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REVIEW ARTICLE

Treatment of glabellar lines with botulinum toxin type A (Speywood Unit): a clinical overview

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Abstract

Azzalure (Galderma) is a newly approved European botulinum neurotoxin type A (BoNT-A) specifically designed for aesthetic usages. It is sourced from Dysport (Ipsen Ltd.), which has a 20-year product consistency and has been used widely for various therapeutic and aesthetic applications. Azzalure and Dysport are collectively referred to as BoNT-A (Speywood Unit; s.U) (or abobotulinumtoxinA in the U.S.) after their biological activity unit, which is unique and not interchangeable with units of other commercial BoNT-A preparations. Azzalure is approved for the treatment of moderate-to-severe glabellar lines, with a total dose of 50 s.U distributed evenly among 5 injection points. To ensure optimal treatment outcomes with BoNT-A (s.U), it is crucial for injectors to adopt proper methods of reconstitution and injection, which can be acquired through training. We review here the method of reconstitution for BoNT-A (s.U), as well as the injection dose, points and techniques for glabellar line treatment. We also review the efficacy and safety results of BoNT-A (s.U) demonstrated in 11 clinical studies, most of which were randomized, double-blind and placebo-controlled. The studies included assessments after single injections as well as after up to 6 repeated treatment sessions. We summarize the clinical efficacy results, which include the responder rate 1 month post-injection, onset of response and duration of action, as well as safety results, which include incidence of treatment-emergent adverse events and specifically eyelid ptosis. The efficacy and safety profiles reported here are unique to BoNT-A (s.U) and cannot be generalized to other BoNT-A products.

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Keywords

botulinum toxin, glabella, efficacy, safety

Conflict of interest

Drs Rzany and Ascher have served as advisors, speakers and investigators for Galderma, investigators and speakers for Ipsen Ltd., clinical advisors and investigators for Merz Pharma, and clinical advisors for Medicis Inc. Dr Monheit has served as an advisor and speaker for Galderma, clinical investigator for Medicis Inc. and Mentor Co. He has also served on the facial advisory board for Allergan Inc.

Introduction

Botulinum neurotoxin (BoNT) is a neurotoxin protein secreted by an anaerobic, Gram-positive bacterium, *Clostridium botulinum*. There are seven serotypes of botulinum toxin (BoNT-A until -G) produced by different bacterium strains, with BoNT-A being the most potent of them all.[1] When ingested in a large quantity, BoNT causes a muscle paralyzing disease called botulism and leads to symptoms such as diplopia, ptosis, muscle weakness and difficulty in swallowing.[1] Internalized toxin complex cleaves proteins responsible for vesicle fusion at the neuromuscular junction, and prevents the release of acetylcholine, a common neurotransmitter which stimulates striated and smooth muscles as well as secretion of glands.[2] The muscle-modulating activity of BoNT was first noticed in 1817 and led to the hypothesis that small doses of BoNT could be beneficial in therapeutic usages. By now, several commercial preparations of BoNT-A products are widely used for the treatment of strabismus,[3] blepharospasm,[4] cervical dystonia[5] and other conditions in which excessive or involuntary muscle contraction is involved.

In the last decade, BoNT has been increasingly used in the field of aesthetics to reduce wrinkles and to rejuvenate skin.[6–8] Other than the commonly treated upper face region, BoNT-A has been gradually used in the perioral, chin and neck regions, either alone or combined with other aesthetic procedures. Azzalure® (Galderma) is a BoNT-A product specifically designed for aesthetic applications and it recently received approvals in 15 European countries

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for the treatment of moderate-to-severe glabellar (frown) lines.[9] It is sourced from Dysport™ (Ipsen Biopharm Ltd, Wrexham, UK), which has consistent formulation and proven efficacy and safety in both therapeutic and aesthetic applications. The biological activities of Azzalure and Dysport are quantified using Speywood Units (s.U), which is unique and not interchangeable with the units of other commercial preparations of BoNT-A products. Therefore, Azzalure and Dysport are collectively referred to as BoNT-A (s.U). Dysport exists in two different quantities: Dysport (300 s.U) is approved as abobotulinumtoxinA in the U.S. for both therapeutic and aesthetic applications; Dysport (500 s.U) is only approved for therapeutic usages in Europe and can be also used in aesthetics applications in several countries outside of Europe.

