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Safety and pharmacodynamics of a novel recombinant botulinum toxin E (rBoNT-E): Results of a phase 1 study in healthy male subjects compared with abobotulinumtoxinA (Dysport®)

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ABSTRACT

Naturally occurring botulinum toxin (BoNT) serotypes have different pharmacological features of therapeutic and aesthetic interest. This phase 1, double-blind, placebo-controlled study (EudraCT: 2016–002609-20) assessed safety, tolerability and pharmacodynamics (PD) of the first recombinant BoNT serotype E (rBoNT-E) versus abobotulinumtoxinA (Dysport®), administered to extensor digitorum brevis (EDB) of healthy males. Subjects were randomised 3:1 (n = 28) to single ascending rBoNT-E (0.04–3.6 ng) doses or placebo. A further 24 subjects received abobotulinumtoxinA (20, 40, or 70 U) or placebo. PD were assessed using compound muscle action potential (CMAP) amplitude. Demographics were similar between groups. All rBoNT-E doses were well tolerated (no severe treatment-emergent adverse events [TEAEs], serious adverse events, or treatment-related toxicities). Most TEAEs were mild/moderate and treatment-unrelated. rBoNT-E had a faster onset of action (days 1–2 post-injection), greater peak effect (> 90% CMAP inhibition), and shorter duration of effect at highest tested doses versus abobotulinumtoxinA (onset of action ≤7 days post-injection; 70% maximal CMAP inhibition). rBoNT-E duration of effect was 2–7 weeks versus > 26 weeks for abobotulinumtoxinA. Dose-dependent effects were observed for magnitude and duration of EDB CMAP inhibition, plateauing at 0.9 and 3.6 ng. rBoNT-E demonstrated a good safety profile and a PD profile that may address unmet therapeutic and aesthetic patient needs.

1. Introduction

Botulinum neurotoxins (BoNTs) are naturally occurring bacterial proteins (produced by *Clostridium* bacteria) that, among other actions, weaken muscles by inhibiting neurotransmitter release at neuromuscular junctions. The effect of BoNT is not permanent and upon clearance of BoNT from the neuromuscular junction, muscles will regain function [1]. BoNTs are classified into seven serotypes (A–G) [2], which differ by up to 70% at the amino acid level and show little antibody cross-reactivity [3]. Thus far, the majority of BoNT products are BoNT serotype A (BoNT-A) and have been used across a wide range of therapeutic indications within adult and paediatric spasticity- (strabismus, blepharospasm, hemifacial spasm, cervical dystonia, axillary hyperhidrosis, chronic migraine, and neurogenic detrusor overactivity); BoNT serotype B is also used clinically for the treatment of cervical dystonia [1]. In addition, BoNT has numerous aesthetic uses, including the treatment of forehead wrinkles and other facial hyperkinetic movements, as well as glabellar, periorbital, and perioral lines [4].

New BoNT products, developed from other BoNT serotypes and having different pharmacological properties, are being investigated for clinical use. Recently, an Ipsen proprietary BoNT serotype E (BoNT-E), manufactured with recombinant technology (rBoNT-E) using the host *Escherichia coli* and containing genes encoding the natural sequence of BoNT-E, has been identified as a drug candidate [5]. Prior pre-clinical research using recombinant technology has been performed in various BoNT serotypes [6]; however, rBoNT-E is the first to reach clinical trials. Faster onset of action and shorter duration of effect, as well as a quick time to peak activity, have been reported in animal studies of rBoNT-E in comparison with established BoNT-A products, including abobotulinumtoxinA (Dysport®) [7,8]. This is likely to be due to differences between the exact mechanisms of action for the two different BoNT serotypes.

rBoNT-E and BoNT-A both bind to synaptic vesicle protein 2 (SV2) [7,8], while BoNT-A can bind to all three isoforms (SV2A, SV2B, and

Abbreviations: BoNT, botulinum toxin; CMAP, compound muscle action potential; rBoNT-E, recombinant BoNT serotype E

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SV2C) and is relatively insensitive to differences in SV2 glycosylation states, suggesting that glycosylation is not essential for BoNT-A binding [7–9]. However, rBoNT-E binds only to the glycosylated forms of SV2A and SV2B [7]. Both BoNTs are internalised via synaptic vesicles [7,10,11], translocate an active protease subunit, and cleave synaptosomal-associated protein 25 k Da (SNAP-25) [12]. The 3-dimensional orientation of the protease subunit in relation to the binding and translocation domain subunit differs between rBoNT-E and BoNT-A [13]. The shape of rBoNT-E has more "folded in" ends compared with BoNT-A [14], and this may contribute to a faster transition from binding and internalisation to translocation [15]. Furthermore, the target amino acid cleavage sites for rBoNT-E and BoNT-A are different [16]. Although both subtypes cleave SNAP25, rBoNT-E targets a site between arginine 180 and isoleucine 181 and BoNT-A targets a site between glutamine 197 and arginine 198, making them pharmacologically different and potentially affecting their clinical activity [16]. While the BoNT-A protease subunit remains inside intoxicated neurons for a long period of time, the rBoNT-E protease is cleared rapidly from the neuron [12]. Therefore, rBoNT-E could address new and different patient needs in therapeutic and aesthetic use, compared to those treated with BoNT-A.

This first-in-human (FIH) study collects preliminary safety, tolerability and pharmacodynamic (PD) information on rBoNT-E. This study aimed to determine the doses of rBoNT-E that yields similar levels of compound muscle action potential (CMAP) amplitude reduction as abobotulinumtoxinA following single intramuscular injection into the extensor digitorum brevis (EDB) muscle of healthy male subjects. This study also aimed to characterise the PD profile of rBoNT-E in terms of time to onset, time to maximal effect, and duration of effect on inhibition of the CMAP amplitude.

2. Subjects and methods

2.1. Subjects

Participants were healthy males (aged 18–49 years) with a body mass index (BMI) of 18–30 kg/m^2 who had not previously been treated with BoNT (any serotype) during the past 6 months. A baseline EDB CMAP total amplitude (peak-to-peak) ≥5 mV during electrophysiological examinations at screening and before study drug administration was required. Subjects who were not vasectomised and who had pregnant female partners or female partners of childbearing potential had to be willing to use condoms with spermicide throughout study participation and for 90 days after the administration of study drug (or until the end of study visit). Non-pregnant female partners of childbearing potential were also required to use highly effective contraceptive measures for the same time period. Subjects provided written informed consent prior to any study-related procedure and could withdraw (investigator/sponsor could withdraw subjects) at any time for any reason.

