: Mean doses at BL, Y2, and Y7 for these muscles. Dose adjusted to incoA units according to reported conversion ratio. Means and standard deviation listed in Supplementary Table 1. Abbreviations: BL, Baseline; Y2, Year 2; Y7, Year 7.

Dose differences between formulations were most pronounced in larger muscles. For example, in the splenius capitis, mean doses differed by approximately 65% between aboA (111.7 U) and incoA (67.5 U) at Year 2, with similar differences at later timepoints. In the sternocleidomastoid, a difference of approximately 20% was observed between aboA (69.8 U) and onaA (52.5 U) at Year 2. For the trapezius, aboA doses were approximately 25% higher than incoA at Years 2 and 7. In contrast, doses were more similar across formulations in the levator scapulae, where mean doses at Year 2 were 32.9, 33.0, and 30.5 U for aboA, incoA, and onaA, respectively. Mean dose changes between timepoints are shown in Supplementary Table 2.

Dose trajectories by muscle and formulation

Figure 2 illustrates muscle-specific dose trajectories as unadjusted means (A) and after adjustment for calendar year (B). In the unadjusted analysis, trajectories were largely parallel across formulations within each muscle, with aboA showing consistently higher dose levels in the splenius capitis.

After adjustment for calendar year, the overall shape and relative positioning of trajectories changed in several muscles. For example, in the splenius capitis, aboA shifted from the highest dose level in the unadjusted analysis to the lowest estimated trajectory in the adjusted model. These differences reflect the impact of accounting for treatment year and indicate that temporal factors can substantially influence estimated dose trajectories.

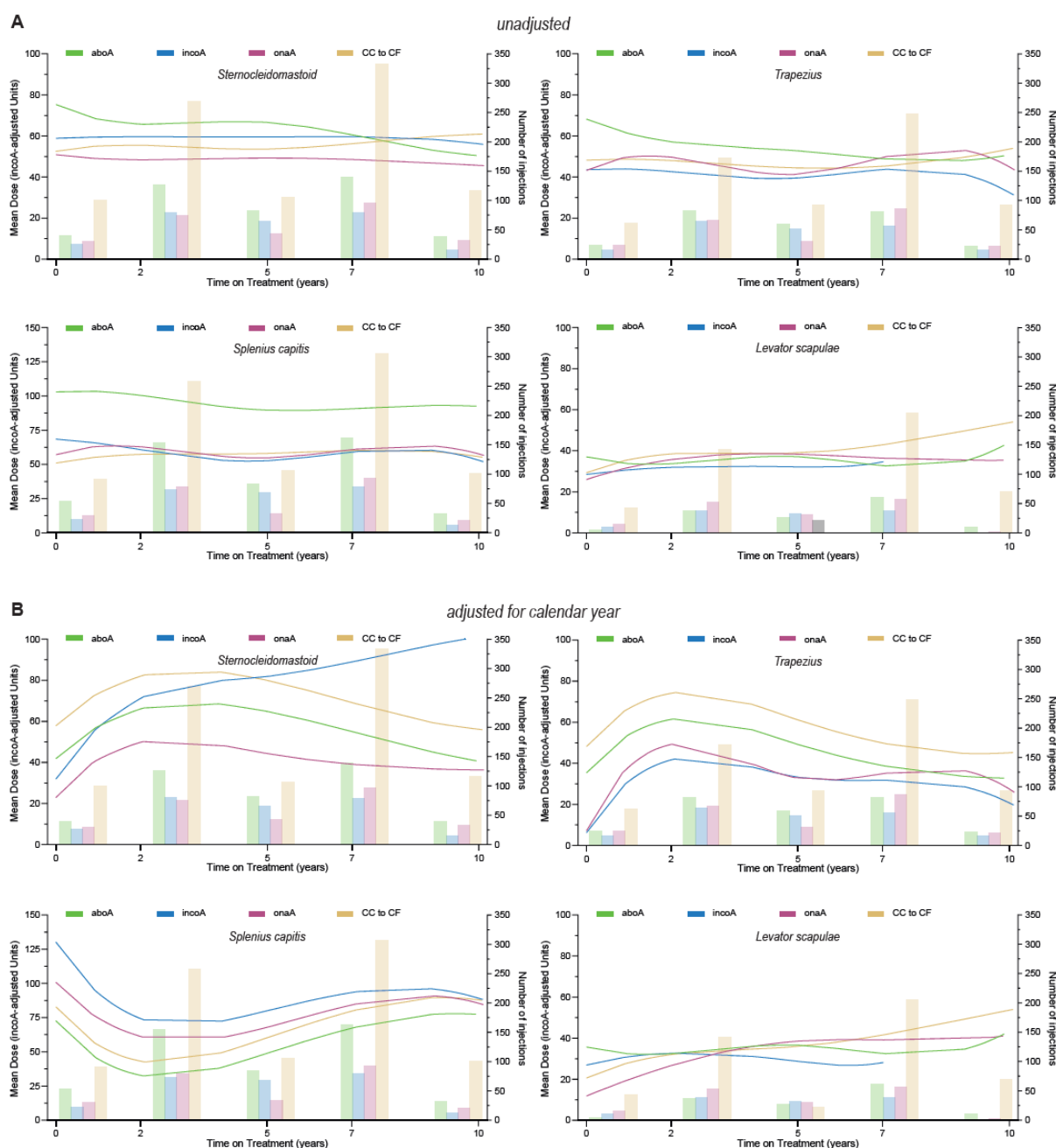


Figure 2. Linear regression model of muscle-specific dose development over time

Linear regression model of dose (left y-axis), unadjusted (A), or accounting for calendar year as covariate (B). “n” represents over number of visits in which the muscle was injected. Bars show number of injections (right y-axis). Dose adjusted to incoA units according to reported conversion ratio. Variability measures (SD/SEM) are not shown for trajectory plots, as lines represent model-based estimates rather than observed means; dispersion of observed data is provided in Table 1. Abbreviations: aboA, abobotulinumtoxinA; incoA, incobotulinumtoxinA; onaA, onabotulinumtoxinA; CC, complex-containing; CF, complex-free.

