doi:10.36849/JDD.6377

 ORIGINAL ARTICLE

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

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ABSTRACT

Background: To further explore clinical trial results indicating increasing doses of botulinum toxin A prolong duration of effect, a 2-stage, phase 2, randomized, double-blind study investigated the duration of effect and safety of incobotulinumtoxinA (INCO; Xeomin®, Bocouture®) doses higher than the US Food and Drug Administration-approved 20 units (U) for glabellar frown lines (GFL). The stage 1 primary efficacy and safety results were reported previously. Here, we report the results of the final analysis (stage 1 and 2), including primary and secondary efficacy and safety endpoints.

Methods: A total of 241 subjects with moderate-to-severe GFL were randomized to receive a single treatment with 20 (N=61), 50 (N=60), 75 (N=61), or 100U (N=59) INCO. The primary efficacy endpoint was duration of ≥ 1-point improvement from baseline assessed by investigator at maximum frown on the Facial Wrinkle Scale.

Results: The median duration of effect was 175 days for the 20U group (95% CI 142, 185), 185 days for the 50U group (95% CI 182, 205), 210 days for the 75U group (95% CI 182, 217), and 215 days for the 100U group (95% CI 183, 237). The incidence of treatment-related adverse events was low across all doses and there were no treatment-related serious adverse events.

Conclusions: These results demonstrate that all INCO doses were well tolerated, consistent with the known safety profile of 20U, and increasing dose prolongs the duration of effect for GFL.

J Drugs Dermatol. 2021;20(10):1052-1060. doi:10.36849/JDD.6377

INTRODUCTION

IncobotulinumtoxinA (INCO; Xeomin®, Bocouture®; Merz Pharmaceuticals GmbH, Frankfurt am Main, Germany) is approved in the United States and worldwide markets for treating glabellar frown lines (GFL) at a dose of 20 units (U) and in the European Union at a dose of 20–30 U. Phase 3 studies that used 20U of INCO demonstrated the duration of effect lasts for at least 4 months.[1–3]

There is an increasing demand for a longer duration of effect from botulinum toxin A (BoNT/A) products. INCO is unique among commercially available products in that it does not contain unnecessary bacterial proteins,[4–6] which may reduce immunogenicity.[7,8,9] The manufacturing process, which includes a 2-step chromatographic purification procedure, yields only the active 150kDa molecule, giving INCO the lowest protein load of available BoNT/A formulations, which is a consideration with overall increasing doses used in aesthetics.[7,10–14]

A randomized, double-blind, investigator-initiated study showed a strong dose-response relationship with doses of 20, 60, and 100U INCO exhibiting a median duration of effect of 120, 180, and 270 days, respectively.[15] Adverse events (AEs) with the higher doses were mild and consistent with the known safety profile of 20U INCO.

A 2-stage dose-ranging phase 2 trial was conducted to assess the safety and duration of escalating INCO doses (20, 50, 75U in stage 1 and 20 and 100U in stage 2) for up to 360 days. The stage 1 primary efficacy and safety results were reported previously.[16] Here, we report the results for the full cohort, including the primary and secondary endpoints for efficacy and safety.

MATERIALS AND METHODS

This was a prospective, randomized, double-blind, dose-ranging, phase 2 clinical study conducted across 4 sites in Germany and 5 in the USA (ClinicalTrials.gov identification number: NCT03806933; EudraCT identification number 2018-002743-28). 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.

The subjects were followed from treatment until return to baseline GFL wrinkle severity as determined by a blinded investigator assessment at maximum frown on the Facial Wrinkle Scale (FWS), a widely-used, 4-point, standardized, 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). Subjects were required to remain in the study for at least 180 days and no longer than 360 days, depending on return to baseline wrinkle severity. This was defined as the main period of the study.

Subjects

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

Treatment

The treatment procedures, which were identical in stage 1 and stage 2, were previously described.[16] Briefly, 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 of the study. In stage 1, subjects were randomized 1:2:2 to receive 20, 50, or 75U INCO. In stage 2, subjects were randomized 1:2 to receive 20 or 100U INCO. Subjects did not cross over from stage 1 to stage 2. Before injection, INCO was reconstituted with unpreserved, sterile 0.9% saline solution. To maintain blinded status, the injection volume was constant across all dose groups. A total injection volume of 0.25 ml was used in blinded syringes. Investigators administered the injections with a 30 G or 32 G needle in equal aliquots of 0.05 mL into each of 5 injection sites of the procerus and corrugator muscles.

Primary Efficacy Endpoint

The primary efficacy endpoint was duration of ≥1-point improvement from baseline on FWS as assessed by investigator at maximum frown.

Secondary Efficacy Endpoints

Secondary efficacy endpoints included:

- The duration of effect of a FWS score as rated by investigator of none (0) or mild (1) at maximum frown from treatment until return to a score of moderate (2) or severe (3).
- The duration of effect of a ≥2-point improvement as rated by investigator from baseline at maximum frown on the FWS.
- The percentage of subjects rated by investigator and the percentage of subjects rated by themselves as none (0) or mild (1) at maximum frown on FWS at day 180.
- The percentage of subjects rated by investigator and the percentage of subjects rated by themselves as at least 1-point improvement from baseline at maximum frown on FWS at day 180.