2.2. Study design

This was a randomised, double-blind, placebo-controlled study (EudraCT: 2016–002609-20), performed at a single study centre in the UK. The centre was accredited by the Medicines and Healthcare products Regulatory Agency. The study was conducted in two parts, which were run in parallel (Fig. 1).

Study cohorts and dose escalation were determined based on the percentage of the CMAP total amplitude relative to baseline (CMAP%), which was calculated as (CMAP total amplitude at the visit/CMAP total amplitude at baseline)x100, where baseline was defined as the average of CMAP values recorded at screening and day −1 (prior to dosing).

In part A, healthy male volunteers were randomised to receive rBoNT-E or placebo (injected into the EDB) in a ratio of 3:1 (three receiving rBoNT-E, one receiving placebo). Randomisation was performed by assigning each eligible subject a randomisation number and allocating them to a treatment cohort in chronological order. The first cohort included only four subjects. Once approximately 50% reduction of the EDB CMAP total amplitude (CMAP inhibition) at three consecutive time points (compared with baseline value) was reached in 2/4 subjects (i.e. 2/3 subjects treated with rBoNT-E), that same dose was then repeated in a further cohort of four subjects. This allows for rapid dose escalation if there are no safety signals and < 50% reduction in the CMAP PD response, but more cautious escalation once 50% reduction in the CMAP PD response is reached. Following this, doses were escalated in further cohorts of four to eight subjects. In each of these cohorts, six subjects received rBoNT-E and two received placebo. Dose escalation and recruitment stopped when the maximal effect was reached within two consecutive cohorts (plateau from at least 85% CMAP inhibition in the EDB CMAP compared with baseline at three consecutive time points in 4/6 subjects receiving rBoNT-E). Decision for dose escalation for a subsequent cohort was agreed by a data review committee upon review of safety and PD data from the previous cohort(s).

For comparative purposes, additional investigations were conducted with abobotulinumtoxinA in a different cohort of healthy male volunteers (part B). Twenty-four subjects (18 receiving abobotulinumtoxinA and six placebo) were randomised to receive a single intramuscular dose of abobotulinumtoxinA (20, 40, or 70 U) or placebo, resulting in a total of six subjects per group.

An independent ethics committee approved the protocol and it was performed in accordance with the Declaration of Helsinki, International Conference on Harmonisation guidelines on Good Clinical Practice, and with local routine medical practice.

2.3. Treatment

Subjects were randomised to receive a single intramuscular injection of either rBoNT-E (Ipsen Biopharm Ltd., Wrexham, UK) or placebo (part A), or abobotulinumtoxinA (Dysport, Ipsen Biopharm Ltd., Wrexham, UK) or placebo (part B) on a single occasion during the study (day 1). Injections were administered into the belly part of the EDB muscle of the right foot (0.2 ml single intramuscular injection), and ultrasound was used to localise the targeted EDB muscles and visualise the injections. Staff administered study treatment under medical supervision. Confinement up to day 4 was mandatory, but subjects could remain in the clinical research unit up to day 7.

Active doses of rBoNT-E were prepared with a solution of rBoNT-E at a label strength of 100 ng/ml (240 U/ml cell-based assay [17] units) diluted to reach the targeted dose/concentration, with a vehicle solution containing only the excipients of the rBoNT-E formulation. AbobotulinumtoxinA active doses were prepared with Dysport marketed lyophilisate 500 U (median lethal dose [LD_{50}] units) reconstituted and further diluted with a sterile sodium chloride (0.9%) solution to reach the target dose/concentration. Placebo was indistinguishable from rBoNT-E or abobotulinumtoxinA. In part A, the placebo contained only the excipients of rBoNT-E, whereas in part B, a sterile and preservative-free sodium chloride (0.9%) solution was used.

2.4. Endpoints, assessments and study duration

The primary endpoint was the safety and tolerability profile of single intramuscular ascending doses of rBoNT-E in healthy male subjects. Safety assessments included monitoring adverse events (AEs) and concomitant medications (potentially administered during the course of the study to resolve AEs), changes from baseline in clinical laboratory parameters, and physical examination findings (vital signs and electrocardiogram [ECG] recordings).