Patient Global Impression of Change

Figure 3A shows PGIC scores across treatment years. All groups demonstrated consistently positive PGIC scores throughout follow-up, indicating high patient-reported improvement. Mean scores increased from Year 2 to Year 7 in all groups. In the CC group, PGIC decreased between Year 7 and Year 10 (1.90 to 1.41), whereas values in the CC to CF group remained elevated at later timepoints. Interpretation of CF at Year 10 is limited due to the small sample size.

Across timepoints, PGIC scores were numerically higher in the CF group compared with the CC group at Years 2, 5, and 7 (1.79, 1.97, and 2.27 vs 1.63, 1.85, and 1.90). The CC to CF group showed the

highest PGIC scores at all timepoints, with significant differences compared with the monotherapy groups at several visits (Figure 3A). The proportion of patients achieving clinically meaningful improvement exceeded 90% at all timepoints across formulations (Supplementary Table 3).

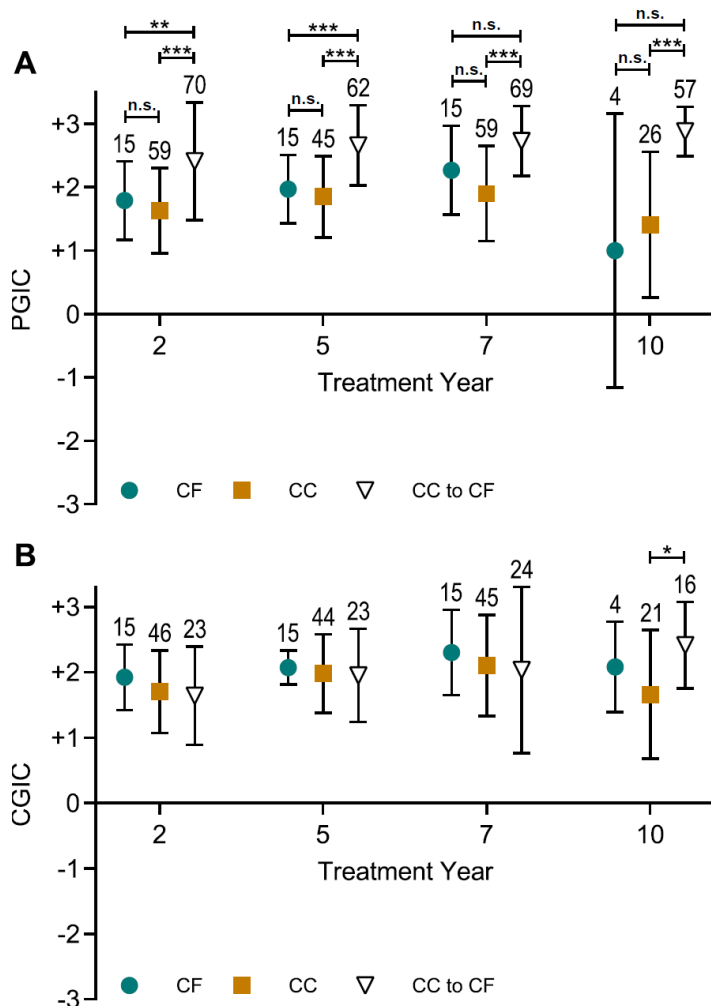
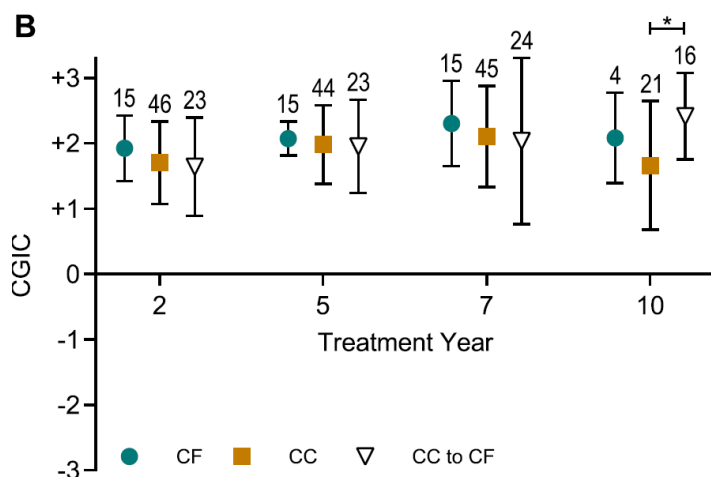


Figure 3. Patient and Clinical Global Impression of Change

PGIC (A) and CGIC (B) for each group (CF, CC, CC to CF) at years 2, 5, 7, and 10. Number of values per year above error bars (Standard Deviation). Significance levels: $p > 0.05$ (n.s.), $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***). Differences in GCIC were not significant except between CC and CC to CF in year 10 (*). Abbreviations: PGIC, Patient Global Impression of Change; CGIC, Clinician Global Impression of Change; CF, complex-free; CC, complex-containing; n.s., not significant.



Clinician Global Impression of Change

Figure 3B shows CGIC scores across treatment years. All groups demonstrated consistently positive CGIC scores, similar to PGIC, although differences between groups were smaller. CGIC scores in the CC to CF group were not consistently higher than in the monotherapy groups at earlier timepoints.

No statistically significant differences between groups were observed at Years 2, 5, and 7. At Year 10, CGIC scores were higher in the CC to CF group compared with the CC group (2.41 vs 1.66, $p < 0.05$).

The proportion of patients achieving clinically meaningful improvement remained high at all timepoints across formulations (Supplementary Table 4).

Discussion

Overview of main findings

This analysis provides a detailed description of long-term dose development and injection patterns in CD and their relationship to patient- and clinician-reported global improvement. Three main findings emerge: first, dose and muscle selection evolve over time in a heterogeneous but structured manner; second, dose differences between formulations are most pronounced in larger muscles; and third, these dynamic treatment adaptations are accompanied by consistently high patient- and clinician-reported improvement.

Muscle-specific dose patterns and clinical targeting

Dose development was not uniform across muscles but followed muscle-specific patterns. Frequently injected muscles such as the splenius capitis, sternocleidomastoid, and trapezius accounted for the highest mean doses, whereas smaller or less frequently targeted muscles contributed less to overall dosing. Notably, the obliquus capitis inferior, despite its small anatomical size, received doses comparable to larger muscles such as the levator scapulae. This observation shows that dose allocation is not solely determined by muscle size but also reflects functional relevance and clinical targeting, consistent with previous reports highlighting the importance of individualized injection strategies in CD [9], [11].