In addition, the percentage of subjects fulfilling the above effect definitions at day 30, 60, 90, 120, and 150 were analyzed as other efficacy variables, as well as the percentage of subjects rated by themselves as improved or better on the Global Aesthetic Improvement Scale (GAIS) from day 30 to 180.

Primary Safety Endpoints

Primary safety endpoints included the occurrence of treatment-emergent AEs (TEAEs), treatment-emergent serious AEs (TESAEs), treatment-emergent AEs of special interest (TEAESI), related TEAEs, and related TESAEs by dose group. TEAE was defined as an AE that began or worsened on or after the date of the first administration of treatment.

Statistical Analysis

Efficacy analyses were conducted on the full analysis set (FAS, all randomized subjects) and safety analyses on the safety evaluation set (SES, all treated subjects) using SAS® version 9.4 (Cary, NC, USA).

Primary and secondary duration of effect variables were analyzed by Kaplan-Meier curves per dose group and the respective medians of times with 2-sided 95% confidence intervals (CIs). Cox proportional hazard regression models with factors dose group, study site, and baseline investigator-assessed FWS score at maximum frown were performed for exploratory purposes. For binary efficacy variables and safety endpoints, descriptive analyses were conducted.

Determination of Sample Size

To detect AEs with an incidence rate of 3% at least once per dose group 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 when estimating median duration of effect. In total, 60 subjects per dose group were deemed necessary.

RESULTS

Participants

A total of 241 subjects were randomized to receive either 20U (stage 1:N=30; stage 2:N=31), 50U (stage 1:N=60), 75U (stage 1: N=61), or 100U (stage 2:N=59) INCO (Table 1). In total, 229 (95.0%) of the 241 randomized subjects completed the study. No subjects discontinued due to AEs or lack of efficacy (Figure 1). All 241 randomized subjects were included in the SES and the FAS.

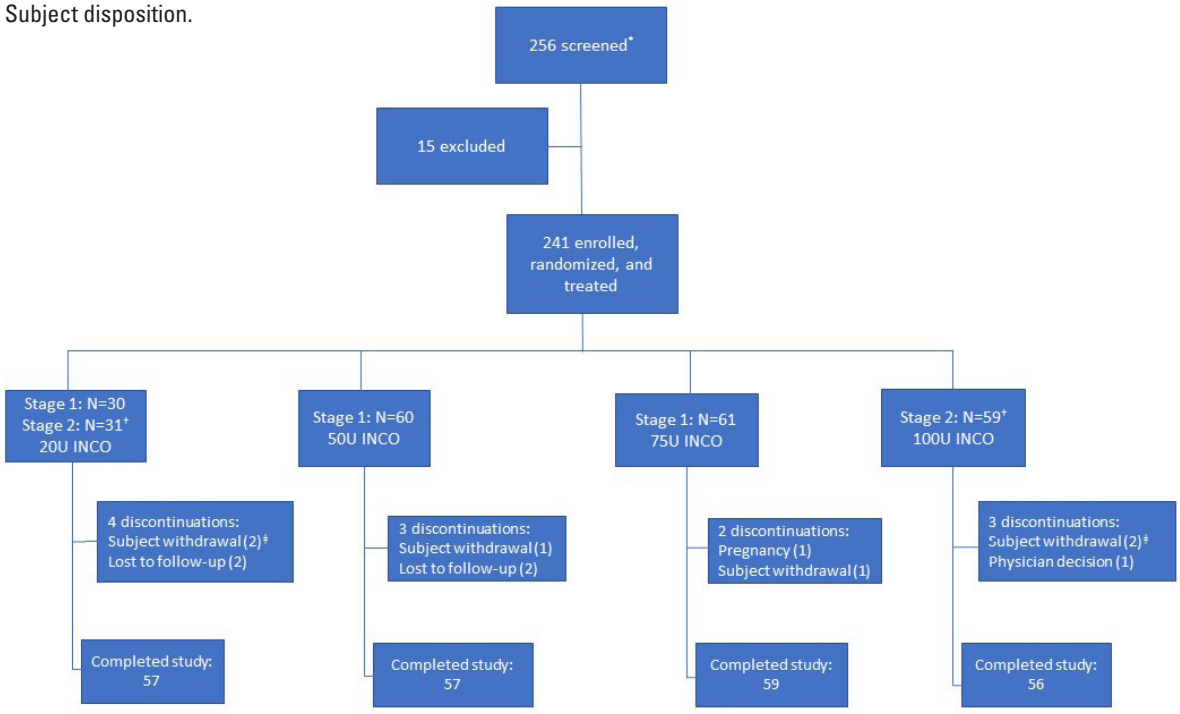
TABLE 1.

