

Incobotulinumtoxina is Effective and Safe for Treating Expression Wrinkles in the Upper Third of the Face: A Phase III, Open-Label Clinical Study

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Introduction

Botulinum neurotoxin type A (BoNT/A) produced by the wild type strain of *Clostridium botulinum* is used for the treatment of several neurologic disorders caused by increased muscle tone, such as cervical dystonia, hemifacial spasm and blepharospasm [1,2]. It is also effective for the treatment of axillary and palmar hyperhidrosis [3] and urologic disorders [4]. The use of botulinum toxin type A for cosmetic purposes was first described by Jean and Alastair Carruthers, who provided clinical evidence of its efficacy in the treatment of facial wrinkles [5]. In the past two decades, it has become evident that BoNT/A could be effective to treat facial wrinkles [6] and successful results have been published showing improvements of expression lines on the upper third of the face [7-9].

The active botulinum toxin (150 kDa) is produced as part of a high molecular weight complex comprising neurotoxin-associated proteins [2,10] that protect the neurotoxins from the harsh environment of the mammalian gastrointestinal tract during bacterial invasion of the host, but do not play any role in inhibiting local neuromuscular cholinergic transmission exerted by the neurotoxin [11,12]. Furthermore, while complexing proteins do not appear to limit diffusion and to be necessary for the stability of botulinum neurotoxin, they are also believed to stimulate the formation of antibodies against botulinum toxin type A, leading to subsequent treatment failure [10-13].

Conventional botulinum toxin type A drugs, including Botox® and Dysport®, contain the active botulinum toxin (150 kDa) as well as complexing proteins. On the other hand, Xeomin® (Incobotulinumtoxina / Xeomin®, Merz Pharmaceuticals GmbH, Frankfurt, Germany), a novel preparation free from complexing proteins, has been developed and, in contrast to other licensed botulinum toxin type A drugs containing complexing proteins, Xeomin® doesn't require refrigerated storage, as demonstrated in long-term storage studies and in short-term temperature stress-studies [14]. Xeomin® was first introduced in Germany in 2005 for the treatment of cervical dystonia and blepharospasm [15,16] and later approved, in 2009, for the treatment of glabellar frown lines [17]. In the present phase III, open label, single centre clinical trial, Xeomin® efficacy and safety is evaluated for the treatment of expression wrinkles in the upper third of the face.

Materials and Methods

Study drug

The botulinum toxin type-A, obtained from *Clostridium botulinum* bacteria culture, is first released as single chain protein and undergoes a cleavage at the last stage of fermentation, forming two polypeptide chains, a heavy chain of 100 kDa and a light chain of 50 kDa, linked by a disulfide bond. The native toxin is formed by the active neurotoxin (150 kDa) and other complexing proteins. The drug used in this study, Xeomin®, is a botulinum toxin type-A preparation where the complexing proteins have been removed by chromatographic

purification, therefore it only consists of the 150 kDa type-A pure neurotoxin.

Study design

One hundred and twenty one female patients were included in this phase III single centre, national, open-label, prospective clinical study between 1st of September 2009 and 9th of February 2010 (trial registered at ClinicalTrials.gov Id, NCT00986570). Inclusion criteria included females aged between 30 and 50 inclusive, with expression wrinkles in the upper third of the face of mild, moderate, or severe degree at maximum frown (severity score of 1, 2 or 3 on the Facial Wrinkle Scale (FWS)) as assessed by the investigator (0 = none, 1 = mild, 2 = moderate, 3 = severe). All patients with childbearing potential agreed to practice an effective contraceptive method. All patients signed the informed consent and the trial was approved by the National Research Ethics Committee and conducted according to the Declaration of Helsinki. Exclusion criteria were previous treatment with botulinum toxin in the upper third of the face in the last 6 months or previous implantation of permanent material and surgery; use of any anticoagulant up to 7 days prior to the investigational product application; concomitant use of aminoglycosides or other drugs that might interfere with neuromuscular transmission; coagulopathies and inflammation/infection at the application site; diseases that interfere with neuromuscular function such as myasthenia gravis or Eaton Lambert Syndrome; pregnancy or breastfeeding; allergy or known sensitivity to any component of the investigational product; participation in another clinical study over the past 12 months.