Formulation differences in dose distribution

Differences between BoNT-A formulations were most evident in larger muscles, particularly the splenius capitis, where higher doses were consistently observed for aboA compared with incoA and onaA. In contrast, doses were similar across formulations in muscles such as the levator scapulae. These findings are consistent with known variability in conversion ratios between aboA and other formulations and likely reflect differences in clinical dosing practice rather than direct pharmacological equivalence [7], [8]. At the same time, trajectories across formulations were largely parallel within each muscle, indicating that relative dose adjustments over time followed similar patterns despite differences in absolute dose levels.

Influence of treatment era and clinical practice patterns

An important methodological observation of this analysis is the impact of calendar-year adjustment on estimated dose trajectories. While unadjusted analyses suggested relatively stable differences between formulations, adjustment for treatment year altered both trajectory shape and relative positioning in several muscles. This indicates that temporal factors, such as evolving clinical practice patterns or dosing strategies, may substantially influence observed dose patterns in long-term observational datasets. Interpretation of later timepoints, particularly Year 10, is limited due to reduced data availability and incomplete follow-up by design. Previous long-term observational studies have described dose development over time and noted variability related to treatment strategy and cohort composition [1], [5], [6], [12], [13], [14], [15]. To our knowledge, calendar-year adjustment of muscle-specific long-term dose trajectories has not been systematically reported in CD cohorts.

Stability of global improvement and treatment adaptation

An important finding of this analysis is the stability of patient- and clinician-reported global improvement despite ongoing changes in dose and injection patterns. PGIC and CGIC scores remained consistently positive across treatment years, with high rates of clinically meaningful improvement at all timepoints. These findings are consistent with long-term observational studies demonstrating sustained treatment benefit with BoNT-A in CD [1], [9]. Notably, higher PGIC scores were observed in patients who switched from CC to CF formulations, suggesting that treatment adaptation, including formulation changes, may contribute to maintained or improved perceived benefit. In the context of previously reported challenges in such patient populations, including reduced treatment response or suspected secondary non-response, these findings support the interpretation that such issues may be related to the clinical condition prior to switching or the underlying reasons for switching, rather than resulting from the switch itself [16], [17], [18], [19].

Dose-efficacy relationship and long-term treatment adaptation

Taken together, these findings indicate that long-term management of CD with BoNT-A is characterized by continuous adjustment of dose and muscle selection while maintaining stable global impressions of treatment effect. In this context, dose and efficacy should not be considered in isolation. The dose-effect parameter (DEff), which was introduced in RELY-CD, reflects the balance between administered dose per muscle and clinical benefit, and changes in dose without corresponding loss of perceived improvement do not necessarily indicate reduced treatment effectiveness [20]. Rather, iterative adjustments of dose and injection patterns appear to represent appropriate clinical responses to evolving disease patterns and treatment needs, supporting the concept of individualized treatment optimization over time, although this balance may be disrupted in situations such as rapid disease progression or immunogenicity-associated secondary treatment failure [16], [20], [21].

Conclusion

In conclusion, BoNT-A treatment in CD over up to 10 years is characterized by dynamic, muscle-specific adaptation of dose and injection patterns. Despite this variability, sustained clinically meaningful improvement in global impression scores indicates effective and appropriate treatment adjustment in routine care. These findings suggest that physicians successfully adapt treatment to evolving disease patterns while maintaining patient-perceived benefit. Furthermore, the results highlight the importance of evaluating dose and efficacy jointly. Integrated measures such as the dose–effect parameter (DEff) provide a more appropriate framework for interpreting longitudinal treatment response and distinguishing adaptive dose changes from potential treatment failure.

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Ethical Compliance Statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Medical Faculty of the University of Düsseldorf, Germany (protocol code 2022-2205, approved 30 January 2023); the Reference Ethics Committee of the Hospital Universitario Clínico San Carlos, Spain (protocol code 23/121-O_M_NoSP, approved 8 March 2023); and the Ethics Committee of the Medical University of Lublin, Poland (protocol code 60/2023/KB/IX, approved 25 April 2023). Written informed consent was obtained from all participants in Germany and Poland. In Spain, patient consent was waived in accordance with the exemption for pseudonymized retrospective clinical studies described in Royal Decree 957/2020 of 3 November. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines.

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Supplementary Tables

Supplementary Table 1. Mean dose per muscle and timepoint

Muscle	Baseline		Timepoint			
	Mean	SD	Year 2		Year 7	
	Mean	SD	Mean	SD	Mean	SD
Levator scapulae	33.34	13.42	38.45	13.72	41.20	16.02
Obliquus capitis inferior	31.25	12.50	41.30	13.00	47.90	16.02
Scalenes	27.64	13.42	31.40	14.00	30.10	11.83
Semispinalis capitis	48.18	27.50	48.00	20.70	52.50	26.30
Semispinalis cervicis	35.28	12.66	43.80	17.30	39.90	19.97
Splenius capitis	73.00	50.30	77.00	41.50	85.50	50.95
Splenius cervicis	45.70	22.30	50.10	21.70	51.70	29.37
Sternocleidomastoid	61.30	31.20	63.00	30.90	65.90	34.10
Trapezius	58.00	29.80	58.50	33.20	60.90	35.38

Doses in incobotulinumtoxinA-adjusted Units. Means per timepoint of all recorded injections across formulations. Abbreviation: SD, standard deviation.