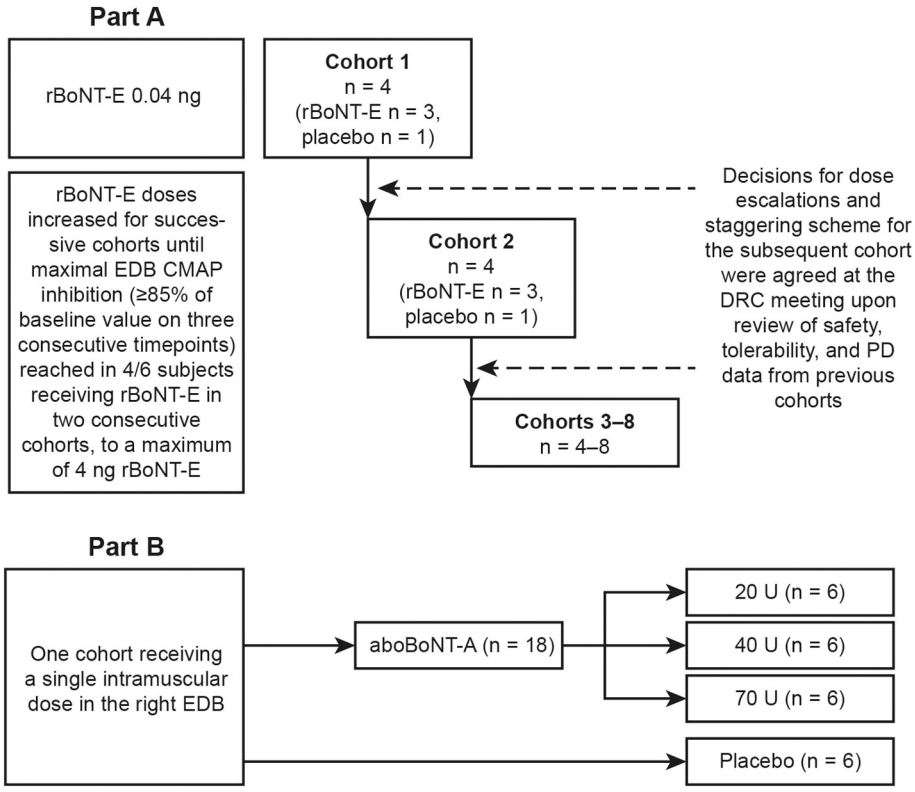

Fig. 1. Study design.
Note: parts A and B run in parallel.
*N = 4 (rBoNT-E *n* = 3, placebo *n* = 1) increasing to 8 (rBoNT-E *n* = 6, placebo *n* = 1) once approximately 50% inhibition of the EDB CMAP on three consecutive time points (when compared with the baseline value) was reached in two of three subjects receiving rBoNT-E.
CMAP, compound muscle action potential; DRC, data review committee, EDB, extensor digitorum brevis; *n*, number of subjects in the group; PD, pharmacodynamic.

Secondary endpoints included:

- Reductions in the stimulated EDB CMAP total amplitude (CMAP inhibition) at various time points post-injection (percentage relative to baseline);
- Dose-effect on amplitude of maximal effect (maximum inhibition reached);
- Time for a maximal effect of rBoNT-E on the EDB CMAP;
- Time to onset of action of rBoNT-E at inhibiting CMAP (first time point post-injection with a 15% inhibition of EDB CMAP total amplitude relative to baseline);
- Dose-effect on duration of effect (time between time to onset and time to recovery) of rBoNT-E on the stimulated EDB CMAP.

To assess time to recovery, the first time point (post-time to onset) where inhibition of EDB CMAP total amplitude returns to within 15% of the baseline value was recorded.

Exploratory endpoints included assessing potential local diffusion of rBoNT-E and abobotulinumtoxinA to adjacent muscles, abductor digiti quinti (ADQ), and abductor hallucis (AH), and exploring the production of putative anti-rBoNT-E antibodies. Changes in CMAP total amplitude in ADQ and AH following supramaximal electrical stimulation of the corresponding tibial nerve were assessed. Blood samples were taken for anti-rBoNT-E antibody assessment (testing for the presence of neutralising antibodies, using the electrochemiluminescent screening assay). Positive results for anti-rBoNT-E antibody assessment were confirmed by a competitive confirmatory assay. Blood samples then underwent a titration assay (to assess antibody level) and were analysed for the presence of neutralising antibodies, using a functional CBA. The PD effects of abobotulinumtoxinA were assessed using the same parameters as those used for rBoNT-E.

AEs were classified as mild (normal functioning was not altered), moderate (function impairment existed, but it was not hazardous, uncomfortable, or embarrassing), or severe (symptoms were hazardous to wellbeing, with significant impairment of function or incapacitation). Serious AEs (SAEs) were any AEs that resulted in death, in-patient hospitalisation, or prolongation of existing hospitalisation, a persistent or significant disability/incapacity, a congenital anomaly/birth defect in the offspring of a patient who received the treatment, or that were life-threatening and placed the subject at immediate risk of death. Treatment emergent AEs (TEAEs) were any AEs that occurred during the active phase (from time of investigational medical product administration to end of study), if they were not present prior to treatment administration, or if intensity increased after treatment administration. AEs of special interest (AESIs) were any AEs that suggested a possible remote spread of the effect of the toxin or hypersensitivity.

The effect of rBoNT-E on the injected EDB muscle was quantified by the electrophysiological evaluation of its CMAP total (peak-to-peak) amplitude (mV), elicited by supramaximal electrical stimulation of the peroneal nerve (using surface electrodes) at the ankle. The distance between surface electrodes (assessed during the screening period in order to determine maximal CMAP response) was marked with indelible ink to allow for consistent recording at the same site for each measurement. Three measurements of EDB CMAP were carried out at approximately 2-min intervals (at each visit, including screening) and averaged to calculate and express a corresponding visit EDB CMAP value.

CMAP of EDB, ADQ and AH was recorded on a daily basis during the first week following dosing, twice a week up to week 4 (day 28), and on a weekly basis from week 5 to week 12 (end of study for part A). In part B, CMAP was also recorded at week 19 and week 26. Subjects enrolled in part A could be discharged from week 5 in the event that EDB CMAP total amplitude showed recovery (CMAP inhibition returned to within 15% of the baseline value). Subjects in whom EDB CMAP inhibition was over 30% of the baseline value at week 12 were followed up weekly until partial recovery (30% CMAP inhibition from baseline) was reached. Baseline was defined as the last available assessment collected prior to treatment administration of rBoNT-E, abobotulinumtoxinA, or placebo. Study duration was a maximum of 12 weeks for part A (rBoNT-E and placebo) and a maximum of 26 weeks for part B (abobotulinumtoxinA and placebo).

Demographics were recorded at baseline. Medical and surgical history, non-drug therapies, and concomitant surgical procedures were coded to MedDRA version 20.0, while prior and concomitant medications were coded to World Health Organization Drug Dictionary June 2017. AEs, monitored from provision of informed consent until the subject's last study assessment, were coded using MedDRA version 20.0 using descriptive information.

2.5. Statistical analyses

An appropriate sample size could not be determined statistically for part A, as no previous human data were available with rBoNT-E. A sample size of eight subjects (six treated with rBoNT-E and two with placebo) was chosen, allowing the demonstration of a statistical difference of 45% between rBoNT-E and placebo with a one-sided type 1 error rate of 0.05 and a power of 80% (assuming a common standard deviation of 20% between rBoNT-E and placebo).

Two different analysis populations were included in this study: the safety population and the PD population. The safety population consisted of all consenting subjects who received at least one dose of study medication. The PD population was all consenting subjects with available PD data and no protocol deviations with relevant impact on PD data.