The study consisted of a single application of Xeomin® during Visit 1 and a follow up period of 4 months with further four visits (at 3,15,90 and 120 days). Xeomin® was supplied as a white lyophilized powder containing 100 U of botulinum toxin type A free from complexing proteins. Figure 1 shows the study design with details of the main procedures for each visit. Xeomin® was reconstituted in 1 ml of saline solution 0.9%, (with a final concentration of 10U/0.1 ml) and injected intramuscularly at Visit1 with the following procedure: 2 U per point, in 4 points in the frontal muscles; 5 points in the glabella (4 U in the procerus muscle, 4 U in the medial part of each corrugator muscle,

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and 4 U in the middle part of each corrugator muscle); 4 U per point, 3 points in the right and 3 points in the left lateral orbital rhytides.

Primary efficacy endpoint was based on wrinkles overall assessment from Visit1 to Visit 5, through standardized photos taken at each visit (Canon™, Omnia System™, Software Mirror™). The evaluation of wrinkles was performed by three independent investigators at all visits and the classification using a 4-point Facial Wrinkle Scale, none (0), mild (1), moderate (2), severe (3), was based upon at least two similar opinions among the investigators. The independent raters assessed encoded photos, so they were blinded concerning to which visit each photo corresponded to. A positive result was defined as a reduction of any degree to a lesser degree, with a reduction of at least 1 point; no change in classification was considered as failure.

Patients' self-assessment of wrinkles status was also carried at Visit 1, 3 and 5; the impact of product application on patients' quality of life was evaluated by the administration of a validated Brazilian version [18] of the Dermatology Life Quality Index (DLQI) questionnaire, at Visit 1, 3 and 5.

Safety evaluation was performed recording adverse events (AE) at all visits based on spontaneous reporting by patients or according to investigator observations. All patients that experienced AE, whether or not considered as associated with the use of study drug, were monitored until symptoms disappeared or a reasonable explanation for such event was found.

Statistical analysis

Probabilistic descriptive statistic was used. Data were analysed by recurrence by binomial distribution and non-parametric tests. Intervals were expressed as confidence intervals; the minimum sample needed to assure 95% confidence would have been 70 patients; a total of 121 patients were enrolled, 119 were considered for efficacy evaluation (Visit 3) and 121 for safety evaluation of study drug; 114 patients completed the study according to the protocol.

Results

Baseline characteristics

The 121 female patients included in this study had a mean age of 42.52 years (CI₉₅: 41.69-43.35 years), with expression wrinkles in the upper third of the face of mild, moderate, or severe degree at maximum frown (severity score of 1, 2 or 3 on the Facial Wrinkle Scale (FWS)) as assessed by the investigator (0 = none, 1 = mild, 2 = moderate, 3 = severe).

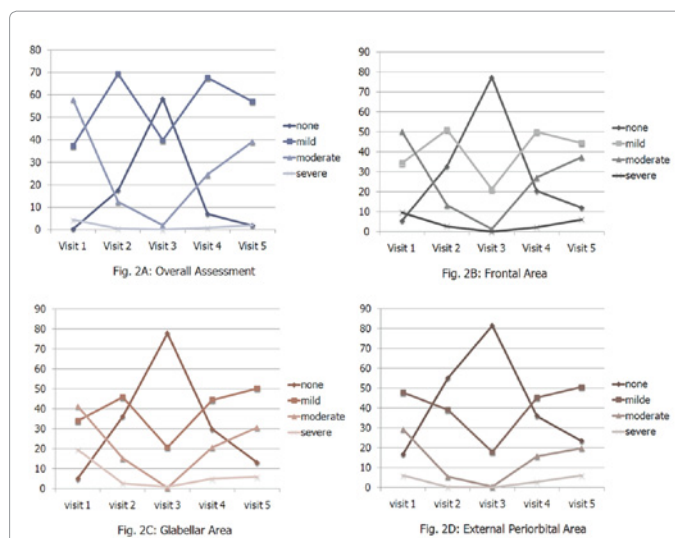
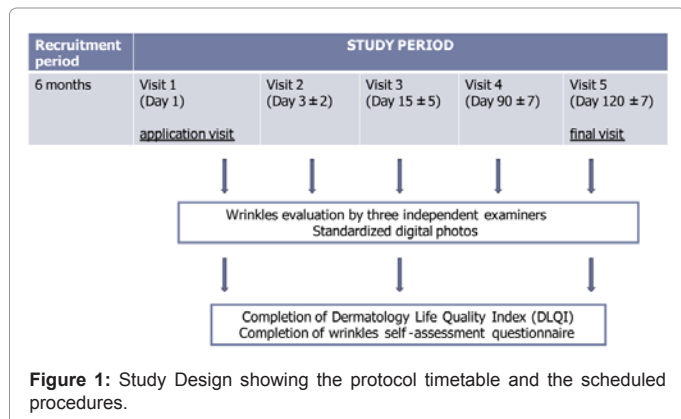


