doi:10.36849/JDD.2020.5454

 ORIGINAL ARTICLE

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

Martina Kerscher MD PhD,[a] Sabrina Fabi MD,[b] Tanja Fischer MD PhD,[c] Michael Gold MD,[d] John Joseph MD,[e] Welf Prager MD,[f] Berthold Rzany MD ScM,[g] Steve Yoelin MD,[h] Susanna Roll Dr. med,[i] Gudrun Klein PhD,[i] Corey Maas MD PhD[j]

[a]Universität Hamburg, Hamburg, Germany
[b]Cosmetic Laser Dermatology, San Diego, CA
[c]Haut- & Lasercentrum, Potsdam, Germany
[d]Gold Skin Care Center, Tennessee Clinical Research Center, Nashville, TN
[e]John Joseph MD, Private Practice, Beverly Hills, CA
[f]Prager and Partner Dermatologische Praxis, Hamburg, Germany
[g]Hautärzte RZANY&HUND, Berlin, Germany
[h]Medical Associates, Inc., Newport Beach, CA
[i]Merz Pharmaceuticals GmbH, Frankfurt am Main, Germany
[j]The Maas Clinic, San Francisco, CA

ABSTRACT

Background: Recently reported clinical data provides evidence that increasing the dose of botulinum toxin A increases the duration of efficacy. A 2-stage Phase 2, randomized, double-blind study investigated the duration of effect and safety of IncobotulinumtoxinA (INCO; Xeomin®, Bocouture®; Merz Pharmaceuticals GmbH, Frankfurt am Main, Germany) at doses higher than the approved 20 units (U) for glabellar frown lines (GFL). Primary safety and efficacy endpoints of Stage 1 are reported here.

Methods: 151 subjects with moderate-to-severe GFL were randomized 1:2:2 to receive a single treatment with 20U, 50U, or 75U INCO. The primary efficacy endpoint was median duration of at least 1-point improvement from baseline as assessed by investigator at maximum frown on the Facial Wrinkle Scale.

Results: The median duration of effect was 185 days for the 50U dose group (95% CI:[182, 205]) and 210 days for the 75U dose group (95% CI:[182, 217]). Duration of effect was significantly longer for 75U vs 50U (P=0.0400) and 20U (P=0.0166) despite the study not being powered for confirmatory statistical significance testing between the dose groups. Duration of effect was also longer for 50U vs 20U, however; statistical significance was not reached (P=0.4349). The incidence of treatment-related adverse events was low across all doses (20U:2[6.7%], 50U:6[10.0%] and 75U:8[13.1%]).

Conclusions: These results demonstrate a dose effect of at least 6 months duration with higher doses in the majority of GFL subjects. All doses were well tolerated and safety was consistent with the known safety profile of 20U INCO for GFL.

J Drugs Dermatol. 2020;19(10):985-991. doi:10.36849/JDD.2020.5454

INTRODUCTION

IncobotulinumtoxinA (INCO; Xeomin®, Bocouture®; Merz Pharmaceuticals GmbH, Frankfurt am Main, Germany) is the only commercially available botulinum toxin A (BoNT/A) preparation free from complexing proteins.[1] INCO is currently approved in worldwide markets and in the United States to treat glabellar frown lines (GFL) at a dose of 20 Units (U) and in the European Union at a dose of 20–30U.

The duration of effect for glabellar lines is approximately 3–4 months in Phase 3 studies investigating the FDA-labeled dose of 20U for INCO, OnabotulinumtoxinA (ONA; Botox®/Vistabel®, Allergan Inc.) and PrabotulinumtoxinA (PRA; Nabota®, Daewong Therapeutics, Korea/ Jeuveau®, Evolus Inc., USA/Nuceiva®, Evolus Inc., Canada, Europe), as well as the FDA-labeled dose of 50U for AbobotulinumtoxinA (ABO; Dysport®/Azzalure®, Ipsen Pharma, Wrexham, UK).[2-4] In the first large, randomized, multicenter, double-blind study to investigate FDA-labeled doses, equivalence between INCO and ONA in the treatment of GFL at the 20U dose in 250 subjects was demonstrated using an investigator-assessed responder rate (≥1-point improvement from baseline on the Facial Wrinkle Scale [FWS] at maximum frown). Similar efficacy profiles were demonstrated at all timepoints (1, 2, 3, and 4 months). Additionally, patient satisfaction was high (>90%) for both treatment groups. In both studies, INCO and ONA were found to be well tolerated.[5] This study supported an earlier head-to-head study demonstrating non-inferiority to ONA in 381 subjects.[6]