Supplementary Table 2. Change in mean dose between years 2 and 7

Muscle	Change Year 7 from 2, Units (%)			
	aboA	incoA	onaA	CC to CF
Levator scapulae	+0.3 (0.9%)	+1.3 (3.9%)	+6.5 (21.3%)	+5.7 (15.4%)
Scalene muscles	-11.6 (-26.9%)	-2.4 (-9.5%)	-0.5 (-1.8%)	+8.9 (50.9%)
Semispinalis capitis	-10.6 (-17.2%)	+9.5 (27.9%)	+9.0 (24.1%)	+1.3 (2.8%)
Splenius capitis	-16.5 (-14.8%)	-7.1 (-10.5%)	-2.9 (-4.3%)	+7.7 (13.5%)
Sternocleidomastoid	-8.8 (-12.6%)	+1.8 (2.8%)	-4.2 (-8.0%)	-1.5 (-2.7%)
Trapezius	-2.9 (-5.2%)	+1.3 (3.0%)	-3.5 (-7.1%)	-5.4 (-11.1%)

Doses in incoA-adjusted Units; the conversion ratios as reported by centers were used. Abbreviations: aboA, abobotulinumtoxinA; incoA, incobotulinumtoxinA; onaA, onabotulinumtoxinA; CC, complex-containing; CF, complex-free.

Supplementary Table 3. Clinically meaningful change in PGIC

Treatment Year	Clinically Meaningful Change, n (%)		
	CF	CC	CC to CF
2	14 (93.3)	45 (97.8)	14 (87.5)
5	15 (100.0)	43 (97.7)	16 (100.0)
7	15 (100.0)	42 (93.3)	15 (93.8)
10	4 (100.0)	19 (90.5)	11 (100.0)

Clinically meaningful change defined as ≥ 1 improvement from "no change" on 7-point scale, rated by the patient at reinjection. Abbreviations: PGIC, Patient Global Impression of Change; CF, complex-free; CC, complex-containing; n, number of visits with PGIC rating.

Supplementary Table 4. Clinically meaningful change in CGIC

Treatment Year	Clinically Meaningful Change, n (%)		
	CF	CC	CC to CF
2	14 (93.3)	57 (96.6)	53 (93.0)
5	14 (93.3)	43 (95.6)	51 (100.0)
7	15 (100.0)	54 (91.5)	56 (100.0)
10	3 (75.0)	22 (84.6)	48 (100.0)

Clinically meaningful change defined as ≥ 1 improvement from "no change" on 7-point scale, rated by the clinician at reinjection. Abbreviations: CGIC, Clinician Global Impression of Change; CF, complex-free; CC, complex-containing; n, number of visits with CGIC rating.

13 DISCUSSION

13.1 INTEGRATION OF THE RELY-CD PUBLICATION FRAMEWORK

The present thesis investigated long-term treatment response and treatment longevity of BoNT/A therapy in CD using a stepwise real-world evidence framework. The overall objective was not limited to describing whether BoNT/A remains effective in CD, since short-term and medium-term efficacy are well established. Instead, the central aim was to develop and apply a clinically meaningful approach for evaluating long-term treatment dynamics under routine conditions, where dose, formulation exposure, muscle selection, injection intervals, and outcome documentation change over time. This objective reflects the everyday reality of CD management: treatment is repeated over many years, injection plans are individualized, and reduced responsiveness rarely appears as a single discrete event. Rather, it may become visible gradually through changing dose requirements, reduced peak effect, shortened duration of benefit, or the need to adapt muscles and formulations.

The five publications and manuscripts included in this thesis form a sequential framework. The two feasibility studies first demonstrated that specialized CD centers routinely document several clinically relevant treatment parameters, but also that documentation is heterogeneous across centers and countries (Waeschle, Stark and Albrecht, 2022; Waeschle *et al.*, 2024). These findings justified the need for a retrospective real-world design that could use data elements commonly found in routine practice rather than relying on one universally applied rating scale. The protocol and baseline manuscript then translated these feasibility findings into the RELY-CD study design and introduced DEff as a structured dose-effect parameter (Waeschle *et al.*, 2025). The main RELY-CD manuscript applied this concept to compare long-term dose-effect development across formulation groups, while the dose-development manuscript examined the underlying muscle-specific treatment patterns and global impressions of change. Together, these studies provide complementary perspectives on the same clinical problem: whether long-term BoNT/A treatment remains stable and how potential deterioration can be recognized in routine care.

The most important conceptual contribution of this thesis is therefore the integration of dose and effect. In CD, dose alone is difficult to interpret because dose escalation may represent either successful treatment optimization or reduced responsiveness. Conversely, effect alone is incomplete because stable clinical scores may be achieved only through increasing dose or broader muscle recruitment. DEff addresses this ambiguity by describing treatment response as a relationship between dose development and clinical effect. This approach does not replace clinical judgment and does not diagnose antibody-mediated resistance. However, it

provides a structured marker of long-term treatment dynamics that can be applied to heterogeneous real-world records.

13.2 FEASIBILITY OF REAL-WORLD LONG-TERM ASSESSMENT IN CERVICAL DYSTONIA

The feasibility studies are central to the thesis because they establish the methodological basis for RELY-CD. They showed that many centers caring for CD patients record dose, efficacy, duration of effect, and safety or tolerability, but that quality-of-life instruments and standardized scales are used inconsistently. This finding is clinically unsurprising but methodologically important. CD treatment is individualized and often occurs in specialized movement-disorder clinics where treatment decisions are based on the physician's longitudinal knowledge of the patient, the patient's reported benefit, and the observed effect of previous injection cycles. Such documentation is highly valuable clinically, but it does not naturally conform to the structure of a controlled trial.

This heterogeneity creates a tension for retrospective research. On one hand, routine records provide access to long treatment periods that would be difficult, expensive, or unrealistic to reproduce prospectively. On the other hand, the lack of standardized assessments limits direct comparability across centers. The RELY-CD framework addresses this tension by using within-patient longitudinal comparisons and by transforming different clinical scales into a standardized metric. This is a pragmatic solution, but it also defines the boundaries of interpretation. RELY-CD can detect unfavorable long-term dose-effect patterns within the available clinical documentation, but it cannot fully harmonize all differences in scale sensitivity, timing of assessment, local documentation habits, or clinical decision-making.

The feasibility work also highlights a broader issue in CD research. Many clinically important outcomes, such as duration of effect, waning before reinjection, pain recurrence, and patient satisfaction, are not consistently captured by traditional motor severity scales. Patient perspectives indicate that end-of-cycle symptom recurrence and the wish for more "good days" are highly relevant aspects of treatment burden (Poliziani, Koch and Liu, 2016). Similarly, treatment satisfaction may vary across the injection cycle even when patients are generally satisfied with BoNT therapy (Leplow *et al.*, 2022; Erro *et al.*, 2023). These observations support the need for long-term data structures that capture more than peak motor improvement. In this respect, RELY-CD contributes to the field by showing that routinely documented dose and effect data can be used to investigate long-term treatment stability, while also demonstrating the need for more systematic prospective documentation in future studies.