Figure 2: Summary of wrinkles degree assessments in the upper third of the face as measured by three independent examiners at each visit. Data are reported overall (A) and divided by area (B, frontal area; C, glabellar area; D, external periorbital area).

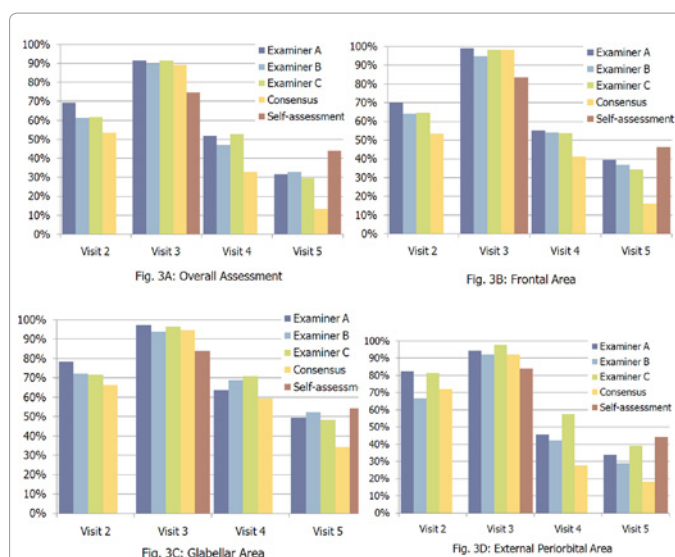


Figure 3: Summary of favourable responses to treatment of wrinkles in the upper third of the face as examined by three independent examiners at each visit, and self-assessed by the patients at visit 3 and 5. Data are reported overall (A) and divided by area (B, frontal area; C, glabellar area; D, external periorbital area).

Efficacy evaluations

Figure 2A shows overall wrinkle severity, as assessed by the 3 examiners. At Visit 1 the highest score was for wrinkles of moderate severity (58.05±4.09%). Three days after drug application (Visit 2), a trend started to emerge whereby wrinkles of moderate severity decreased (12.5±3.88%) while both none and mild severity wrinkles started to increase (17.5±1.27 and 69.44±2.9%, respectively). The peak of efficacy in this study was observed 15 days after neurotoxin injection (Visit 3) when the severity of wrinkles was none for 58.26±3.11% of patients. During the following two visits, 90 and 120 days post injection, the efficacy of the neurotoxin could still be observed. When

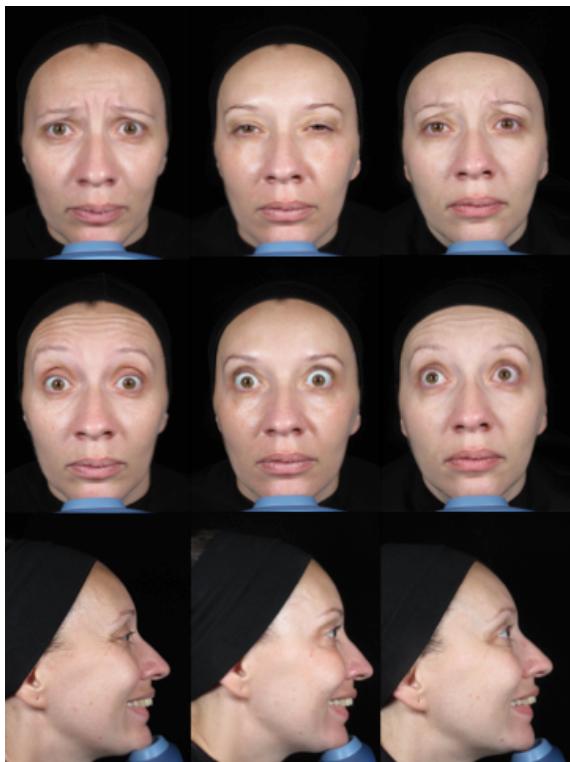


Figure 4: Clinical photographs at Visit 1 before treatment, at Visit 3 (15 days) and Visit 5 (120 days).