In pivotal clinical studies, the duration of effect of 20U INCO for the treatment of GFL was not explored beyond 4 months.[7-9] However, a recent randomized, double-blind, investigator-initiated study showed a strong dose-response relationship with doses of 20, 60, or 100U INCO exhibiting a median duration of effect of 120, 180, and 270 days, respectively. All adverse events (AEs) were mild and consistent with the known safety profile of INCO.[10]

To investigate the duration of effect further for INCO treatment, a prospective, randomized, controlled trial was initiated for the treatment of GFL. This 2-stage study aimed to assess the safety and duration of escalating INCO doses (20U, 50U, 75U in stage 1 and 20U, 100U in stage 2) for up to 360 days. We investigated dose steps of 25 and 30U as these offer practical application to clinical practice and are readily administered from commercially available 50 and 100U vials; 75U was chosen as a suitable intermediate dose. Results for the primary efficacy and safety endpoints from the first stage of this study (dose groups: 20, 50, and 75U) are reported.

SUBJECTS AND METHODS

Study Design

This is a prospective, randomized, double-blind, dose-ranging, Phase 2 clinical study with two stages, conducted across 4 sites in Germany and 5 sites in the USA (ClinicalTrials.gov identification number: NCT03806933; EudraCT identification number 2018-002743-28) as of January 2019. The study was conducted in compliance with the Declaration of Helsinki and Good Clinical Practice principles. All subjects provided written informed consent prior to beginning any study-related procedures.

In Stage 1, 151 subjects were randomized 1:2:2 to receive GFL treatment with 20, 50, or 75U INCO and followed from treatment until return to baseline severity of GFL wrinkle severity according to the blinded investigator assessment on the FWS at maximum frown. Subjects were required to remain in the study for at least 180±7 days and no longer than 360±7 days, depending on return to baseline.

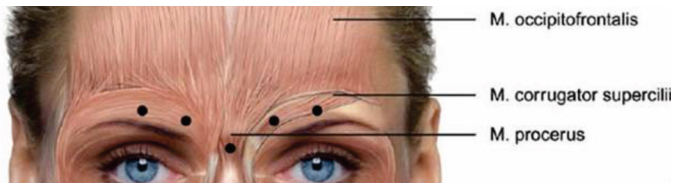
Study Subjects

Male and female subjects (≥18 of age) with moderate (score=2) to severe (score=3) GFL at maximum frown according to both subject and investigator assessment on the 4-point FWS were eligible for this study. Key exclusion criteria included: treatment with BoNT (any serotype) in the facial area ≤12 months before injection; treatment with any facial cosmetic procedure in the glabella area ≤12 months before injection; treatment with any biodegradable filler in the glabella area ≤12 months before injection; any previous insertion of permanent material in the glabella area; and planned cosmetic treatment of the face during the study period.

Study Treatment

Subjects received a single GFL treatment on day 1, with an optional follow-up treatment (20U INCO) for subjects who had completed the main period (MP) of the study. INCO was reconstituted with unpreserved, sterile 0.9% saline solution. The injection volume was constant across all dose groups. Blinded syringes were loaded with a total injection volume of 0.25 mL and administered by the investigator with a 30 or 32G needle in equal aliquots of 0.05 mL into each of 5 injection sites of the procerus and corrugator muscles (Figure 1).

FIGURE 1. Injection scheme. Holding the needle at a 45 degree angle, one injection (0.05 mL) in the procerus muscle at the crossing of two lines connecting the medial part of the eyebrow and the contralateral caruncle; one injection (0.05 mL) on each side of the medial (inner) part of the corrugator muscle, at least 1 cm above the bony orbital rim on an imaginary line drawn vertically from the caruncle; and one injection (0.05 mL) on each side lateral to the previous site in the middle part of the corrugator muscle, at least 1 cm above the bony orbital rim on an imaginary line drawn vertically from the midpupillary line.



Primary Efficacy Endpoint

The primary efficacy endpoint was duration of effect, assessed as the time between treatment and return to baseline severity. Effect was defined as ≥1-point improvement compared to baseline at maximum frown by investigator's live assessment at maximum frown using the FWS, a widely used 4-point standardized photonumeric assessment scale for glabellar line severity (0=no muscle action at all; 1=some even slight muscle action possible; 2=moderately strong muscle action possible; 3=strong muscle action possible that may cause local pallor).

Primary Safety Endpoints

Primary safety endpoints were the occurrence of treatment-emergent AEs (TEAEs), treatment-emergent serious AEs (TESAEs), treatment-emergent AEs of special interest (TEAESIs), related TEAEs, and related TESAEs by dose group as reported by patient and/or investigator. TEAE was defined as an AE emergent with onset or worsening on or after date of the first administration of treatment.