Baseline Characteristics of Study Participants (randomized subjects/full analysis set)

		INCO 20U* N = 61	INCO 50U N = 60	INCO 75U N = 61	INCO 100U* N = 59	Total N = 241
Gender (n [%])	Male	8 (13.1)	9 (15.0)	7 (11.5)	9 (15.3)	33 (13.7)
	Female	53 (86.9)	51 (85.0)	54 (88.5)	50 (84.7)	208 (86.3)
Age (years)	Mean (SD)	52.0 (11.43)	46.9 (10.27)	49.2 (13.75)	49.4 (11.19)	49.4 (11.81)
	Median	54.0	45.5	49.0	51.0	50.0
	Min, max	25, 74	27, 76	22, 74	25, 72	22, 76
Race (n [%])	Hispanic or Latino	3 (4.9)	9 (15.0)	3 (4.9)	13 (22.0)	28 (11.6)
	Not Hispanic or Latino	58 (95.1)	51 (85.0)	58 (95.1)	46 (78.0)	213 (88.4)
	White	56 (91.8)	59 (98.3)	59 (96.7)	54 (91.5)	228 (94.6)
	Black or African American	4 (6.6)	0	1 (1.6)	3 (5.1)	8 (3.3)
	Asian	0	1 (1.7)	1 (1.6)	0	2 (0.8)
	American Indian or Alaska Native	1 (1.6)	1 (1.7)	1 (1.6)	2 (3.4)	5 (2.1)
	Native Hawaiian or Other Pacific Islander	1 (1.6)	0	0	0	1 (0.4)
Baseline FWS† (n [%])	Moderate (2)	8 (13.1)	9 (15.0)	9 (14.8)	8 (13.6)	34 (14.1)
	Severe (3)	53 (86.9)	51 (85.0)	52 (85.2)	51 (86.4)	207 (85.9)

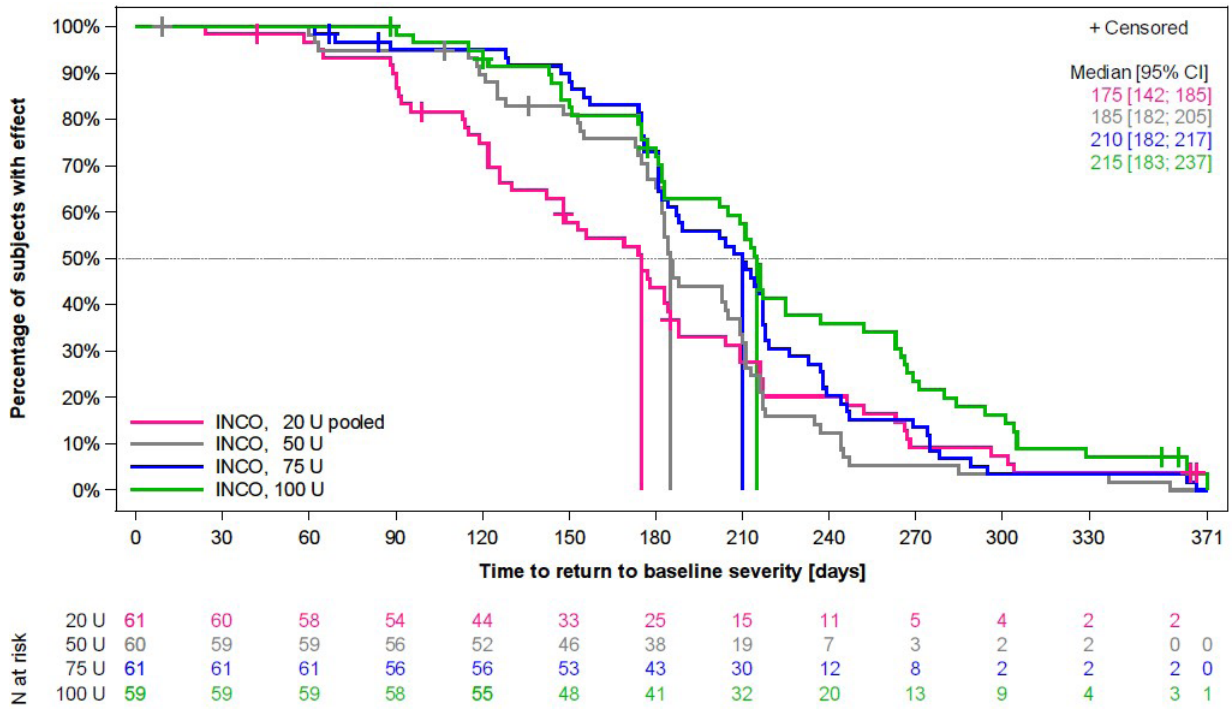
Abbreviations: FWS, Facial Wrinkle Scale; SD, standard deviation. *One subject randomized to the 100U group was treated with 20U. †As assessed by investigator at maximum frown.

FIGURE 1. Subject disposition.



*Three subjects were re-screened once, resulting in a total of 259 screenings. One subject was a screening failure while the other two subjects were randomized after second screening.
†One subject (Stage 2) was randomized to 100U but was treated with 20U.
‡There were two discontinuations related to the COVID-19 pandemic (one subject in the 20U group and one in the 100U group).

FIGURE 2. Investigator-assessed duration of effect for 20, 50, 75, and 100U INCO dose groups.