comparing the percentages of the various severity scores at Visit 5 with the assessment at Visit 1, the improvement was still visible as mild wrinkles represent $57.02 \pm 3.82\%$ at Visit 5 vs. $37.22 \pm 6.62\%$ at visit 1 and moderate wrinkles represent $39.18 \pm 4.06\%$ at Visit 5 vs. $58.05 \pm 4.09\%$ at Visit 1. The same trend was observed when frontal, glabellar and periorbicular external wrinkles were assessed separately (Figure 2 B-D). Interestingly, the glabellar wrinkles showed a higher rate of severity degree at Visit 1 ($19.72 \pm 4.74\%$) compared to the other areas and neurotoxin treatment effectively decreased and maintained the rate low until the end of the study observation period ($0.84 \pm 0.00\%$ at Visit 3 and $5.85 \pm 1.91\%$ at Visit 5).

The efficacy of treatment was then analysed in terms of favourable response defined as a reduction of any degree to a lesser degree, with a reduction of at least 1 point in wrinkles severity assessment. In Figure 3A-D, efficacy results are shown for the overall assessment and divided by area. Results from each examiner were compared and a consensus result was determined with an agreement between at least two of them. For visit 3 and 5 results of patients self-assessment are also reported. The results of the primary efficacy analysis showed high response rates in the treatment of the three muscle groups. Two weeks after injection (15 ± 3 days), the response rate regarding the FWS at maximum frown was 98.15% in the frontal muscles, 94.55% in the glabella, and 92.05% in the lateral orbital rhytides, as assessed by the panel of independent raters from standardized digital photographs. Four months (120 ± 7 days) after injection, the response rate at maximum frown was 16.04% in the frontal muscles, 34.29% in the glabella, and 18.09% in the lateral orbital rhytides. As shown in Figure 3A, the consensus efficacy results indicated a 53.33% of overall improvement at Visit 2 that rose to 89.08% by Visit 3 and then started to decrease with values of 32.74% at Visit 4 and 13.27% at Visit 5. It is interesting to notice that, although patients

self-assessment is broadly in agreement with the examiners assessment, this is slightly underestimated at the peak of efficacy with patients self-reported efficacy being 74.79% vs 89.08% of the examiners at Visit 3; conversely, at the end of treatment patients self-assessed improvement was still significant compared to the examiners consensus (43.86% vs. 13.27%) at Visit 5. The same trend was found when results are reported by area (Figure 2B-D). Clinical photographs are shown in Figure 4 at Visit 1 before treatment, at Visit 3 (15 days) and 5 (120 days).

Quality of life evaluation was performed through a self-administered questionnaire (Dermatology Life Quality Index, DLQI) before treatment, during Visit 1, and after treatment, at Visit 3 and Visit 5. The results of the DLQI were scored as recommended by the instrument developer [19] as reported in Table 1. Results shown in Table 2 indicated that, before treatment, a consistent number of patients was affected to some extent by wrinkles in terms of quality of life, with 55% of patients allocated in band 1 to 4. However, at Visit 3 quality of life resulted greatly improved, with 84.87% of patients being in band 0 (a two fold increase compared to visit 1) and all other bands showing a decreased rate. These results were also maintained at Visit 5. In fact, the rate of patients in band 0 is higher compared to Visit 1 while all the other bands were lower.

In addition, DLQI results performed at Visit 3 and 5 were compared with baseline results (Visit 1) and a favourable response was considered as a decrease in at least one band after treatment. As shown in Table 3, 93.94% of patients had a favourable response at Visit 3 and 79.69% at Visit 5.

Safety evaluations

Safety evaluation included all participants who receive treatment. There was no need to interrupt or postpone the treatment due to adverse events.

Systemic non-serious adverse events with causal relationship with treatment occurred in approximately one-fifth of the study population, mostly due to mild to moderate headache.