Statistical Analysis

Statistical analyses were performed using SAS® version 9.4 (Cary, NC, USA). Efficacy analyses were conducted on the full analysis set (FAS; all subjects who received study treatment and have a baseline and at least one post-baseline value of any efficacy variable). Duration of effect was described by Kaplan-Meier curves per group and the respective medians of times with associated 2-sided 95% confidence interval (CI). A Cox

proportional hazard regression model was applied with factors dose group, study site, and baseline investigator-assessed FWS score at maximum frown. For safety endpoints, descriptive analyses were conducted for the safety evaluation set (SES; all subjects who received study treatment).

Determination of Sample Size

To detect AEs at least once per group with an incidence rate of 3% with a probability of approximately 80%, a sample size of 53 subjects per group was necessary. Assuming an exponential distribution, a median duration of effect of 3 months and a censoring rate of 5%, a minimum of 55 subjects per group, were needed to obtain a precision of 1.1 months with 80% probability and a precision of 1.5 months with 90% probability. In total, approximately 60 subjects per dose group (20U: 30 subjects in both Stage 1 and 2) were deemed necessary.

RESULTS

Participants

In Stage 1, a total of 161 subjects were screened at 4 sites in Germany and 5 sites in the US, with 151 subjects randomized to receive either 20U (N=30), 50U (N=60) or 75U (N=61) INCO (Figure 2). Six subjects discontinued prior to day 180; all 151 randomized subjects were included in the SES and the FAS according to the intention-to-treat principle. Subject demographics and baseline severity of the GFL were similar for all dose groups. The majority of subjects (N=128, 84.8%) had a baseline score of severe (Table 1).

Primary Efficacy Analysis

The median duration of effect was 185 days for the 50U dose group (95% CI:[182, 205]) and 210 days for the 75U dose group (95% CI:[182, 217]). The Kaplan-Meier plot (Figure 3) reveals a dose effect that was most pronounced between day 120 and day 210. Nearly 100% of subjects had not returned to baseline severity within the first 120 days. From day 210 onward, an increasing number of subjects from all three dose groups returned to baseline. For the 20U dose group, the median duration of effect was 177 days (95% CI: [126, 188] in this first cohort (second 20U cohort to be reported in Stage 2) and nearly 100% of subjects had not returned to baseline severity within the first 90 days. Hazard ratios (HRs) from a Cox proportional hazard regression performed over the entire 360-day follow-up period reached statistical significance for 2 out of 3 pairwise comparisons of dose groups despite the study not being powered for confirmatory statistical significance testing between the groups. HRs indicated significant differences in duration of effect: HR=0.67 (95%-CI: [0.46, 0.98]); *P*=0.0400) for 75U vs 50U and HR= 0.56 (95%-CI: [0.34, 0.90]; *P*=0.0166) for 75U vs 20U. A longer duration of effect for 50U vs. 20U was also observed; however, the HR did not reach statistical significance for this comparison (HR=0.83; 95%-CI: [0.51; 1.34]; *P*=0.4394), likely due to the smaller number of subjects randomized to the 20U group in Stage 1 (30 vs 60 subjects) than to the higher dose groups.

FIGURE 2. Subject disposition [main period (MP) Stage 1].