Effect defined by ≥1-point improvement from baseline severity at maximum frown on the Facial Wrinkle Scale. Kaplan-Meier plot, FAS. Final results for 20U (N=61), 50U (N=60), 75U (N=61), and 100U (N=59) groups. Numbers in legend denote median time to return to baseline severity and 95 % confidence interval.

Primary Efficacy Endpoint

Duration of effect as assessed by the primary efficacy variable increased with increasing dose of INCO (for Kaplan-Meier curves, see Figure 2). The median duration of ≥1 point improvement from baseline was 175 days (25 weeks) for the 20U group, 185 days (26 weeks) for the 50U group, 210 days (30 weeks) for the 75U group, and 215 days (31 weeks) for the 100U group (see Table 2 for median durations with 95% CIs).

Pairwise comparisons of dose groups based on hazard ratios (HRs) from Cox proportional hazard regression performed over the entire 360-day follow-up period indicated significantly longer duration of effect for 100U vs 20U INCO (HR=0.56 [95% CI 0.38, 0.83]; *P*=0.0035) and for 100U vs 50U (HR=0.55 [95% CI 0.37, 0.81]; *P*=0.0023) despite the study not being powered for confirmatory statistical significance testing between the dose groups.

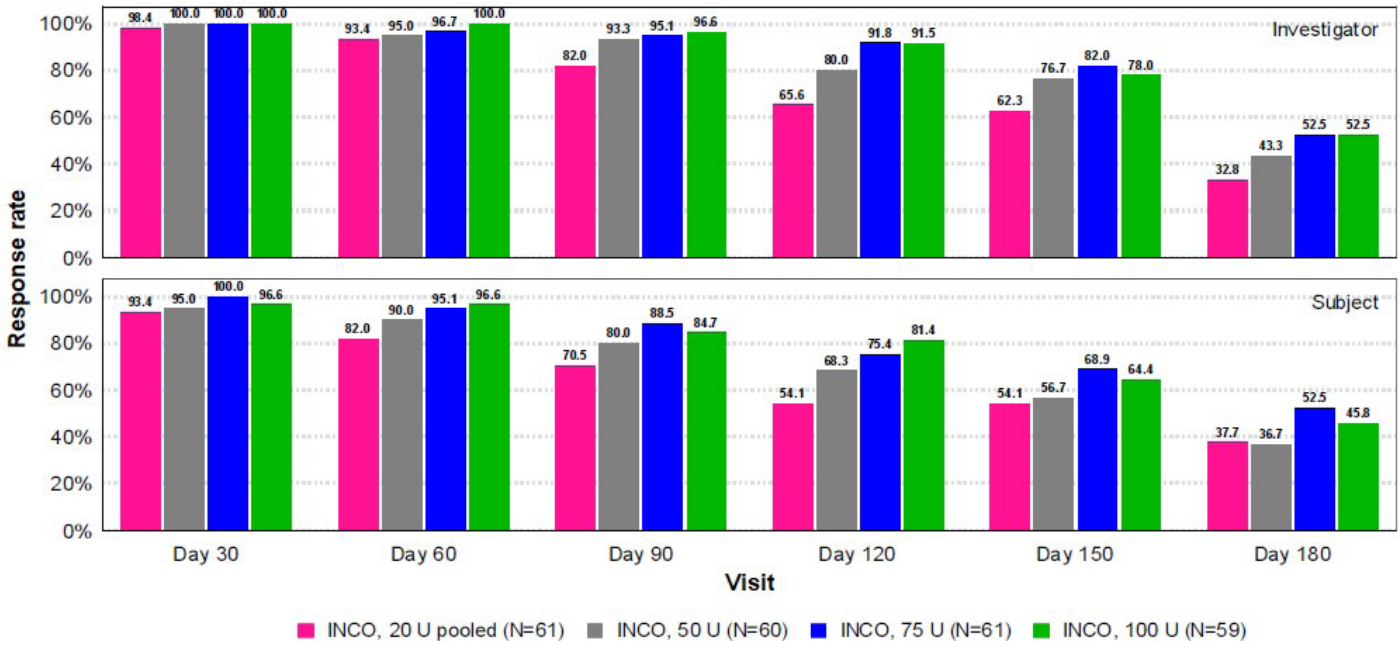
Secondary and Other Efficacy Endpoints

The median duration of an effect defined as FWS score of none (0) or mild (1) at maximum frown and as 2-point improvement on the FWS for each INCO dose group is shown in Table 2. In line with the primary efficacy variable, both secondary duration of effect variables consistently increased with increasing dose. For the response rate defined as ≥ 1 point improvement as determined by the investigator (Figure 3, top) and subject (Figure 3, bottom), there was a clear dose-response relationship across INCO dose groups with increasing doses resulting in a greater percentage of responders over time for higher doses.

TABLE 2.