13.3 DEff AS A MARKER OF LONG-TERM TREATMENT DYNAMICS

DEff was developed to capture a clinically familiar but methodologically difficult phenomenon: worsening of the dose-effect relationship over time. The parameter combines a dose coefficient and an effect coefficient, comparing later treatment years with a post-optimization reference year. This is clinically reasonable because the initial treatment period in CD often involves dose finding, muscle selection, and adjustment of injection strategy (Albanese *et al.*, 2015; Contarino *et al.*, 2017; Castagna and Albanese, 2019). A comparison with an early but already optimized reference period may therefore better reflect long-term treatment stability than a comparison with the first ever injection cycle.

The main RELY-CD analysis showed that most patients maintained stable or improved DEff values, supporting the general conclusion that BoNT/A treatment remains beneficial for many patients over long periods. This finding is consistent with previous long-term cohorts and reviews showing sustained efficacy and acceptable safety of BoNT treatment in CD over many years (Haussermann *et al.*, 2004; Bentivoglio *et al.*, 2017; Jochim *et al.*, 2019; Colosimo *et al.*, 2020; Marsili *et al.*, 2021; Petracca *et al.*, 2023). However, the presence of a subgroup with clinically meaningful DEff worsening emphasizes that long-term treatment is not uniformly stable. At treatment year 7, clinically meaningful worsening defined as $DEff > 1.2$ was observed in 11.1% of complex-free monotherapy patients, 24.2% of complex-containing monotherapy patients, and 24.8% of switcher patients (Wäschle *et al.*, 2026). These proportions should not be interpreted as rates of immunogenic secondary treatment failure. Rather, they describe the proportion of patients in whom the relationship between administered dose and documented clinical effect became less favorable.

This distinction is essential. DEff is intentionally broader than antibody-mediated secondary treatment failure. It may capture immunogenicity, but also disease progression, spread or change of dystonic pattern, suboptimal muscle targeting, changes in injection technique, altered expectations, comorbidities, psychological factors, or documentation variability. The observation that $DEff > 1.2$ occurred more often than expected rates of neutralizing antibody positivity or immunogenic secondary treatment failure is therefore not a weakness of the parameter; it reflects its broader clinical scope. DEff should be understood as a signal of long-term treatment dynamics that requires interpretation, not as a diagnostic endpoint.

At the same time, the 20% threshold used to define clinically meaningful worsening should be interpreted cautiously. The threshold was predefined in the RELY-CD protocol as a pragmatic marker of clinically meaningful dose-effect worsening and was supported indirectly by published work on minimal clinically important change in cervical dystonia and dystonia rating scales, together with clinical experience (Dashtipour *et al.*, 2019; Pintér, Janszky and Kovács, 2020). However, these references support the broader concept of clinically meaningful change

in dystonia outcomes rather than validating DEff specifically. The clustering of values around stable ranges supports the practical use of the threshold, and the threshold is clinically plausible, but it was not derived from formal sensitivity and specificity testing against an external gold standard such as antibody testing, blinded clinical reassessment, or prospective patient-reported outcomes. Future validation should examine whether DEff thresholds predict clinically meaningful outcomes such as patient dissatisfaction, shortened duration of benefit, physician decision to switch formulation, or laboratory-confirmed neutralizing antibodies.

13.4 FORMULATION-RELATED FINDINGS AND IMMUNOGENICITY

INTERPRETATION

The formulation-related findings of RELY-CD are clinically relevant but require careful interpretation. Patients treated with complex-free incoA monotherapy had the lowest observed proportion of DEff > 1.2 at year 7, whereas complex-containing monotherapy patients and switchers showed numerically higher proportions of clinically meaningful worsening (Wäschle *et al.*, 2026). This pattern is consistent with the biological rationale that foreign protein exposure, formulation composition, dose, and repeated treatment may contribute to immunogenicity risk. It is also compatible with clinical and systematic evidence suggesting lower rates of neutralizing antibodies or immunogenic secondary treatment failure with incoA compared with onaA or aboA in some study settings (Albrecht *et al.*, 2019; Walter *et al.*, 2020, 2025).

However, the present data does not establish a causal immunogenicity mechanism. RELY-CD did not include systematic antibody testing, and the primary analysis was descriptive because the planned monotherapy sample size was not reached (Waeschle *et al.*, 2025; Wäschle *et al.*, 2026). Moreover, formulation groups in real-world datasets are not randomized. Patients treated with different products may differ in baseline characteristics, local practice environment, physician preference, dose-conversion habits, prior treatment exposure, and probability of switching. Differences in baseline characteristics were not observed, but the impact of the other potential covariates remains unknown (Waeschle *et al.*, 2025). Switcher patients are particularly difficult to interpret because switching may occur for insufficient response, tolerability, availability, reimbursement, or physician preference (Marion *et al.*, 2016; Hefter *et al.*, 2022). Therefore, the higher proportion of DEff worsening among switchers should not be interpreted as evidence that switching itself causes worse outcomes. The trajectory analysis indicates that effect had already worsened before the switch and improved thereafter, suggesting that switching was more likely a response to pre-existing treatment concerns than the cause of deterioration. This interpretation is in line with previous evidence from Hefter *et al.*, who reported clinical improvement after switching to incoA in cervical dystonia patients with secondary treatment failure (Hefter *et al.*, 2022). Thus, the switcher

group may represent a clinically enriched population rather than a group in which the switch itself explains the unfavorable dose-effect pattern.

The thesis therefore supports a balanced conclusion. The RELY-CD findings are directionally consistent with the hypothesis that formulation composition and immunogenicity risk matter in long-term BoNT/A treatment, but they do not prove antibody-mediated resistance. This distinction is important both scientifically and clinically. Overstating the immunogenicity interpretation would ignore alternative explanations for reduced dose-effect performance. Understating it would disregard the substantial mechanistic, preclinical, and clinical literature showing that neutralizing antibodies can impair BoNT/A treatment in a subset of patients. The most evidence-based interpretation is that immunogenicity remains a plausible and clinically relevant contributor to long-term deterioration, but one that should be evaluated alongside other causes of changing treatment response. *Figure 8* summarizes the main factors that need to be considered when a DEff worsening is observed.