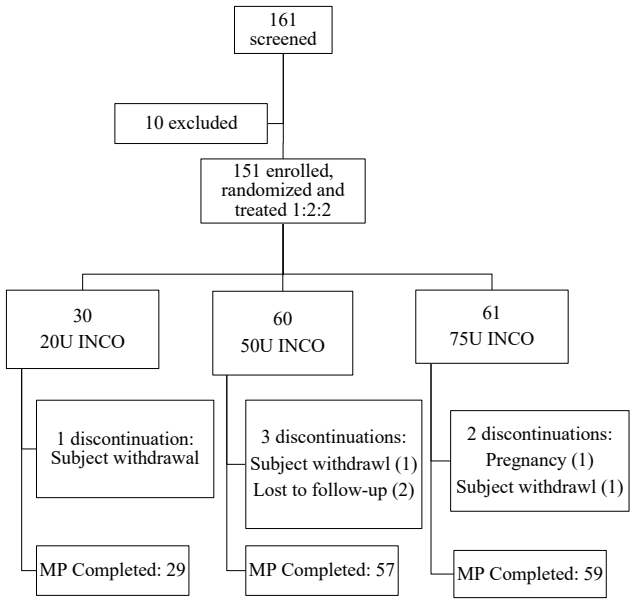
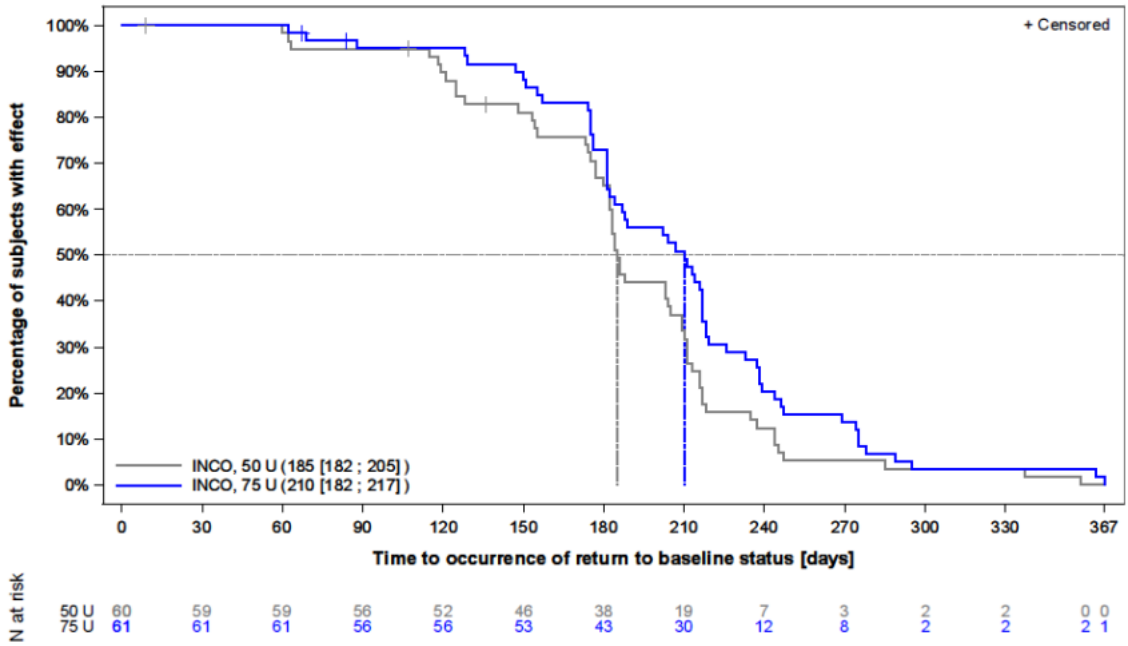


TABLE 1.

Baseline Characteristics (SES/FAS)	INCO 20U (N=30)	INCO 50U (N=60)	INCO 75U (N=61)	Total (N=151)
Sex (n [%])				
Male	3 (10.0)	9 (15.0)	7 (11.5)	19 (12.6)
Female	27 (90.0)	51 (85.0)	54 (88.5)	132 (87.4)
Age (years)				
n	30	60	61	151
Mean (SD)	52.3 (11.68)	46.9 (10.27)	49.2 (13.75)	48.9 (12.14)
Median	52.0	45.5	49.0	48.0
Min, max	25, 74	27, 76	22, 74	22, 76
Race (n [%])				
White	28 (93.3)	59 (98.3)	59 (96.7)	146 (96.7)
Black or African American	2 (6.7)	0	1 (1.6)	3 (2.0)
Asian	0	1 (1.7)	1 (1.6)	3 (2.0)
American Indian or Alaska Native	1 (0.3)	1 (1.7)	1 (1.6)	3 (2.0)
Baseline FWS[1] (n [%])				
Moderate (2)	5 (16.7)	9 (15.0)	9 (14.8)	23 (15.2)
Severe (3)	25 (83.3)	51 (85.0)	52 (85.2)	128 (84.8)

[1]Baseline FWS for GFL at maximum frown determined by treating investigator at the baseline visit.
FWS, Facial Wrinkle Scale (2=moderate; moderately strong muscle action possible to 3=severe; strong muscle action possible which may cause local pallor); SD, standard deviation

FIGURE 3. Investigator-assessed duration of effect for 50 and 75U dose groups.



Effect defined by ≥1-point improvement to baseline severity at maximum frown on the FWS. Kaplan-Meier plot, FAS. Final results for 50U (N=60) and 75U (N=61) groups. Numbers in legend denote median time to occurence and 95 % confidence interval.
*Each step of the curve depicts time to return to baseline severity of 1 or more individual subjects (steeper steps indicate more subjects from the respective dose group returned to baseline on that specific day).
+=time of censoring, ie, when time to return to baseline severity could not be observed, eg, in case of subjects dropping out from the study before returning to baseline severity.
N at risk=subjects who are still having an effect.