Duration of Effect Across INCO Dose Groups				
Primary efficacy variable	**INCO 20U**	**INCO 50U**	**INCO 75U**	**INCO 100U**
Time to return to baseline severity Median in days [95% CI]	175 [142; 185]	185 [182; 205]	210 [182; 217]	215 [183; 237]
Secondary duration of effect variables	**INCO 20U**	**INCO 50U**	**INCO 75U**	**INCO 100U**
Duration of effect – "none or mild" Median in days [95% CI]	113 [91; 134]	121 [113; 149]	129 [122; 157]	148 [125; 173]
Duration of effect – 2-point improvement Median in days [95% CI]	96 [90; 118]	118 [91; 127]	122 [119; 127]	145 [120; 149]

FIGURE 3. Response rate ≥ 1-point improvement from baseline on the Facial Wrinkle Scale at maximum frown as assessed by investigator and by subject over time.



For the response rates defined as "none or mild" as determined by the investigator (Figure 4, top) and the subject (Figure 4, bottom), there was a dose-response relationship with higher response rates with increasing INCO dose. The 75U group was comparable to 100U at some of the timepoints. High response rates were reached in all four dose groups after treatment even though most subjects (85.0%-86.9% per dose group) had a baseline FWS score of "severe" (Table 1) and thus needed at least a 2-point improvement (from a score of 2 or 3 to 0 or 1) to reach this response criterion.

FIGURE 4. Response rate "none or mild" on the Facial Wrinkle Scale at maximum frown as assessed by investigator and by subject over time.

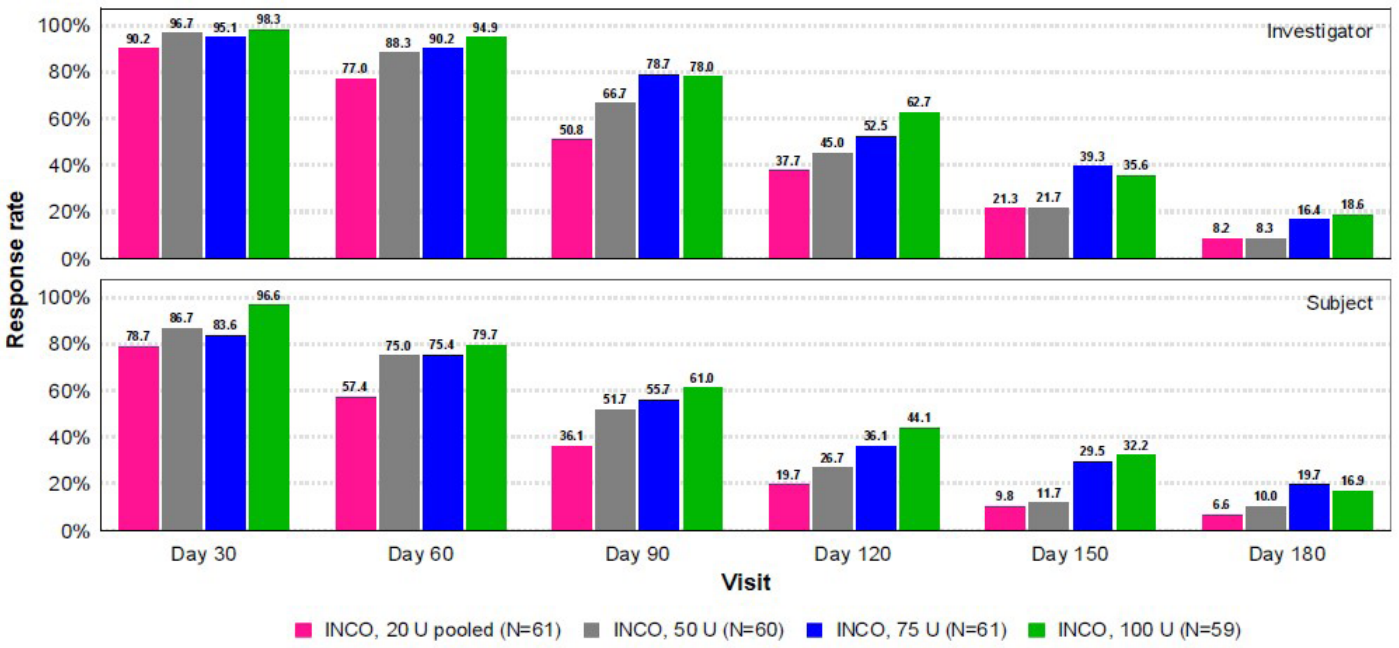
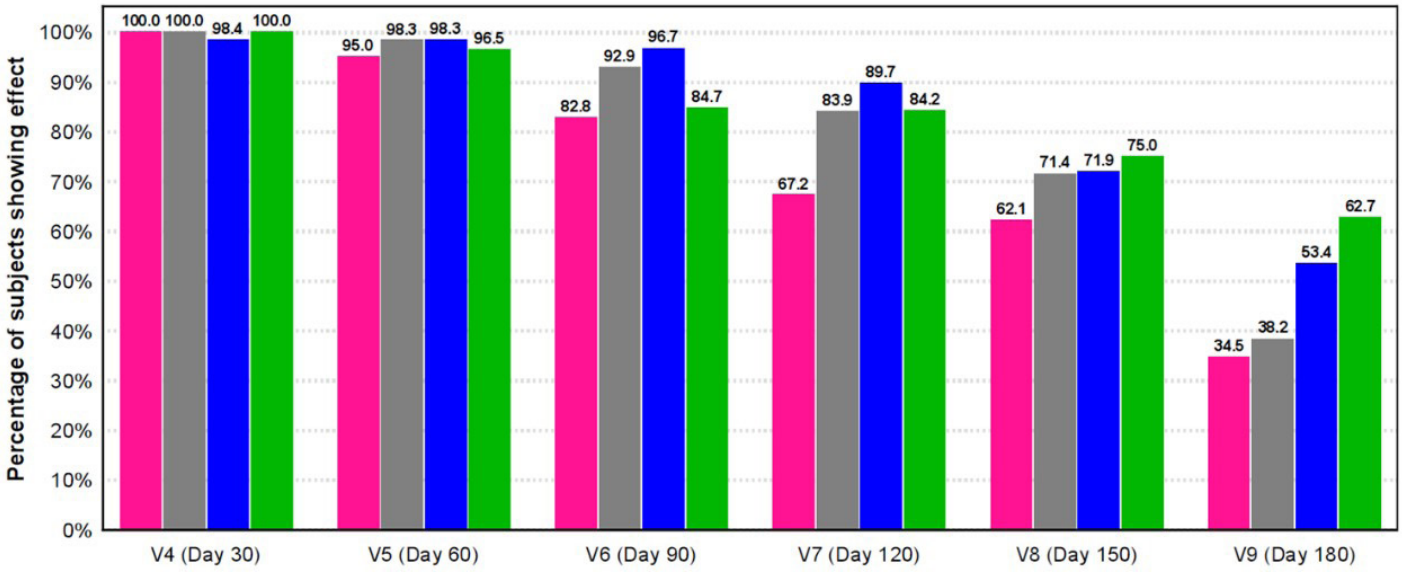