Descriptive statistics were used to present raw EDB/ADQ/AH CMAP, percentage of EDB/ADQ/AH CMAP total amplitude relative to baseline (CMAP%), and percentage CMAP inhibition relative to baseline. Graphs are presented for mean maximal inhibition of the EDB and least-squares means of percentage CMAP inhibition over time for EDB, ADQ, and AH. In addition, the PD effect of rBoNT-E and abobotulinumtoxinA to reduce the CMAP of the EDB was analysed using a mixed-effect model with repeat measurement approach. Maximal inhibition and time to maximal effect, time to onset, time to recovery, and duration of effect were also described for the 15% and 50% EDB CMAP inhibition thresholds. Only subjects who reached onset (15% or 50% EDB CMAP inhibition) were included in the description of time to onset and only subjects who reached onset and recovery (i.e. return to 15% or 50% EDB CMAP inhibition) were included in the description of time to recovery. Only descriptive statistics were used to describe safety endpoints.

3. Results

3.1. Subjects

Overall, 65 subjects were screened for inclusion in part A and 28 were randomised to receive rBoNT-E or placebo (21 and seven subjects, respectively). Of these, four subjects were randomised to each of cohorts 1 (0.04 ng), 2, and 3 (both 0.2 ng), and eight subjects were randomised to each of cohorts 4 (0.9 ng) and 5 (3.6 ng). Subjects failed screening in part A as a result of adverse events ($n = 2$), consent withdrawal ($n = 3$), not meeting entry criteria ($n = 27$), lost to follow-up ($n = 2$) and other ($n = 3$). Sixty-one subjects were screened for inclusion in part B and 24 were randomised to receive abobotulinumtoxinA or placebo (18 versus six, respectively) (Fig. 1). Of these, six subjects each were randomised to receive 20, 40, or 70 U (six received placebo). Screening failures in part B were due to consent withdrawal ($n = 4$), not meeting entry criteria ($n = 27$), lost to follow-up ($n = 4$) and other ($n = 2$). All randomised subjects received a single treatment dose, completed the study, and were included in the safety and PD population.

Demographics and baseline characteristics are presented in Table 1. The majority of subjects were Caucasian, and mean age and BMI were similar across all treatment groups at baseline. Medical and surgical history (Table S1) and prior and concomitant medications of the enrolled subjects were consistent with that expected of the healthy study population. None of the history reported had an impact on the study. There were no prior or concomitant non-drug therapies or concomitant surgical procedures for subjects receiving rBoNT-E and only one for abobotulinumtoxinA. Baseline serology assessments were negative for all subjects.

The 28 subjects randomised for rBoNT-E or placebo had a mean (± SD) study duration ranging from 38.3 (± 4.9) days to 74.8 (± 22.3) days. Meanwhile, the 24 subjects randomised for abobotulinumtoxinA or placebo had a mean (± SD) study duration ranging from 171.8 (± 40.5) days to 186.5 (± 6.4) days.

3.2. Safety

Overall, rBoNT-E was well tolerated at the assessed doses (Table 2). TEAEs were experienced by 15/28 subjects in part A (11/21 rBoNT-*E*-treated subjects and 4/7 placebo-treated subjects) and 20/24 subjects in part B (15/18 abobotulinumtoxinA-treated subjects and 5/6 placebo-treated subjects). More subjects experienced AEs with rBoNT-E in the 0.9 ng group, while for abobotulinumtoxinA, the number of subjects experiencing AEs was comparable between groups (Table 2).

TEAEs occurred within the first 14 days post-treatment for part A (rBoNT-E, $n = 8$; placebo, $n = 4$). The majority of TEAEs were resolved without administration of concomitant medication within 2 days. For part B, TEAEs occurred throughout the study, with a similar number occurring within 14 days after treatment and later than 30 days post-treatment. The vast majority of these TEAEs recovered, with approximately equal numbers resolving in < 2 days, > 2–10 days, and > 10 days, and approximately one-third requiring concomitant medications.

The majority of TEAEs were considered unrelated to treatment. A summary of TEAEs reported in Part A and Part B is presented in Tables S2 and S3, respectively. The most frequently reported TEAEs in part A were pain in extremity, paraesthesia, injection-site paraesthesia, and muscular weakness, reported in two subjects each. Muscular weakness was reported as ‘weak left hip’ in one patient in 0.9 ng group, and as ‘right halluces extensor longus muscle feels weak’ in another patient who received placebo, for both patients the intensity was noted as mild by the investigator. In part B, the most frequently reported TEAE was headache ($n = 4$), which was considered by the investigator to be unrelated to study treatment. The majority of TEAEs were mild or moderate in intensity. In part B, three severe TEAEs were experienced by two subjects, including increased levels of blood creatinine phosphokinase and transaminase (one subject, both events also considered SAEs) and tooth fracture (one subject, unrelated to study treatment). No TEAEs led to withdrawal of a study subject and no deaths were reported.

There was no evidence of local diffusion to adjacent muscles (ADQ and AH) for rBoNT-E or abobotulinumtoxinA at any dose level (Fig. 2). There were no findings of note for clinical laboratory parameters, vital signs, physical examination, or ECG. All 74 serum samples (from 28 subjects in part A) were negative for the presence of binding antibodies to rBoNT-E; therefore, no subject seroconverted following rBoNT-E injection.

3.3. Pharmacodynamics

Time to onset (Fig. 3 and Table 3) in subjects receiving rBoNT-E ranged from day 1 to day 2, which was faster than the time to onset in subjects receiving abobotulinumtoxinA (range: day 1 to day 7). As shown in Fig. 3 and Table 4, a clear dose effect was observed in the maximal EDB CMAP inhibition, increasing with rBoNT-E 0.04 and 0.2 ng up to a plateau of > 90% inhibition observed with 0.9 and 3.6 ng. AbobotulinumtoxinA had a similar PD profile across all doses (inhibition plateaued at approximately 1 to 3 weeks post-injection and remained consistent for several weeks after treatment), with a lower maximal inhibition observed with 20 U (52.2%) compared with 40 U (72.5%) and 70 U (70.3%) (Table 4). Maximal effect was achieved

Table 1
Subject demographics at baseline and extent of exposure.