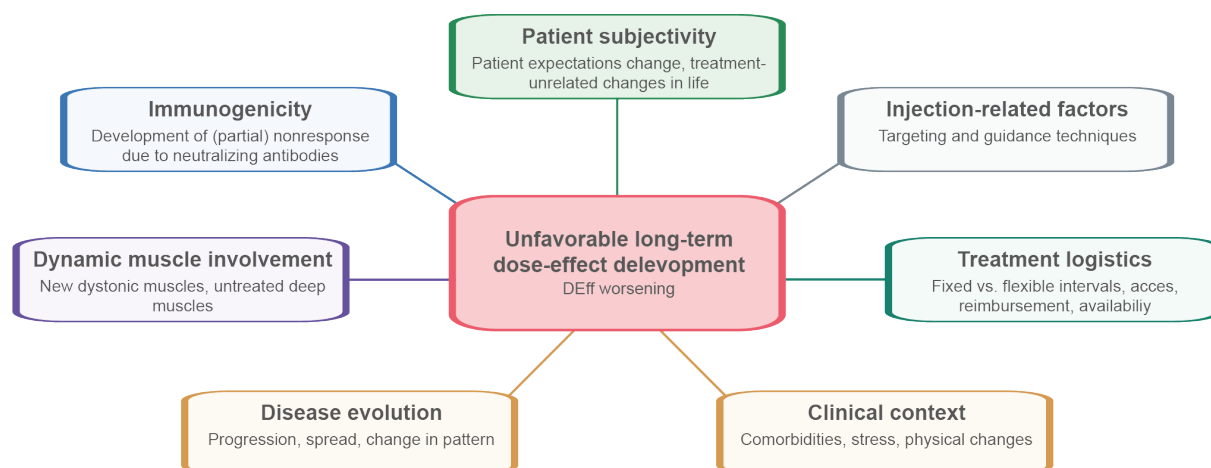


Figure 8. Multifactorial interpretation of DEff worsening

DEff worsening indicates an unfavorable long-term dose-effect relationship, but it does not identify a single underlying mechanism. Potential contributors include immunogenicity, patient subjectivity, injection-related factors, treatment logistics, clinical context, disease evolution, and dynamic muscle involvement. Clinical interpretation is therefore required before attributing DEff worsening to antibody-mediated resistance. Abbreviation: DEff, dose-effect parameter.

The preclinical evidence summarized in this thesis supports this cautious interpretation. BoNT/A contains multiple antigenic regions on the LC, HN, and Hc domains, and neutralizing antibodies can target more than one functional domain (Mullaney, Pallavicini and Marks, 2001; Wu *et al.*, 2001; Dolimbek *et al.*, 2007; Atassi *et al.*, 2011; Garcia-Rodriguez *et al.*, 2011). Accessory proteins found in the complex-containing formulations (aboA and onaA), such as HA-33/Hn-33 are antigenic in experimental systems and may contribute to binding-antibody responses against BoNT/A complexes (Kukreja *et al.*, 2009; Bryant, Cai and Singh, 2013; Sayadmanesh *et al.*, 2013). Animal repeated-dose experiments further support formulation-dependent immunogenicity under certain conditions (Torii *et al.*, 2014). Nevertheless, binding-antibody data and animal experiments cannot be directly equated with clinical secondary non-

response after therapeutic intramuscular injection. This reinforces the need to treat DEff as a clinical signal rather than a surrogate antibody assay.

13.5 LONG-TERM STABILITY, DOSE ADAPTATION AND MUSCLE-SPECIFIC INTERPRETATION

The dose-development manuscript adds an important layer to the interpretation of the main DEff findings. It shows that long-term BoNT/A treatment in CD is not only a matter of total dose escalation or formulation exposure, but also of muscle-specific treatment adaptation. Sternocleidomastoid and splenius capitis were the most frequently injected muscles, which is consistent with the common clinical relevance of rotational and lateral components in CD and previously reported frequencies (Jost *et al.*, 2021). At the same time, doses per muscle remained mostly stable over long-term treatment, and Global Impression of Change Scale (GICS) score means indicated stable improvement across treatment years. These findings suggest that many patients can maintain clinical benefit through repeated individualized adjustments without progressive global dose escalation.

This is clinically important because total dose trajectories can be misleading. A stable total dose may hide substantial redistribution between muscles, while a higher total dose may reflect the addition of clinically relevant muscles rather than declining toxin responsiveness. Conversely, a stable or modestly increasing dose does not exclude waning effect if clinical outcomes worsen. Muscle-specific dose analysis therefore provides an intermediate layer between the clinical phenotype and global treatment outcomes. It helps clarify whether long-term changes reflect broad escalation, selective adaptation, changes in muscle pattern, or stable individualized treatment.

The recent literature further supports the importance of muscle targeting and guidance. Evidence-based recommendations emphasize individualized muscle selection, appropriate dosing, and the use of guidance techniques when clinically indicated (Contarino *et al.*, 2017; Castagna and Albanese, 2019; Llorente Peris, Miranda Bautista and Menéndez Fernández-Miranda, 2025). Recent data on ultrasound-guided treatment suggest that the guidance method can substantially influence injection strategy, including the number of injected muscles, total dose, and dose per muscle (Jost *et al.*, 2021; Kreisler *et al.*, 2024). This aligns with the RELY-CD perspective that dose cannot be interpreted independently from injection pattern and targeting strategy. In routine practice, improved recognition of deep or previously undertreated muscles may increase the number of injected muscles while improving the rationality of treatment. Such changes should be interpreted as clinical optimization rather than automatically as loss of response.

The dose manuscript also strengthens the interpretation that long-term BoNT/A treatment can remain clinically stable despite individual variability. Stable GICS means across treatment years suggest sustained perceived benefit at the group level. However, global impression scales have limitations. They are pragmatic and widely used, but they may be less sensitive to specific changes in pain, tremor, disability, quality of life, or end-of-cycle waning. Therefore, stable GICS findings should be interpreted as supportive evidence of durable benefit, not as proof that all clinically relevant dimensions remained unchanged.