FIGURE 5. Response rates of subjects rating themselves improved or better on Global Aesthetic Improvement Scale.*



*Percentage of subjects rating themselves with a score of 1 or greater on Global Aesthetic Improvement Scale (-3 = very much worse, -2 = much worse, -1 = worse, 0 = no change, 1 = improved, 2 = much improved, 3 = very much improved).

FIGURE 6. Subjects treated with (a) 50U, (b) 75U, and (c) 100U of INCO. Subjects are shown at baseline, day 30, day 180, day 240, and day of return to baseline.

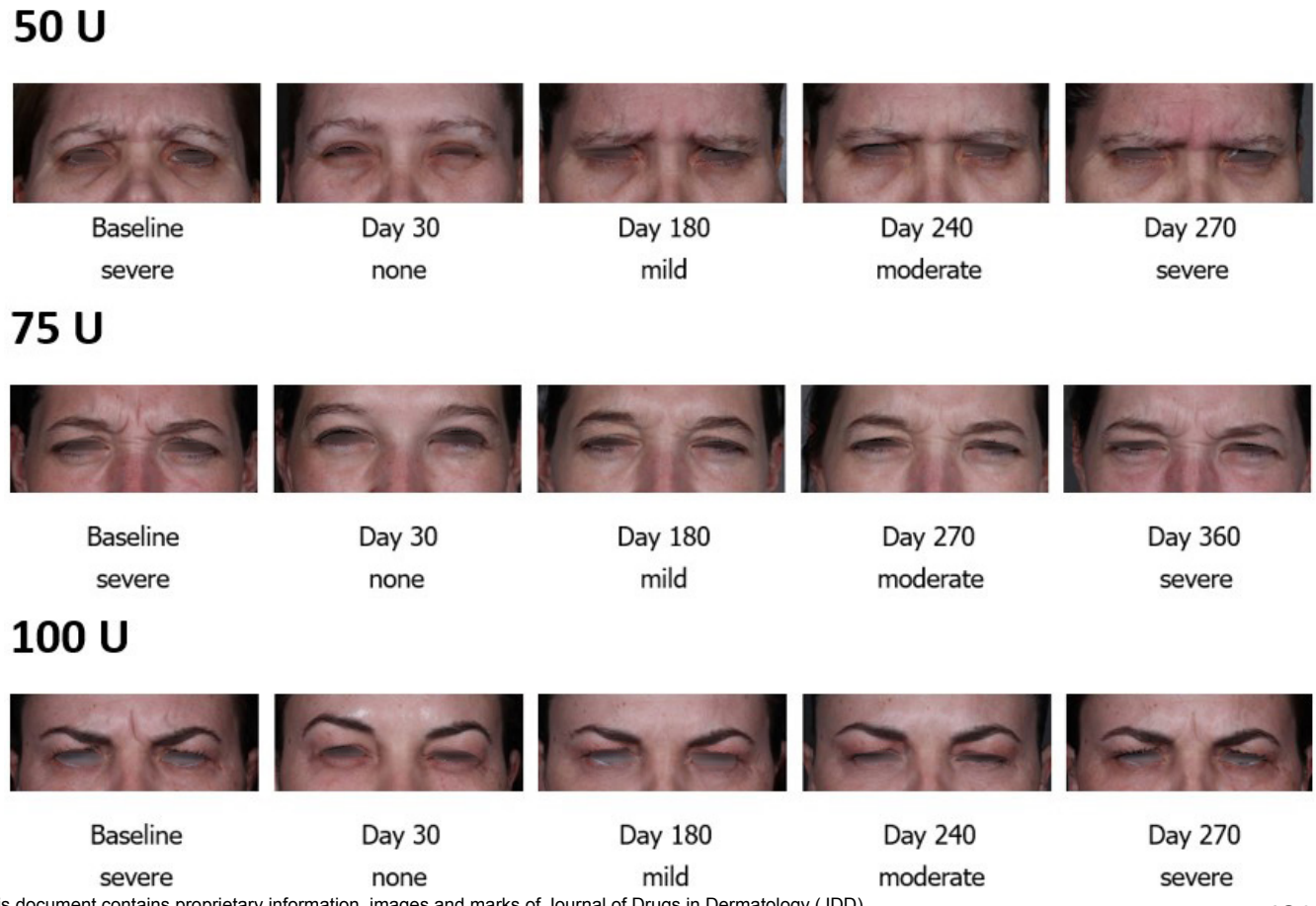


TABLE 3.

Incidence of Treatment-Emergent Adverse Events by Dose Group Over the Entire Main Period of up to 360 Days (SES)

	Stage 1 and 2	Main Period (up to 360 Days)			
Dose Group	Adverse event	INCO 20U (N=62[b])	INCO 50U (N=60)	INCO 75U (N=61)	INCO 100U (N=58[b])
Any TEAE (n [%])	--	23 (37.1)	23 (38.3)	26 (42.6)	22 (37.9)
Any TESAE	--	0	0	0	1(1.7)[a]
Related TESAE (n [%])	--	0	0	0	0
TEAEs with incidence rate ≥ 5% (n [%])	Nasopharyngitis: Headache:	11 (17.7) 6 (9.7)	6 (10.0) 6 (10.0)	10 (16.4) 1 (1.6)	8 (13.8) 4 (6.9)
Related TEAEs (n [%])	--	7 (11.3)	6 (10)	8 (13.1)	7 (12.1)
TEAESI (n [%])	Eyelid ptosis: Constipation:	0 1 (1.6)	0 0	2 (3.3) 0	2 (3.4) 0

[a]One subject in the 100U group developed appendicitis that the investigator determined was not related to treatment.
[b]One subject randomized to the 100U group was treated with 20U.
Abbreviations: TEAE, treatment-emergent adverse event; TESAE, treatment-emergent serious adverse event; TEAESI, treatment-emergent adverse event of special interest.

TABLE 4.

Median Duration of Effect in the Treatment of GFL With Higher Doses[1]

	INCO			ABO	ONA			DAXI		
Dose	50U	75U	100U	120U	40U	60U	80U	40U	40U	40U
N	60	61	59	30	50	49	52	39	201	204
Study type	Ph 2	Ph 2	Ph 2	IIS	Ph 1b	Ph 1b	Ph 1b	Ph 2	Ph 3	Ph 3