FIGURE 4. Subjects treated with (a) 50U and (b) 75U of INCO.

TABLE 2.

Incidence of Treatment-Emergent Adverse Events by Dose Group over the entire Main Period of up to 360 days (SES)				
	INCO 20U (N=30)	INCO 50U (N=60)	INCO 75U (N=61)	Total (N=151)
	n (%)	n (%)	n (%)	n (%)
Subjects with ≥ 1 TEAE	10 (33.3)	23 (38.3)	26 (42.6)	59 (39.1)
Subjects with ≥ 1 TEAE related to treatment	2 (6.7)	6 (10.0)	8 (13.1)	16 (10.6)
Subjects with ≥ 1 serious TEAE	0 (0)	0 (0)	0 (0)	0 (0)

Primary Safety Analyses

The incidence of TEAEs over the entire MP of up to 360 days was 10 (33.3%) for the INCO 20U group (N=30), 23 (38.3%) for the 50U group (N=60), 26 (42.6%) for the 75U group (N=61), and 59 (39.1%) in total (n=151). No serious TEAEs occurred (Table 2). One TEAE, a pregnancy, led to premature discontinuation of the study. The outcome was a healthy baby.

The incidence of treatment-related TEAEs was 2 (6.7%), 6 (10.0%) and 8 (13.1%) for the 20, 50 and 75U groups, respectively, and 16 (10.6%) in total (n=151). All treatment-related TEAEs were transient and mild to moderate in severity. In the entire SES, incidence of >1% was reported for only 4 Preferred Terms (MedDRA version 22.1): nodule (2[1.3%]), hypoaesthesia (2[1.3%]), headache (6[4.0%]), and eyelid ptosis (2[1.3%]). Over all three dose groups, only 3 subjects (2.0%) reported TEAESIs: eyelid ptosis (2[1.3%]; both related to treatment) and constipation (1[0.7%]; unrelated to treatment). The incidence rates of related TEAEs and of TEAESIs on Preferred Term level are very low and thus not reported by dose group to avoid unblinding of investigators prior to completion of Stage 2.

DISCUSSION

Efficacy

The results of this study demonstrate a dose effect of at least 6 months duration with higher doses for the majority of GFL subjects as assessed by ≥1-point improvement to baseline severity at maximum frown on the FWS. Of note is the high proportion of subjects in this study with GFL rated "severe" at baseline (83–85% of subjects in all dose groups), indicating that such long duration can be achieved with INCO, even for difficult-to-treat patients with severe GFL. In similar studies investigating duration of effect for other neuromodulators, only 35%–41% of subjects per active treatment group had severe GFL at baseline.[12-14]

Preclinical studies demonstrate higher doses result in a longer duration of effect because more BoNT binds to motor endplates, allowing more light chain molecules can reach the cytosol of the neuron.[15] The degradation of more light chain molecules takes a longer time, consequently, the duration is prolonged.[16] Clinical studies with other commercially available neuromodulators have also demonstrated longer duration of effect with increasing dose in the treatment of GFL as described in Table 3. It is important to note that dosing should be based on the clinical data for each product. In an open-label, investigator-initiated study, 30 subjects treated with 120U ABO exhibited a median duration of response of 150 days (21.4 weeks; 95% CI: 120, 180) compared to the 85 days (12.1 weeks) reported in pivotal studies for 50U ABO.[14] A recent pharmacology (Phase 1b) study investigating duration for 40, 60, and 80U vs 20U ONA determined a median duration of response of 19.7 weeks (137.9 days; 95% CI: 16.1, 20.3) for the 20U group and 24 weeks (168 days; 95% CI: 20.1, 24.4) for the 40U group.[17] Duration of response for investigational drug DAXI was explored for the 20, 40, and 60U doses compared to 20U ONA and placebo in a Phase 2 dose-ranging study.[18] A statistically significant difference in median duration of response was observed for 40U DAXI vs 20U ONA, but not for 20U DAXI vs 20U ONA in this Phase 2 trial. Two subsequent Phase 3 studies investigated duration of response for the 40U dose vs placebo and confirmed the Phase 2 median of 24 weeks (168 days).[19]

TABLE 3.