To ensure optimal treatment results with BoNT-A (s.U), it is crucial to use proper methods of reconstitution and injection, which can be acquired through trainings. The clinical efficacy and safety of glabellar line treatment with BoNT-A (s.U) have been demonstrated in 11 clinical studies, most of which were randomized, double-blind and controlled (Table 1).[10–20] In this review, we summarize the reconstitution and injection techniques for treatment with Azzalure, as well as the demonstrated efficacy and safety after single injections and after long-term repeated treatments.

How to treat glabellar lines with Azzalure?

Azzalure is indicated for the treatment of moderate-to-severe glabellar lines, which are hyperfunctional vertical wrinkles between the eyebrows. These lines are exaggerated by facial expressions such as anger, fear or anxiety. The presence of glabellar lines can have important psychological impacts on the patients: many patients notice being considered as angry when they are not.[7,8] Injection of Azzalure can temporarily relax the responsible muscles and improve patient's appearance.

The effectiveness and safety of the treatment with Azzalure is ensured when proper techniques of reconstitution and injection are adopted. Here, we review the treatment procedure, which was validated in 10 large-scale international clinical studies.[10–19] The procedure is specific to BoNT-A (s.U) and cannot be generalized to other commercial BoNT-A preparations, which have distinct chemical and biological properties, as well as different safety and efficacy profiles.

Reconstitution

According to prescribing information, the vial containing lyophilized white powder of Azzalure should be stored at 2–8 °C. Once reconstituted, Azzalure should be used within 4 h for a single patient during a single treatment session.[9]

Each vial containing 125 s.U Azzalure should be reconstituted with 0.63 mL of sterile non-preserved physiological saline to reach a final concentration of 200 s.U/mL (or 10 s.U/0.05 mL). This concentration of BoNT-A (s.U) was used in all fixed-dose clinical studies, achieved by reconstituting Dysport (500 s.U) with 2.5 mL saline solution (Table 2).[11,13,14,16–19] The corresponding volume of reconstitution should be 1.5 mL for Dysport (300 s.U). The saline solution is pulled into the vial by partial vacuum. The vial should be gently rotated (not shaken) until the white powder is fully dissolved. To avoid losing product, the syringe should be detached briefly to eliminate the vacuum and to allow an easy uptake of solution. A 1cc insulin-type syringe bearing graduations of 10 s.U and 0.01 mL was specifically designed for reconstitution and injection of Azzalure. The syringe can be attached to a 21G-40 mm needle for reconstitution and to a 30G-13 mm needle for injection. For Dysport (500 and 300 s.U), a syringe of larger volume should be adopted based on the volume of reconstitution.

Injection points and technique

The glabellar lines arise mostly from the activity of the procerus and the corrugator supercilii, two muscles that can be identified visually and by palpation when patients frown. The procerus, a

Table 1 Summary of clinical studies on the treatment of glabellar lines with botulinum neurotoxin type A (BoNT-A; Speywood Unit)

	Randomized, double-blind and controlled	Dose (s.U)	Study duration (month)	No. patients receiving BoNT-A	No. BoNT-A treatments
Single treatment					
Ascher[10]	X	25, 50 and 75	6	102	102
Rzany[11]	X	30 and 50	4	145	145
Monheit[12]	X	20, 50 and 75	4	279	279
Study 718[13]	X	50	5	200	200
Brandt[14]	X	50	6	105	105
Kane[15]	X	50–80*	5	544	544
Repeated treatment					
Ascher[16]	X	50	9	50	150
Moy[17]		50	13	1200	4214
Rubin[18]	X	50	23	311	743
Monheit[19]		50	17	768	2259
Rzany[20]		~100 for the upper third face	3–5 cycles	945	4103

*In Kane *et al.* study,[15] variable dose was used based on investigator's assessment on the muscle mass of each patient.