13.6 RELATIONSHIP TO PREVIOUS LONG-TERM EVIDENCE

The RELY-CD findings extend previous long-term CD literature in several ways. Earlier long-term studies showed that BoNT/A treatment can remain effective and safe over many years, including observational cohorts with follow-up periods extending over one or two decades (Haussermann *et al.*, 2004; Jochim *et al.*, 2019; Yahalom *et al.*, 2021; Petracca *et al.*, 2023). Prospective observational studies such as INTEREST IN CD2 demonstrated sustained improvement and patient satisfaction over several years in routine care (Colosimo *et al.*, 2020). Reviews have consistently concluded that BoNT treatment is an effective long-term therapy for CD, although the available evidence is heterogeneous with respect to serotype, formulation, dose, outcome measures, and follow-up duration (Marsili *et al.*, 2021).

RELY-CD does not contradict this literature. On the contrary, the finding that most patients maintained stable or improved DEff supports the overall durability of BoNT/A treatment. The added value of RELY-CD lies in shifting the analytical focus from efficacy alone to treatment dynamics. Existing studies often report total dose, intervals, safety, satisfaction, or clinical severity, but they less often integrate dose adaptation with clinical effect across long follow-up. RELY-CD addresses this gap by analyzing whether the amount of treatment required to maintain effect changes over time.

The thesis also connects long-term efficacy literature with immunogenicity literature. Studies on neutralizing antibodies and secondary treatment failure demonstrate that antibody-mediated reduced response exists and is clinically relevant in a subset of patients (Hefter, Spiess and Rosenthal, 2014; Hefter, Rosenthal and Moll, 2016; Albrecht *et al.*, 2019; Hefter *et al.*, 2019; Walter *et al.*, 2020, 2025). However, these studies often depend on specialized antibody assays, selected populations, or suspected non-response cohorts. In contrast, efficacy studies often include broader long-term cohorts but may not systematically investigate immunogenicity. RELY-CD sits between these approaches: it does not measure antibodies, but it provides a structured real-world measure of reduced dose-effect performance that may include immunogenic and non-immunogenic mechanisms.

The importance of duration of effect also deserves emphasis. A recent scoping review noted that BoNT treatment in CD usually requires repeated injections every three to four months and that maintaining efficacy throughout the injection cycle is clinically important (Castagna, Jinnah and Albanese, 2024). This is highly relevant to RELY-CD because dose-effect deterioration may reflect not only worse peak response but also shorter duration of benefit or greater end-of-cycle symptom recurrence. If future datasets systematically capture peak effect, trough effect, and duration of benefit, DEff or related parameters could be refined to distinguish these dimensions more precisely.

13.7 CLINICAL IMPLICATIONS

The main clinical implication of this thesis is that long-term BoNT/A management in CD should be evaluated dynamically. A single dose value, a single severity score, or a single patient report cannot adequately describe treatment stability over years. Clinicians should interpret dose changes in relation to clinical effect, duration of benefit, muscle selection, adverse effects, and patient goals. This is already how experienced clinicians often practice, but RELY-CD provides a structured framework for analyzing this process.

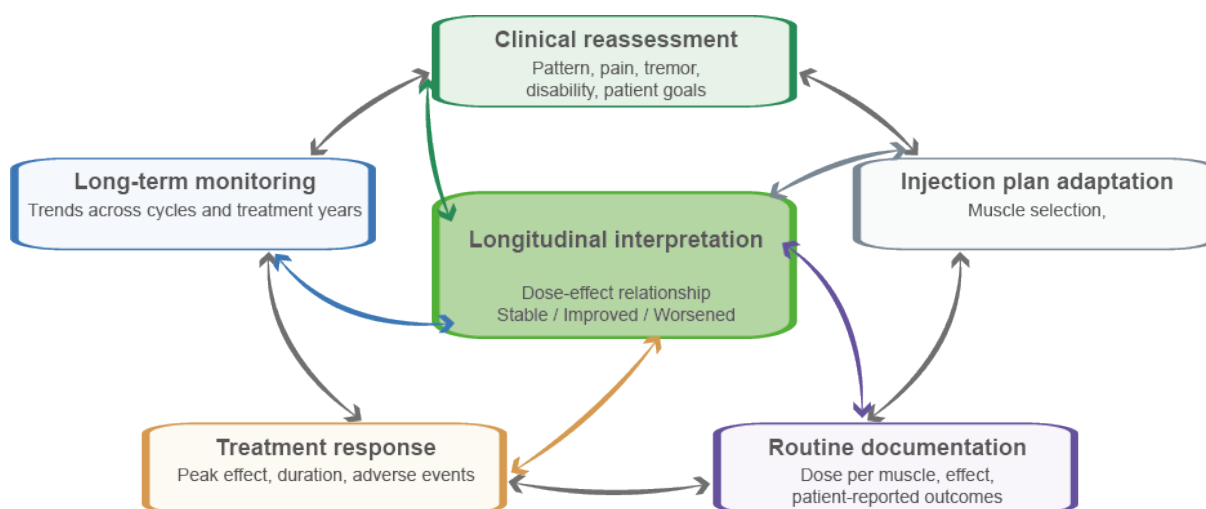


Figure 9. Dynamic framework for long-term BoNT/A management in cervical dystonia

Long-term BoNT/A treatment in cervical dystonia involves repeated clinical reassessment, individualized injection-plan adaptation, documentation of treatment response, and longitudinal interpretation of dose-effect dynamics. If DEff worsens, the clinical review should consider disease evolution, targeting, guidance, dose redistribution, formulation history, duration of effect, patient goals, comorbidity, and possible immunogenicity. Abbreviations: BoNT/A, botulinum neurotoxin type A; DEff, dose-effect parameter.

The findings support several practical considerations. First, stable long-term benefit is achievable for many patients. The majority of patients in RELY-CD did not show clinically meaningful worsening by DEff (Wäschle *et al.*, 2026). Second, a subgroup of patients develops an unfavorable dose-effect relationship, and this should prompt systematic clinical review, illustrated in Figure 9. Such review should include assessment of disease pattern evolution,

muscle targeting, guidance techniques, adherence to injection intervals, dose distribution, patient expectations, pain and tremor control, comorbidities, and potential immunogenicity. Third, formulation history may be relevant when interpreting long-term response, particularly in patients requiring high cumulative doses or showing signs of reduced benefit (Albrecht *et al.*, 2019; Walter *et al.*, 2020). However, formulation choice should be individualized and interpreted within the full clinical context rather than reduced to a single immunogenicity argument.