Median Duration of Effect in the Treatment of GFL With Higher Doses.[1]							
	INCO		ABO	ONA	DAXI		
	50U	75U	120U	40U	40U	40U	40U
N	60	61	30	50	39	201	204
Study Type	Ph 2	Ph 2	IIS	Ph 1b	Ph 2	Ph 3	Ph 3
Median (95% CI) duration of effect, days	185 (182–205)	210 (182–217)	150† (120–180)	168 (140.7–170.8)	165.2* (137.2–172.9)	168.7* (168–175)	168.7* (168–172.2)

[1]Median duration of ≥1-point improvement from baseline. †Return to baseline severity of Grade 2 or 3. Assessments by investigator at maximum frown on a 4-point scale (INCO, ONA:Facial Wrinkle Scale; ABO:Glabellar Line Severity Score; DAXI:IGA-FWS. Median (95% CI) was based on Kaplan–Meier method.
*[17,32]reported duration of effect in weeks.
CI, confidence interval; Ph, Phase; IIS, Investigator-Initiated Study. Direct study comparisons cannot be made, as studies differ in several aspects.

While a dose effect was demonstrated in our study, the amount of diluent/injection volume may also play a role when it comes to duration of effect. The combination of higher doses and low injection volumes resulted in a high degree of efficacy with sustained duration of effect while maintaining a favorable safety profile. Sustained efficacy beyond 4 months for the 20U dose group is notable for the majority of subjects in this first cohort of 30 subjects. Previous INCO studies have demonstrated efficacy up to 4 months for the majority of patients; however, these previous studies did not extend beyond day 120 or utilize a time to event study design to investigate longer duration.[5,6,9,20]

Safety

The results of this Phase 2, dose-ranging study demonstrate INCO doses of 50U and 75U are safe, well tolerated, and safety is consistent with the known safety profile of the 20U dose for GFL. Notably, the incidence of eyelid ptosis (N=2) in the total SES (N=151) was 1.3%, and no other TEAESIs related to treatment occurred. This favorable safety profile was not unexpected given a previous dose-escalation study in which patients with spasticity received 3 consecutive injection cycles of 400U, 600U, and 600–800U INCO, respectively, without emergence of new TEAESIs.[21]

INCO has demonstrated an excellent safety profile across all large, well-controlled clinical studies, as determined by a pooled safety analysis of 13 studies evaluating more than 6,000 INCO treatments in 2,547 aesthetic patients.[22] Furthermore, INCO is the only BoNT/A with no subjects in clinical studies who have developed neutralizing antibodies and demonstrated a secondary lack of treatment response as outlined in recent updates to the INCO prescribing information.[1] A recent pharmacovigilance analysis of the US FDA AE reporting system database assessed a total of 23,789 BoNT/A cases reported for therapeutic and aesthetic patients. The rate of AEs that involved decreased effect when on treatment for at least 1 year was 0% for INCO compared to other BoNT/As. Causal relationships cannot be established from pharmacovigilance analyses; however, an association was identified in this analysis of one of the largest BoNT/A safety data sets.[23]

By utilizing the only state-of-the-art manufacturing process that employs a 2-step chromatographic purification process to extract from the complexing proteins and leave just the active 150kDa molecule, INCO provides the lowest protein load available compared to other BoNT/A formulations.[1,24-29] INCO contains 0.44ng 150kDa neurotoxin per 100U (0.088 ng/20U dose) and has a high specific activity of 227U/ng, consistent with no denaturing of the BoNT complex during the INCO chromatographic purification process.[24-29] In contrast, ONA, ABO, and PRA all contain complexing proteins and/or denatured BoNT protein that may initiate an immune response, leading to production of neutralizing antibodies that can be associated with decreased effect over time or treatment non-response.[24,31] Investigational drug DaxibotulinumtoxinA (DAXI; RT002, Revance Therapeutics Inc.) contains a virally-derived protein transduction domain (PTD), as part of an added excipient and stabilizer (RTP004). While PTDs have shown promise in pre-clinical efforts for transporting cargo across the cellular membrane, many PTDs have demonstrated immunogenic potential.[29] In line with these findings, DAXI was shown to induce detectable antibody titers to RTP004 within 3 months of use in 30% of tested monkeys, but no long-term immunogenicity data in humans is available at this time.[30,33]

Limitations of our study include low sample size (n=30) in the 20U group for Stage 1, that results in limited power for statistical comparisons to the higher doses. Strengths of our study include a robust observation period of 360 days and high subject retention. Additional strengths include enrollment of males (12.6%), and >80% of subjects with GFL rated "severe" at baseline and across each dose group indicating that even difficult-to-treat patients can achieve long duration with INCO.