	Part A					Part B			
	Placebo (n = 7)	rBoNT-E				Placebo (n = 6)	aboBoNT-A		
		0.04 ng (n = 3)	0.2 ng (n = 6)	0.9 ng (n = 6)	3.6 ng (n = 6)		20 U (n = 6)	40 U (n = 6)	70 U (n = 6)
Randomised population	7	3	6	6	6	6	6	6	6
Safety population, n (%)	7 (100)	3 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)
PD population, n (%)	7 (100)	3 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)
Age (years)									
Mean (SD)	26.4 (8.3)	27.7 (8.1)	28.3 (13.2)	30.8 (10.0)	28.7 (9.6)	24.3 (4.2)	29.7 (8.6)	29.0 (7.4)	33.2 (10.5)
Median [range]	27.0 [18;40]	24.0 [22;37]	21.5 [18;49]	33.5 [18;44]	24.5 [21;43]	25.0 [19;30]	29.0 [21;44]	27.5 [22;43]	36.5 [20;45]
Race, n (%)									
Asian	0	0	0	0	0	0	0	0	1 (16.7)
Black/African-American	0	0	0	0	0	1 (16.7)	1 (16.7)	0	0
Caucasian/White	7 (100)	3 (100)	6 (100)	6 (100)	6 (100)	5 (83.3)	5 (83.3)	6 (100)	5 (83.3)
BMI									
Mean (SD)	24.9 (2.3)	23.3 (1.9)	23.6 (2.6)	24.7 (2.3)	24.9 (2.2)	24.3 (2.3)	24.6 (2.9)	26.5 (2.9)	25.9 (3.8)
Median [range]	24.2 [22.3;29.3]	23.2 [21.5;25.2]	23.1 [20.8;27.3]	24.3 [22.4;28.0]	25.2 [21.0;27.2]	25.1 [20.2;26.8]	24.7 [20.8;28.8]	27.3 [21.8;29.3]	26.8 [19.9;29.8]
Study duration (days)									
Mean (SD)	38.3 (5.1)	38.3 (4.9)	46.0 (7.9)	74.8 (22.3)	67.7 (17.0)	186.5 (6.4)	171.8 (40.5)	184.5 (5.1)	186.0 (3.8)
Median [range]	36.0 [33; 46]	36.0 [35; 44]	49.5 [30; 50]	74.5 [47; 107]	63.0 [50; 97]	184.0 [180; 195]	185.5 [90; 200]	184.0 [179; 191]	187.0 [180; 190]

aboBoNT-A, abobotulinumtoxinA; BMI, body mass index; n, number of subjects; PD, pharmacodynamic; rBoNT-E, recombinant botulinum neurotoxin E; SD, standard deviation.

Table 2
Incidence of AEs (safety population): A) for rBoNT-E and placebo (part A); B) for aboBoNT-A and placebo (part B).

	Part A					Part B			
	Placebo (n = 7)	rBoNT-E				Placebo (n = 6)	aboBoNT-A		
		0.04 ng (n = 3)	0.2 ng (n = 6)	0.9 ng (n = 6)	3.6 ng (n = 6)		20 U (n = 6)	40 U (n = 6)	70 U (n = 6)
Any AEs	5 (71.4) [8]	0	3 (50.0) [4]	5 (83.3) [14]	5 (83.3) [7]	5 (83.3) [14]	5 (83.3) [18]	5 (83.3) [18]	5 (83.3) [14]
Any TEAEs	4 (57.1) [6]	0	3 (50.0) [3]	5 (83.3) [13]	3 (50.0) [4]	5 (83.3) [14]	5 (83.3) [17]	5 (83.3) [18]	5 (83.3) [10]
Intensity of TEAEs									
At least one severe	0	0	0	0	0	0	0	2 (33.3) [3]	0
At least one moderate	2 (28.6) [2]	0	0	3 (50.0) [3]	1 (16.7) [1]	0	1 (16.7) [1]	3 (50.0) [4]	0
At least one mild	4 (57.1) [4]	0	3 (50.0) [3]	5 (83.3) [10]	2 (33.3) [3]	5 (83.3) [14]	5 (83.3) [16]	4 (66.7) [11]	5 (83.3) [10]
Causality of TEAEs									
At least one related	2 (28.6) [2]	0	1 (16.7) [1]	3 (50.0) [4]	1 (16.7) [2]	2 (33.3) [4]	1 (16.7) [1]	2 (33.3) [4]	0
At least one not related	3 (42.9) [4]	0	2 (33.3) [2]	5 (83.3) [9]	2 (33.3) [2]	4 (66.7) [10]	5 (83.3) [16]	5 (83.3) [14]	5 (83.3) [10]
Any serious AEs	0	0	0	0	0	0	0	1 (16.7) [2]	0
Any serious TEAEs[a]	0	0	0	0	0	0	0	1 (16.7) [2]	0

Note: format is n (%) [m]: the number of subjects (the percentage of subjects) [the number of occurrences]. When calculating n, if a subject experienced more than one event in a category, the subject was counted only once in that category. Percentages are calculated based on the number of subjects in the safety population. For summaries of intensity and causality, individual subjects may be reported in more than one category.
aboBoNT-A, abobotulinumtoxinA; AE, adverse event; n, number of subjects; rBoNT-E, recombinant botulinum neurotoxin E; TEAE, treatment-emergent adverse event.
[a] No TEAE led to withdrawal or death.

within approximately 1 week following rBoNT-E injections (at all doses), while abobotulinumtoxinA took 2–6 weeks to reach maximal effect (dose-dependent response, Table 4). Median time to maximal inhibition was day 8 (range: days 7–9) for 0.04 ng, days 9 and 10 (range: days 6–12) for 0.2 ng, days 5 and 6 (range: days 3–9) for 0.9 ng and days 7 and 8 (range: days 5–10) for 3.6 ng. For abobotulinumtoxinA, median time to maximal inhibition was shorter with higher doses; days 41 and 42 (range: days 19–128) for 20 U, days 20 and 21 (range: days 5–51) for 40 U and days 14 and 15 (range: days 9–25) for 70 U.

Median duration of effect when considering the 15% EDB CMAP inhibition threshold was shorter for rBoNT-E compared with abobotulinumtoxinA (Table 3). A clear dose effect was observed in the median duration of effect, increasing from ~10 days for 0.04 ng to ~31 days for 0.2 ng, and plateauing to ~50 days for 0.9 and 3.6 ng. One subject in each rBoNT-E dose level group did not reach recovery at the end of the 12-week (3-month) reporting period, whereas 16/18 subjects had not reached recovery by the end of the 26-week (6-month) follow-up for abobotulinumtoxinA. The two subjects who reached recovery were both from the 20 U group (duration was 36 and 52 days).