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Table 2 Reconstitution of botulinum neurotoxin type A (Speywood Unit)

	Quantity (s.U)	Volume of reconstitution (mL)	Final concentration	Injection volume for	
				10 s.U	5 s.U
Azzalure	125	0.63	10 s.U/0.05 mL (200 s.U/mL)	50 μL (0.05 mL)	25 μL (0.025 mL)
Dysport	300	1.5			
Dysport	500	2.5			

thin muscle that lies vertically between the eyebrows, originates from the nose bridge and inserts into the skin of the glabella.[7,8] Contracting the procerus induces a horizontal line. The corrugator supercilii is a narrow and strong paired muscle with deep medial insertion at the glabellar periosteum and another more superficial lateral insertion into the skin above the midbrow region. Contraction of the corrugator narrows the distance between eyebrows and creates vertical lines in between.

Five injections of 10 s.U/0.05 mL each were used in all prospective clinical studies except one.[10,12–19] The middle injection point targeted the procerus and was located between the level of eyebrows and the root of the nose. The other four injection points were symmetrical, with two points targeting each corrugator. The more medial points were administered directly above the inner canthus. The exact positions of the lateral points were not identical in all studies. In the U.S. studies, the two lateral injection points were at the mid-pupillary lines,[12–15,17–19] whereas in the European studies, they were more medial to the mid-pupillary lines, targeting the second third of the corrugator (Fig. 1).[10,16] The latter approach targets the medial part of the corrugator, reduces the potential risk of eyebrow ptosis and was adopted recently in a North American study using another commercial preparation of BoNT-A.[21] To reduce the risk of eyelid ptosis, it is recommended to avoid injecting near the levator palpebrae superioris, particularly in patients with larger brow depressor complexes (depressor supercilii), by placing the corrugator injection points at least 1 cm above the bony supraorbital ridge and by ensuring accurate injection volume and dose.[9]

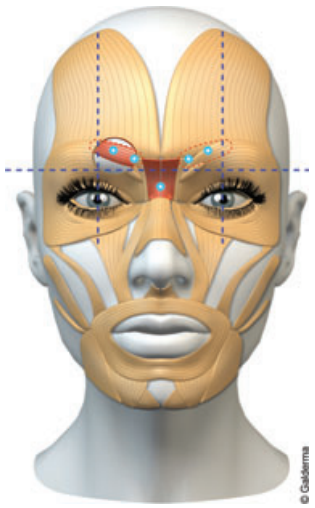

Figure 1 The injection points for glabellar line treatment with botulinum neurotoxin type A (Speywood Unit) used in the European studies[10,16]

The medial fibres of the frontalis are intertwined with the corrugator and may also contribute to the wrinkle formation in the glabellar region. However, two additional injection points in the frontalis did not increase the treatment efficacy, compared with a three-point injection targeting the procerus and each corrugator.[11] The responder rates of the two treatment groups at maximum frown at week 4 were similar (86.1% and 86.3%), and were significantly higher than the responder rate observed in placebo groups ($P < 0.001$). At week 16, the proportion of patients who were at least moderately satisfied with the treatment (61.8% and 67.1%) was also comparable between the two treatment groups.