CONCLUSION

The results of this study demonstrate a dose effect of at least 6 months duration with higher doses in the majority of GFL subjects. Remarkably, this prolonged duration of effect with INCO was achieved even for difficult-to-treat patients with severe GFL. All doses were well tolerated, and safety was consistent with the known safety profile of 20U INCO for GFL.

DISCLOSURES

All authors except Drs. Roll and Klein have been consultants and/or investigators for Merz Pharmaceuticals GmbH and Merz North America, Inc. Dr. Roll, Lead Medical Expert, and Dr. Klein, Senior Biostatistician, are employees of Merz Pharmaceuticals GmbH. This study and publication were sponsored by Merz Pharmaceuticals GmbH.

ACKNOWLEDGMENT

Medical writing support was provided for the first draft by Emma Robertson, formerly of Merz Pharmaceuticals GmbH, in accordance with Good Publication Practice (GPP3) guidelines.

REFERENCES

1. Merz Pharmaceuticals GmbH. Xeomin - US Highlights of Prescribing Information. Available at: https://www.xeominaesthetic.com/wp-content/uploads/2019/05/XEOMIN-Full-Prescribing-Information-including-MedGuide.pdf. Accessed April, 2020. Merz Pharmaceuticals GmbH. Bocouture - Summary of Product Characteristics. Available at: https://www.medicines.org.uk/emc/product/7418/smpc. Accessed April, 2020.
2. Allergan Inc. Highlights of prescribing information - Botox®. 2017. Available from:https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/103000s5302lbl.pdf. Accessed 2 October 2018
3. Jeuveau Highlights of prescribing information. 2019. Availablefrom:https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/761085s000lbl.pdf. Accessed 1 July 2020
4. Galderma (U.K.) Ltd. Azzalure® Summary of Product Characteristics. 2018. Available from:https://www.medicines.org.uk/emc/product/6584/smpc. Accessed 2 October 2018.