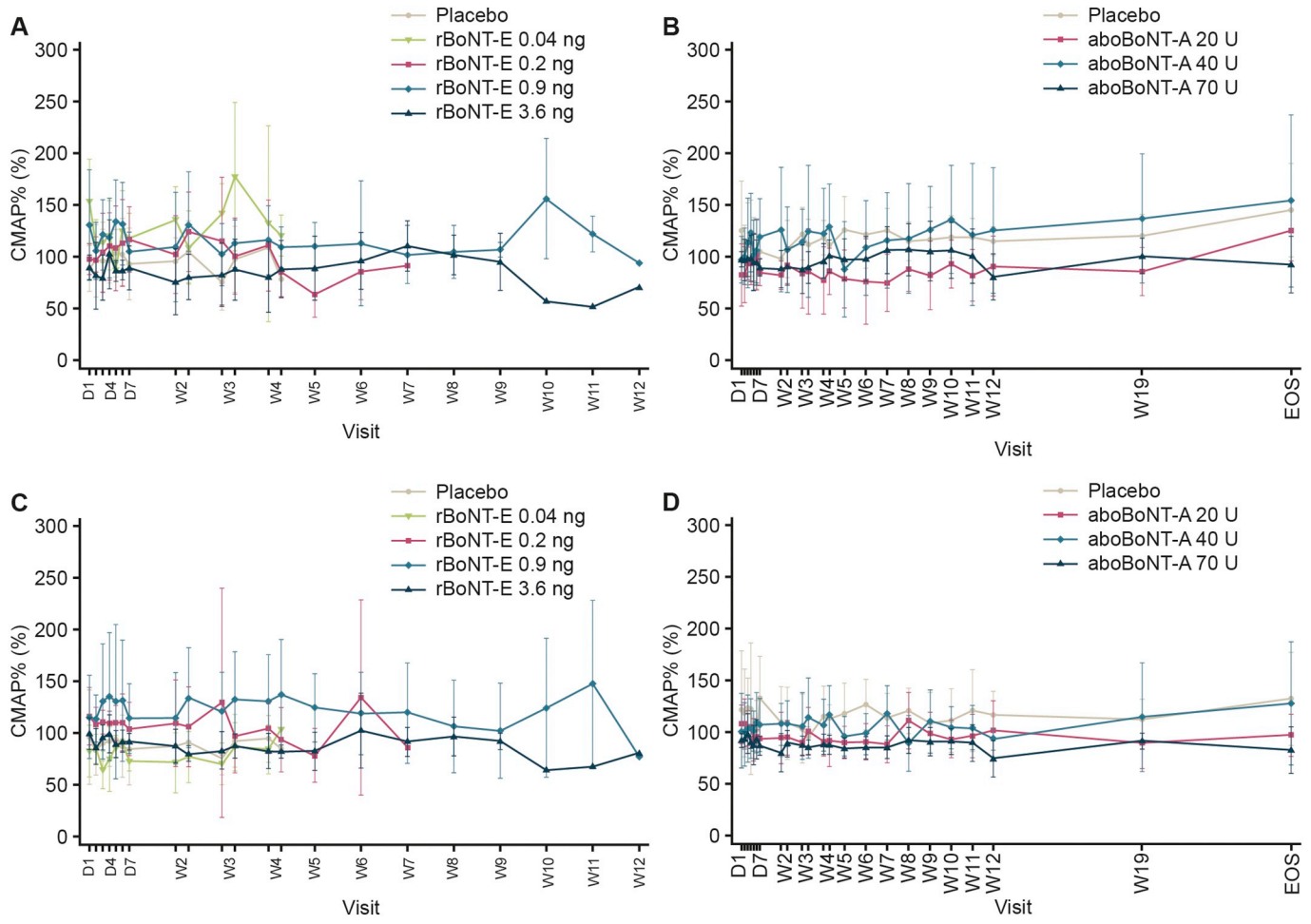

Fig. 2. Local diffusion to extensor digitorum brevis-adjacent muscles – means (± SD) CMAP% over time (PD population).
CMAP of ADQ for (A) rBoNT-E (part A) and (B) aboBoNT-A (part B). CMAP of AH for (C) rBoNT-E (part A) and (D) aboBoNT-A (part B).
Note: percentage of the CMAP total amplitude relative to baseline (CMAP%) is calculated as (CMAP at the visit/CMAP at baseline)*100. Baseline is defined as the average of CMAP values on day −1.
aboBoNT-A, abobotulinumtoxinA; ADQ, abductor digiti quinti; AH, abductor hallucis; CMAP, compound muscle action potential; D, day; PD, pharmacodynamic; rBoNT-E, recombinant botulinum neurotoxin E; SD, standard deviation; W, week.