The most frequent treatment-related local adverse event was bleeding at the site of the application (particularly in the external periorbicular area). Regarding the intensity, adverse events were considered mild and disappeared with no need of any specific treatment.

Score	DLQI band	Effect on quality of life
0	0-1	No effect
1	2-5	Low effect
2	6-10	Moderate effect
3	11-20	Severe effect
4	21-30	Extremely severe effect

Table 1: Scores for interpretation of the Dermatology Life Quality Index (DLQI).

	n	Band 0	Band 1	Band 2	Band 3	Band 4
Initial evaluation	120	45.00%	27.50%	16.67%	8.33%	2.50%
Visit 3 (15±5 days)	119	84.87%	12.61%	1.68%	0.84%	0.00%
Visit 5 (120±7 days)	114	73.68%	17.54%	4.39%	3.51%	0.88%

Table 2: Summary of results of the Dermatology Life Quality Index (DLQI) by band of interpretation.

	n	Band reduction	C _{1gs}
Visit 3 (15±5 days)	66	93.94%	88.18%-99.70%
Visit 5 (120±7 days)	64	79.69%	69.83%-89.55%

Table 3: Reduction in the band of the Dermatology Life Quality Index (DLQI) post-treatment.

Only one serious adverse event was reported during the study; this was a severe gastroenterocolitis that required hospitalization and then resolved; however, this event was unrelated to treatment. No incidences of blepharoptosis or eyebrow ptosis were reported.

Discussion

Ageing is a dynamic and unchangeable process affecting several systems of the body, the more noticeable manifestations of which include wrinkles and flaccidity [20]. Psychological and physiological effects of skin ageing have generated a huge demand for a better understanding of the ageing process in order to provide effective interventions [21].

The use of BoNT/A for aesthetic indications was the result of clinical observations in patients treated with different facial dystonia who reported additional improvements of expression lines [6]. This has led to further investigations and several papers have published successful treatments of expression lines in the upper third of the face with BoNT/A [22-24].

In the present study, we have demonstrated the efficacy and safety of a novel BoNT/A preparation free of complexing proteins, Xeomin[®], in the treatment of expression lines. A rapid improvement of expression wrinkles was visible already 3 days after injection (Visit 2) and reached the maximal peak of action at Visit 3 (15±3 days after injection) with significant reduction of wrinkles of all severity degrees; a response was still evident in about half of the patients at Visit 4, and in one third of patients at Visit 5. Glabellar lines showed even better results and were still decreased in half of the patients at Visit 5; moreover the response rate in this area (34.29% after 120 days) is higher compared to data from literature (approximately 25% after 120 days) [8,9].

A report comparing Xeomin[®] with previous botulinum toxin preparation, Botox[®], showed similar efficacy results [17]. It is important to notice that although the initial patient self assessment was more stringent and a higher percent of patients judged their wrinkles as being severe or moderate compared to the examiners; conversely, patients self assessment at Visit 5 was much more positive compared to the examiners assessments, with half of the patients considering that the improvement was still present.

The same trend was observed when the effect of treatment on quality of life was analysed, showing a significative improvement in the quality of life throughout Visit 5, exceeding the effect independently assessed by the examiners. Since the indication of treatment for this study was mainly cosmetic, the improvement in quality of life can be considered as indication of treatment success.

Our data also reaffirm the need for an independent assessment in this type of studies, where there is a large subjectivity and treatment effects can easily be underestimated or overestimated, especially when psychological effects are also playing a role.

Systemic adverse events with causal relationship with study treatment occurred in approximately one-fifth of the study population, mostly due to mild to moderate headache. Bruising on application site was the main local adverse event reported and occurring mainly in periorbicular and frontal areas, showing anatomical difficulty for treatment application in these areas.

No incidences of blepharoptosis or eyebrow ptosis were reported. There was no report of treatment related to a serious adverse event.

In conclusion, this study demonstrated that Xeomin[®] represents

a fast acting, effective and safe treatment for expression wrinkles in the upper third of the face. The high satisfaction rates reported by the patients support the independent panel's and investigators' assessments of the high treatment success. An improvement in patient's quality of life, as assessed by Dermatology Life Quality Index questionnaire, was also reported.

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