Median (95% CI) duration of effect, days	185 (182–205)	210 (182–217)	215 (183–237)	150[†] (120–180)	168.7[†,*]	168.7[†,*]	168[†,*]	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.
[*]Reported duration of effect in weeks.[19,21,22]
[†]95% CI not provided.
ABO, abobotulinumtoxinA; CI, confidence interval; DAXI, daxibotulinumtoxinA; IIS, Investigator-Initiated Study; INCO, incobotulinumtoxinA; ONA, onabotulinumtoxinA; Ph, Phase.
Direct study comparisons cannot be made, as studies differ in several aspects.

The percentage of subjects rating themselves as improved or better on the GAIS was 95% or greater across all dose groups at days 30 and 60 (Figure 5). Over time, the percentage of subjects in the higher dose groups who rated themselves as improved or better was greater than that of the lower dose groups with a clear dose-response relationship evident at day 180. Subjects treated with 50, 75, and 100U INCO are shown at baseline, days 30, 180, and 240, and at visit when return to baseline was observed in Figure 6.

Primary Safety Endpoints

The incidence of TEAEs over the main period was 23 (37.1%) for the 20U group (N=62), 23 (38.3%) for the 50U group (N=60), 26 (42.6%) for the 75U group (N=61), and 22 (37.9%) for the 100U group (N=58) (Table 3). The TEAEs with incidences rate ≥ 5% were nasopharyngitis (14.5%) and headache (7.1%). Only one serious TEAE was reported (an appendicitis in the 100U group that the investigator determined was not related to treatment). No new or unexpected adverse events were observed.

The incidence of treatment-related adverse events was low across all doses (20U:7[11.3%], 50U:6[10.0%], 75U:8[13.1%], and 100U:7[12.1%]) (Table 3). All treatment-related TEAEs were mild to moderate in intensity. No treatment related serious TEAEs were reported. Only five subjects (2.1%) reported TEAESIs: eyelid ptosis (4[1.7%], all related to treatment with duration ranging from 44–108 days); and constipation (1[0.4%], unrelated to treatment).