The thesis also emphasizes the need for regular routine documentation. If dose per muscle, efficacy scale, duration of effect, adverse events, formulation exposure, and reason for treatment changes were captured consistently, real-world datasets could support more precise monitoring of long-term response. Digital tools or structured electronic treatment records may improve this process by reducing reliance on patient recall and by capturing response across the injection cycle. Such developments would be particularly valuable in CD, where symptoms fluctuate and patient priorities may differ from clinician-rated motor severity.

13.8 METHODOLOGICAL IMPLICATIONS AND FUTURE VALIDATION

DEff should be regarded as a promising but still evolving methodological concept. Its strength lies in its pragmatic applicability to heterogeneous retrospective data. It uses information that is often available in routine records and converts it into a longitudinal marker of dose-effect stability. This makes it suitable for multicenter real-world research where perfectly standardized data collection is unavailable.

However, future validation is required. First, DEff should be tested prospectively against standardized clinical outcomes, patient-reported outcomes, and duration-of-effect measures. Second, its relationship with laboratory-confirmed neutralizing antibodies should be evaluated in cohorts where antibody testing is available. Third, different thresholds for clinically meaningful worsening should be examined to determine whether the current >20% threshold optimally identifies relevant deterioration. Fourth, the parameter should be tested in larger samples with sufficient power for formulation comparisons and with adjustment for confounding factors such as baseline severity, dose, treatment duration, injection interval, comorbidity, and center effects.

The handling of muscle changes also warrants further refinement. In RELY-CD, dose coefficients were calculated for muscles injected in both comparator years, while muscles added or discontinued were handled conservatively (Waeschle *et al.*, 2025). This approach reduces confounding by changing injection patterns but may also underrepresent clinically meaningful changes in muscle recruitment. Future versions of dose-effect methodology could incorporate additional measures such as number of injected muscles, addition of deep

muscles, changes in guidance technique, or redistribution indices. These refinements could help distinguish dose escalation due to broader pattern involvement from dose escalation within stable muscle patterns.

Finally, future research should integrate immunogenicity more directly. A prospective long-term study combining standardized clinical outcomes, structured dose/muscle documentation, patient-reported duration of effect, and periodic antibody testing would be ideal. Such a study would be logistically challenging, but it would help determine which components of DEff worsening are attributable to immunogenicity and which are explained by clinical or technical factors. Until such data are available, the interpretation of long-term dose-effect deterioration should remain cautious and multifactorial.

13.9 INTEGRATIVE INTERPRETATION OF THE FINDINGS

The findings of this thesis are best interpreted within a multifactorial model of long-term BoNT/A treatment in CD. Across the included studies, treatment stability did not appear to be determined by a single factor such as formulation, dose, muscle selection, or immunogenicity alone. Instead, the results suggest that long-term outcome reflects the interaction between repeated individualized treatment adaptation, available routine documentation, formulation exposure, patient-specific clinical course, and potential biological mechanisms of reduced responsiveness.

Within this model, the feasibility studies define the real-world data environment in which long-term treatment decisions are made and documented. They show that routine documentation is heterogeneous, but that key variables such as dose, efficacy, duration of effect, and safety or tolerability are sufficiently available to support structured retrospective analysis. The RELY-CD protocol then operationalized this information through DEff, allowing long-term response to be examined as a relationship between dose and effect rather than as either parameter alone. The main RELY-CD analysis applied this concept and identified a subgroup of patients with clinically meaningful worsening of dose-effect development, while also showing that most patients maintained stable or improved long-term treatment dynamics. The dose-development analysis added an important clinical layer by showing that muscle-specific dose patterns and global improvement must be considered when interpreting treatment stability.

14 CONCLUSION & OUTLOOK

Long-term BoNT/A treatment in cervical dystonia is generally durable, but clinically dynamic. In RELY-CD, most patients maintained stable or improved treatment response over long-term follow-up, while a clinically relevant subgroup showed worsening of the dose-effect relationship. This supports a nuanced view of long-term treatment: sustained benefit is

common, but it depends on repeated clinical reassessment, individualized dose adaptation, and careful interpretation of treatment response over time.

Dose and clinical effect should not be interpreted in isolation. Dose escalation may indicate reduced responsiveness, but it may also reflect appropriate optimization, changing muscle involvement, altered injection strategy, or improved recognition of the dystonic pattern. Conversely, stable dosing may be insufficient if the clinical effect declines. DEff provides a pragmatic way to describe these long-term dose-effect dynamics in real-world data, without replacing clinical judgment or functioning as a diagnostic test for antibody-mediated resistance.

Muscle-specific interpretation is essential. CD treatment is individualized at the level of target muscles and dose distribution, and stable global improvement may coexist with relevant changes in muscle-specific dosing. Long-term response therefore has to be understood as the result of repeated treatment adaptation rather than as the consequence of a fixed injection pattern.

The formulation-related findings suggest potential long-term differences between complex-free and complex-containing BoNT/A preparations. Numerically lower rates of clinically meaningful DEff worsening were observed under complex-free monotherapy than under complex-containing monotherapy or in switchers. This pattern is compatible with the biological and clinical relevance of immunogenicity, but it does not prove antibody-mediated secondary treatment failure, because systematic antibody testing was not part of RELY-CD. Formulation history should therefore be considered as one factor in long-term monitoring, alongside dose, effect, muscle selection, treatment interval, and the broader clinical context.

Future research should prospectively validate DEff against standardized clinical outcomes, patient-reported outcomes, duration-of-effect measures, and laboratory testing for neutralizing antibodies where feasible. More consistent routine documentation of dose per muscle, formulation exposure, injection interval, guidance technique, duration of effect, adverse events, and patient-reported outcomes would improve both clinical care and real-world research. Such structured long-term monitoring may help identify unfavorable treatment development earlier and support more individualized management of BoNT/A-treated cervical dystonia.

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Part of the RELY-CD project:

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