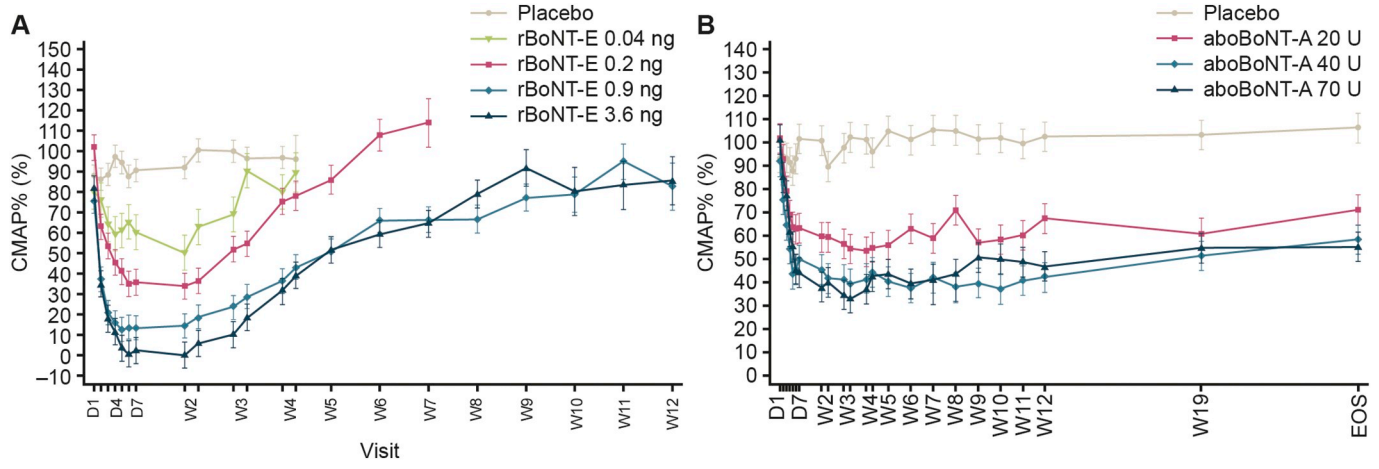

Fig. 3. LS means (± SE) CMAP% over time (PD population).
A) CMAP of EDB for rBoNT-E and placebo (part A). B) CMAP of EDB for aboBoNT-A and placebo (part B).
Note: Percentage of the CMAP total amplitude relative to baseline (CMAP%) is calculated as (CMAP at the visit/CMAP at baseline)*100. Baseline is defined as the average of CMAP values at screening and on day −1.
aboBoNT-A, abobotulinumtoxinA; CMAP, compound muscle action potential; D, day; EDB, extensor digitorum brevis; LS mean, least-squares mean; PD, pharmacodynamic; rBoNT-E, recombinant botulinum neurotoxin E; SE, standard error; W, week.

Table 3
Duration of CMAP inhibition for rBoNT-E and placebo (part A), and aboBoNT-A and placebo (part B) with a 15% inhibition cut-off (PD population).

	Part A					Part B			
	Placebo (n = 7)	rBoNT-E				Placebo (n = 6)	aboBoNT-A		
		0.04 ng (n = 3)	0.2 ng (n = 6)	0.9 ng (n = 6)	3.6 ng (n = 6)		20 U (n = 6)	40 U (n = 6)	70 U (n = 6)
Subjects who reach onset (cut-off 15% inhibition)									
Onset, n (%)	4 (57.1)	3 (100)	6 (100)	6 (100)	6 (100)	5 (83.3)	6 (100)	6 (100)	6 (100)
Time to onset (days)									
Mean (SD)	2.8 (2.4)	1.7 (0.6)	1.7 (0.5)	1.2 (0.4)	1.5 (0.5)	4.0 (4.2)	3.5 (2.1)	2.3 (1.0)	3.0 (1.7)
Median [range]	2.0 [1;6]	2.0 [1;2]	2.0 [1;2]	1.0 [1;2]	1.5 [1;2]	2.0 [1;11]	3.5 [1;7]	2.0 [1;4]	3.0 [1;6]
Recovery, n (%)	4 (100)	2 (66.7)	5 (83.3)	5 (83.3)	5 (83.3)	5 (100)	2 (33.3)	0	0
Time to recovery (days)									
Mean (SD)	8.5 (7.9)	10.5 (12.0)	34.0 (7.7)	52.2 (15.1)	50.6 (8.6)	5.8 (4.8)	45.0 (11.3)	–	–
Median [range]	5.5 [3;20]	10.5 [2;19]	32.0 [24;44]	57.0 [37;72]	51.0 [37;61]	4.0 [2;14]	45.0 [37;53]	–	–
Duration of effect (days) for subjects who reach onset and recovery									
Mean (SD)	6.8 (8.8)	10.0 (11.3)	33.2 (8.0)	52.0 (15.0)	50.0 (9.0)	2.8 (0.8)	40.5 (13.4)	–	–
Median [range]	2.5 [2;20]	10.0 [2;18]	31.0 [23;44]	56.0 [37;72]	50.0 [36;61]	3.0 [2;4]	40.5 [31;50]	–	–

aboBoNT-A, abobotulinumtoxinA; CMAP, compound muscle action potential; n, number of subjects; PD, pharmacodynamic; rBoNT-E, recombinant botulinum neurotoxin E; SD, standard deviation.

Amplitude of inhibition began to plateau at ~1–3 weeks after dosing with abobotulinumtoxinA and the inhibitory effect was maintained for several weeks, and in some cases still observed at the end of the 26-week (6-month) reporting period (Fig. 3).

Median duration of effect was still shorter for rBoNT-E compared with abobotulinumtoxinA when considering the 50% EDB CMAP inhibition threshold (Table S4). With the exception of one subject in the 0.04 ng group, all subjects given rBoNT-E experienced > 50% EDM CMAP inhibition. Recovery was within the 12-week (3-month) study period for all dose levels (Table S4). For subjects treated with abobotulinumtoxinA, 50% EDM CMAP inhibition was reached by 4/6 subjects in the 20 U group, and all subjects from the 40 and 70 U groups. At the end of the 26-week (6-month) reporting period, all subjects except one (in the 40 U group) recovered to 50% of baseline.

No noticeable effect of rBoNT-E on ADQ or AH CMAP was observed at any dose level when compared with baseline (Fig. 2).

4. Discussion

This study was a FIH, single-centre, randomised, double-blind, placebo-controlled study to assess the safety, tolerability, and PD profile of rBoNT-E injected into the EDB of healthy male subjects (compared with established abobotulinumtoxinA treatment). Overall, subjects were well matched in terms of demographic characteristics, as all subjects were male, with similar mean ages across treatment groups. Furthermore, all randomised subjects received study treatment and completed the study. Mean EDB CMAP data from placebo-treated subjects was consistent with the literature [18].

Single doses of rBoNT-E were well tolerated and rBoNT-E showed a good safety profile (with no dose-related effect) within the dose range investigated (up to 3.6 ng). All TEAEs were of mild or moderate intensity (mostly considered unrelated to study treatment) and were consistent with those expected and/or events in individual subjects. No unexpected TEAEs were identified during this study and there were no severe TEAEs, SAEs, or TEAEs leading to premature withdrawal or death. The vast majority of TEAEs occurred within 14 days of rBoNT-E treatment and most events recovered without treatment, within 2 days. Furthermore, there was no evidence of local diffusion to adjacent muscles (ADQ and AH) and no antibodies were reported. TEAEs observed in the abobotulinumtoxinA group occurred throughout the study (in line with the known safety profile of abobotulinumtoxinA), perhaps reflecting the longer duration of effect.