5. Kane MA, Gold MH, Coleman WP, 3rd, et al. A Randomized, Double-Blind Trial to Investigate the Equivalence of IncobotulinumtoxinA and OnabotulinumtoxinA for Glabellar Frown Lines. *Dermatol Surg.* 2015;41(11):1310-1319.
6. Sattler G, Callander MJ, Grablowitz D, et al. Noninferiority of incobotulinumtoxinA, free from complexing proteins, compared with another botulinum toxin type A in the treatment of glabellar frown lines. *Dermatol Surg.* 2010;36 Suppl 4:2146-2154.
7. Carruthers A, Carruthers J, Coleman WP, 3rd, et al. Multicenter, randomized, phase III study of a single dose of incobotulinumtoxinA, free from complexing proteins, in the treatment of glabellar frown lines. *Dermatol Surg.* 2013;39(4):551-558.
8. Hanke CW, Narins RS, Brandt F, et al. A randomized, placebo-controlled, double-blind phase III trial investigating the efficacy and safety of incobotulinumtoxinA in the treatment of glabellar frown lines using a stringent composite endpoint. *Dermatol Surg.* 2013;39(6):891-899.
9. Jones D, Carruthers J, Narins RS, et al. Efficacy of incobotulinumtoxinA for treatment of glabellar frown lines: a post hoc pooled analysis of 2 randomized, placebo-controlled, phase 3 trials. *Dermatol Surg.* 2014;40(7):776-785.
10. Polacco M, Singleton A, Barnes C, MD, et al. A Double-Blind, Randomized Clinical Trial to Determine Effects of Increasing Doses and Dose-Response Relationship of IncobotulinumtoxinA in the Treatment of Glabellar Rhytids. *Aesthet Surg J.* 2020 Jul 28; sjaa220. doi: 10.1093/asj/sjaa220. Online ahead of print.
11. Honeck P, Weiss C, Sterry W, et al. Reproducibility of a fourpoint clinical severity score for glabellar frown lines. *Br J Dermatol.* 2003;149:306–10.
12. Sloop RR, Escutin RO, Matus JA, et al. Dose-response curve of human extensor digitorum brevis muscle function to intramuscularly injected botulinum toxin type A. *Neurology.* 1996;46(5):1382-6.
13. Wohlfarth K, Muller C, Sassin I, et al. Neurophysiological double-blind trial of a botulinum neurotoxin type a free of complexing proteins. *Clin Neuropharmacol.* 2007;30(2):86-94.
14. Joseph JH, Eaton LL, Robinson J, et al. Does increasing the dose of abobotulinumtoxin A impact the duration of effectiveness for the treatment of moderate to severe glabellar lines? *J Drugs Dermatol.* 2016;15(2):1544-1549.
15. Keller JE. Recovery from botulinum neurotoxin poisoning in vivo. *Neuroscience.* 2006;139(2):629-37.
16. Pellett S, Bradshaw M, Tepp WH, et al. The light chain defines the duration of action of botulinum toxin serotype A subtypes. *mBio.* 2018;9(2):e00089-18.
17. Allergan Aesthetics Day Press Release September 14, 2018.
18. Carruthers J, Solish N, Humphrey S, et al. Injectable daxibotulinumtoxinA for the treatment of glabellar lines: a phase 2, randomized, dose-ranging, double-blind, multicenter comparison with onabotulinumtoxinA and placebo. *Dermatol Surg.* 2017;43(11):1321-1331.
19. Carruthers JD, Fagien S, Joseph JH, Humphrey SD at al. DaxibotulinumtoxinA for injection for the treatment of glabellar lines: results from each of two multicenter, randomized, double-blind, placebo-controlled, phase 3 studies (SAKURA 1 and SAKURA 2). *Plast Reconstr Surg.* 2020;145(1):45-58.
20. Prager W, Rappl T. Phase IV study comparing incobotulinumtoxinA and onabotulinumtoxinA using a 1:1.5 dose-conversion ratio for the treatment of glabellar frown lines. *J Cosmet Dermatol.* 2012 Dec;11(4):267-71.
21. Wissel J, Bensmail D, Ferreira JJ, et al. Safety and efficacy of incobotulinumtoxinA doses up to 800U in limb spasticity: The TOWER study. *Neurology.* 2017;88(14):1321-1328.
22. Coleman WP, Sattler G, Weissenberger P et al. Safety of incobotulinumtoxinA in the Treatment of Facial Lines: Results From a Pooled Analysis of randomized, prospective, controlled clinical studies. *Dermatol Surg.* 2017;43:S293–S303.
23. Kazerooni, R. Decreased therapeutic effect over time amongst botulinum toxin type A agents. *Mov Disord Clin Pract.* 2020;7(S1):S10(110).
24. Frevert J. Pharmaceutical, biological, and clinical properties of botulinum neurotoxin type A products. *Drugs R D.* 2015;15(1):1-9.
25. Frevert J, Ahn KY, Park MY, et al. Comparison of botulinum neurotoxin type A formulations in Asia. *Clin Cosmet Investig Dermatol.* 2018;11:327-331.
26. Wanitphakdeedecha R, Ungaksornpairote C, Kaewkes A, et al. A pilot study comparing the efficacy of two formulations of botulinum toxin type A for muscular calves contouring. *J Cosmet Dermatol.* 2018;17(6):984-990.
27. Zhang L, Lin WJ, Li S, Aoki KR. Complete DNA sequences of the botulinum neurotoxin complex of clostridium botulinum type A-Hall (Allergan) strain. Gene. 2003;315:21-32.Frevert J. *Drugs R D.* 2010;10(2):67-73.
28. Kerscher M, Wanitphakdeedecha R, Trindade de Almeida A, et al. IncobotulinumtoxinA: a highly purified and precisely manufactured botulinum neurotoxin type A. *J Drugs Dermatol.* 2019;18(1):52-57.
29. Xie J, Bi Y, Zhang H, et al. Cell-penetrating peptides in diagnosis and treatment of human diseases: from preclinical research to clinical application. *Front Pharmacol.* 2020;11:697.
30. Prawdzik G, Oliyai C, Joshi A. Nonclinical overview of daxibotulinumtoxinA for injection to support registration for human use. Poster presented at: "TOXINS 2019 Basic Science and Clinical Aspects of Botulinum and Other Neurotoxins." 2019;Copenhagen, Denmark.
31. Jankovic J, Schwartz K. Response and immunoresistance to botulinum toxin injections. *Neurology.* 1995;45(9):1743-1746.
32. Solish N, Bertucci V, Humphrey S, et al. Two phase 3, randomized double-blind, placebo controlled, multi-center trials to evaluate the efficacy and safety of daxibotulinumtoxinA for injection to treat moderate to severe glabellar lines (SAKURA 1 and 2). Annual Meeting of the American Academy of Dermatology. February 16-20, 2018: Poster 7490.
33. Fabi S, Cohen J, Green L, et al. DaxibotulinumtoxinA for injection for the treatment of glabellar lines: efficacy results from SAKURA 3, a large, open-label, phase 3 safety study. *Dermatol Surg.* 2020; Online ahead of print.

AUTHOR CORRESPONDENCE

Martina Kerscher MD PhD

E-mail:..... martina.kerscher@uni-hamburg.de