DISCUSSION

These results demonstrate a clear INCO dose effect of at least 6 months duration for the majority of GFL subjects as assessed by ≥1-point improvement from baseline severity at maximum frown on the FWS. There was a consistent prolongation in the median duration of effect with increasing doses. For the primary endpoint, effect was observed in all dose groups up to day 90 and a strong dose effect became apparent thereafter. From day 120 there was a clear differentiation in duration between 20U, 50U and the two higher dose groups. After day 180, the best

efficacy results were seen in the 100U group. Sustained efficacy at the 20U labeled INCO dose was also notable, including a median duration of effect for none or mild of 113 days.

Preclinical studies have demonstrated that increased INCO doses result in more BoNT binding to motor endplates, allowing more light chain molecules to reach the cytosol of the neuron.[17] It takes longer to degrade additional light chain molecules, and hence, the duration of effect is prolonged. Clinical studies with increased doses of other BoNT products have demonstrated the same effect for treatment of GFL (Table 4).[18–22]

The duration of effect of INCO achieved in this study is especially notable because a large proportion of subjects (85.9%) were rated as severe by the investigator on the FWS at maximum frown at baseline. For secondary variables assessing duration of effect and response rates for effect defined by a score of none or mild, these subjects had to achieve at least a 2-point improvement, indicating that a greater duration of effect with INCO can be achieved even in these difficult to treat patients. The large proportion of severe subjects makes it difficult to compare these results to other trials involving large doses of BoNT/A products because these typically have included much lower proportions of subjects with severe GFL (31%–53%) per treatment group.[18–20]

In pairwise comparison of INCO dose groups, the increase in dose may not have been large enough to induce a statistically significant difference in duration of effect since the pharmacodynamics of BoNT/A are not dose-proportional. Differences in median time to return to baseline between dose groups might also be limited due to the predefined interval period between visits. Return to baseline could only be recorded at visits, which were performed every 30 days according to the clinical study protocol.

The safety analysis results were in line with the previous stage 1 findings that doses of INCO greater than 20U do not pose an increased risk of AEs and are as safe as 20U. Notably, the incidence of eyelid ptosis (N=4) in the total SES (N=241) was 1.7% (20U:0[0%], 50U:0[0%], 75U:2[3.3%], and 100U:2[3.4%]). The ptosis incidence was low and both the incidence and duration (44–108 days) were within the range for the approved 20U dose and comparable to other BoNT/A products.

The use of higher doses in aesthetic treatment is becoming more common. These results support the safety of using INCO in such an approach (eg, for large areas that require multiple injections up to 75 or 100U INCO, such as the full face), particularly in the context of the potentially lower immunogenicity of INCO.[4–9] OnabotulinumtoxinA (Botox®/Vistabel®, Allergan Inc.), prabotulinumtoxinA (Nabota®, Daewong Therapeutics, Korea/ Jeuveau®, Evolus Inc., USA/Nuceiva®, Evolus Inc., Canada, Europe), and abobotulinumtoxinA (Dysport®/Azzalure®, Ipsen Pharma, Wrexham, UK) contain complexing proteins and/ or denatured BoNT protein that may induce the production of neutralizing antibodies that can lead to a decreased effect over time or treatment non-response.[10,24] DaxibotulinumtoxinA (Revance Therapeutics, Inc., Nashville, TN, USA), which contains a virally-derived protein transduction domain (PTD), has been shown to induce detectable antibody titers in monkeys, but no real-world evidence in humans is available at this time.[25,26]

There is also an increasing desire from clinicians for more flexibility in BoNT dosing and intervals between treatments.[27] These results support an increased flexibility with INCO (eg, a lower dose more often or a higher dose less often) to achieve different patient needs and preferences, which are paramount in determining treatment approach.

Limitations of the study include the large proportion of subjects with severe grade GFL, which may not be representative of a clinician's patient population. Additionally, although this phase 2 study was not designed for confirmatory comparisons between dose groups, the Kaplan-Meier plot (Figure 2) revealed clear differences in duration of effect between all pairs of dose groups when looking at different time intervals between day 90 and day 300. Few subjects in any dose group returned to baseline severity by day 90 (month 3), and nearly all subjects had returned to baseline by day 300 (month 10). Interpretation of results should focus on overall trends in the Kaplan-Meier plot, which clearly shows that time to return to baseline severity increases with increasing the INCO dose. While a clear dose-response was demonstrated in our study, the dilution ratio may also play a role when it comes to duration of effect; however, further research would be required to confirm the role that the amount of diluent/injection volume plays.

CONCLUSION

These results clearly demonstrate that doses of INCO up to 100U are well tolerated, consistent with the known safety profile of a 20U dose, and increasing doses of INCO prolong the duration of effect for GFL.

DISCLOSURES

All authors except SR and GK have been consultants and/or investigators for Merz Pharmaceuticals GmbH and Merz North America, Inc. SR and GK are employees of Merz Pharmaceuticals GmbH. This study and publication were sponsored by Merz Aesthetics.

ACKNOWLEDGMENT

We wish to thank all study investigators and the R&D team for conducting the study. Medical writing support was provided for the first draft by Steve Mitchell, Merz North America, Inc., in accordance with Good Publication Practice (GPP3) guidelines.

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