In this study, rBoNT-E induced EDB CMAP inhibition at all dose levels tested. Data suggest a dose relationship in maximal inhibition (~55% at 0.04 ng, 75% at 0.2 ng, and > 90% at 0.9 and 3.6 ng) and duration of effect of EDB CMAP inhibition up to 0.9 ng (with 0.9 and 3.6 ng having comparable effects). A fast onset of action was observed with all rBoNT-E doses (from day 1 to day 2). Time to maximal inhibition was relatively consistent between dose levels (approximately 1 week), with median time ranging from around day 9 with 0.2 ng, to day 5/6 with 0.9 ng. Subjects given rBoNT-E had a faster onset and a shorter duration of effect than those treated with abobotulinumtoxinA.

Table 4
Maximal inhibition and time to maximal effect of CMAP EDB for rBoNT-E and placebo (part A) and for aboBoNT-A and placebo (part B) (PD population).

	Part A					Part B			
	Placebo (n = 7)	rBoNT-E				Placebo (n = 6)	aboBoNT-A		
		0.04 ng (n = 3)	0.2 ng (n = 6)	0.9 ng (n = 6)	3.6 ng (n = 6)		20 U (n = 6)	40 U (n = 6)	70 U (n = 6)
Maximal inhibition (%)									
Mean (SD)	22.7 (19.2)	53.7 (9.1)	77.4 (6.8)	91.5 (7.6)	95.8 (3.1)	24.2 (9.9)	52.2 (13.3)	72.5 (12.9)	70.3 (7.8)
Median [range]	20.3 [8.7;63.1]	55.9 [43.7;61.6]	76.6 [69.8;87.8]	92.7 [77.7;100.0]	97.2 [91.6;98.7]	26.2 [6.8;34.6]	51.3 [36.4;70.6]	71.3 [58.3;92.1]	72.6 [57.1;77.9]
Time to maximal effect (days)									
Mean (SD)	6.3 (6.5)	8.0 (1.0)	9.2 (2.3)	5.7 (2.2)	7.5 (2.1)	6.5 (4.0)	53.0 (41.6)	26.5 (17.4)	15.0 (6.2)
Median [range]	6.0 [1.0;20.0]	8.0 [7.0;9.0]	9.5 [6.0;12.0]	5.5 [3.0;9.0]	7.5 [5.0;10.0]	5.0 [3.0;12.0]	41.5 [19.0;128.0]	20.5 [5.0;51.0]	14.5 [9.0;25.0]

aboBoNT-A, abobotulinumtoxinA; CMAP, compound muscle action potential; EDB, extensor digitorum brevis; n, number of subjects; PD, pharmacodynamic; rBoNT-E, recombinant botulinum neurotoxin E; SD, standard deviation.

While the highest doses of rBoNT-E had a duration of effect of around 50 days, with abobotulinumtoxinA all doses resulted in the EDB CMAP inhibition plateauing for several weeks, and persisting in most subjects at the end of the 26-week (6-month) reporting period.

The faster onset and shorter effect duration of rBoNT-E compared with abobotulinumtoxinA is consistent with the literature [19,20]. Botulinum toxin serotype A (BoNT-A) has a 3–9-month duration of effect in human muscles [21,22], much longer than BoNT-E (< 60-day duration of effect in human skeletal muscles [23]). In rodent models of skeletal muscle function, BoNT-A has a half-life of around 10–20 days [24] (> 78 days in vitro [12,25]), compared with < 3 days with BoNT-E (5.8 days in vitro [12], in-house data). The difference in onset and effect duration is also consistent with the mechanism of action, as abobotulinumtoxinA protease subunit activity persists inside intoxicated neurons for long periods of time, whereas rBoNT-E protease activity is cleared rapidly [12].

The EDB muscle is superficial and is involved in the extension of the second to the fourth digits of the foot. The EDB muscle was chosen because any weakening of this muscle would have no impact on the daily life of the subjects. This model is widely used by the scientific community to investigate BoNTs, including the treatment effects of BoNTs and quantifying the onset of human muscle relaxation following BoNT injection [18,26–28]. In addition, the method has been previously used to compare different BoNT serotypes [23,29–31] and to evaluate the potency of BoNT formulations [29]. Future studies should focus on rBoNT-E in different muscle groups and in small cohorts of patients with hypertonic or spastic muscles.

Based on the above-mentioned properties, it is anticipated that rBoNT-E may be able to address patient needs currently unmet by BoNT-A products. rBoNT-E may be used to rapidly improve hypertonicity and muscle over activity in patients with different conditions, including strabismus and cervical dystonia, and in adults or children with spastic paresis. In patients who have not previously received rBoNT-E, it may be used to achieve a decrease in muscle tone to start rehab and quickly achieve functional outcomes. rBoNT-E may also be beneficial for patients with spasticity who have already received BoNT-A but who may have alternative treatment goals or a change in pattern impairments over time. As such, clinicians may need to reassess the overall BoNT injection paradigm by starting treatment with a fast-onset toxin.

4.1. Conclusion

This study highlights the differences between the PD profile of rBoNT-E compared with abobotulinumtoxinA when injected into the EDB of healthy male subjects, including a faster onset of action, greater peak effect at the highest dose tested, and a shorter duration of activity. The treatment doses of rBoNT-E that were tested were well tolerated. Further studies are required to explore the effect of larger doses over hypertonic/spastic muscles in patients to establish potential therapeutic and aesthetic uses.

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Declaration of interest

LP, CV, MV, and PP are employed by Ipsen.

Data sharing

Where such data can be anonymised, Ipsen will share all individual participant data that underlie the results reported in this article with qualified researchers who provide a valid research question. Study documents, such as the study protocol and clinical study report, are not always available. Proposals should be submitted to DataSharing@Ipsen.com and will be assessed by a scientific review board. Data are available beginning 6 months and ending 5 years after publication; after this time, only raw data may be available.

Prior presentation

Data from this manuscript have been previously been presented at Toxins 2019, IMCAS 2019, DPG/AkBONT 2019 and AMWC 2019, and will shortly be presented at ICTD 2019, AACPDM 2019, MDS 2019 and SOFMER 2019.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jns.2019.116516.

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