A similar injection technique was used in all clinical studies. A 30G-13mm needle was used in most of the studies.[12–15,17–19] Patients were in half-seated position, and intramuscular injection was performed perpendicularly to the skin, with no prior test, local anaesthesia or other pre-medication.[10,16] Injection at the two lateral points targeting the corrugator may be subdermal to better target the muscle and to avoid the supraorbital vessels.[15]

Dose

The optimal total dose for the glabellar line treatment with BoNT-A (s.U) is 50 s.U, established in two dose-finding studies performed in France and in the U.S. respectively.[10,12] Both studies were double-blind, randomized and placebo-controlled, with three doses of BoNT-A (s.U) tested. The responder rate, defined as percentage of patients having a Glabellar Line Severity Score (GLSS) of 0 or 1 (on a scale of 0–3) post-injection, was assessed at rest and at maximum frown by blinded investigators in both studies (Table 3). The responder rates on Day 30 observed in all treatment groups were significantly higher than that in the placebo group ($P < 0.016$), suggesting that the BoNT-A (s.U) injection was efficacious for the treatment of moderate-to-severe glabellar lines. Overall, the treatment efficacy of the 50 s.U and 75 s.U groups was very similar, although higher responder rate was observed in the 50 s.U group at rest in the Ascher *et al.* study and in the 75 s.U group at maximum frown and at rest in the Monheit

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Table 3 The investigator-assessed responder rate (defined as percentage of patients with no or mild wrinkles) on Day 30 post-injection reported in two dose-finding studies[10,22]

	Ascher[10]				Monheit[22]			
	25 s.U (*n* = 34)	50 s.U (*n* = 34)	75 s.U (*n* = 34)	Placebo (*n* = 17)	20 s.U (*n* = 91)	50 s.U (*n* = 93)	75 s.U (*n* = 95)	Placebo (*n* = 94)
At maximum frown (%)	52	76	76	7	65	77	85	6
P-value (vs. placebo)	<0.016	<0.001	<0.001	–	<0.001	<0.001	<0.001	–
At rest (%)	72	93	76	13	63	68	76	17
P-value (vs. placebo)	<0.001	<0.001	<0.001	–	<0.001	<0.001	<0.001	–

et al. study. The discrepancy could be a result of slight variations in distribution of baseline GLSS, age and gender, all of which could affect responder rate.[15]

The duration of response was shorter for groups of 20 or 25 s.U. On Day 90, the responder rate at maximum frown in the groups of 50 s.U and 75 s.U (48% and 52% respectively) was much higher compared to that in the group of 25 s.U (32%), although the responder rate of all treatment groups remained significantly higher compared to that of placebo ($P < 0.016$).[10] Results of the same trend were observed in the Monheit *et al.* study. By Day 120, the responder rate at maximum frown in the groups of 50 s.U and 75 s.U (26% and 27% respectively) remained significantly higher compared with placebo (1.1%, $P < 0.001$); However, the responder rate in the 20 s.U group (5.7%) was no longer significantly different from that of placebo ($P = 0.071$).[22] Thus, treatment effect with 50 s.U or 75 s.U lasted at least 4 months, much longer than the duration of response with 20 s.U or 25 s.U.

All tested doses were well tolerated. The percentage of patients experiencing treatment-emergent adverse events (TEAE) was similar between all treatment groups and placebo in both studies.[10,12] Eyelid ptosis was absent in one study,[10] and mild ptosis was reported by only 0.8% of patients in the other study.[12]

Patient satisfaction was assessed in Ascher *et al.*[10] At Month 1, 86% of patients in the 50 s.U group reported to be 'satisfied' or 'completely satisfied'. This rate was sustained throughout the study and 93% were satisfied 6 months after injection, compared to 69% and 62% in the groups of 25 s.U and 75 s.U respectively.

Taken together, a total dose of 50 s.U with 10 s.U/0.05 mL per injection point is optimal for the treatment of glabellar lines with BoNT-A (s.U). Injection with 50 s.U of BoNT-A (s.U) provided high responder rate, long duration of response, good safety and great patient satisfaction.

Although a total dose of 50 s.U was deemed optimal based on results of clinical studies, the optimal dose for each individual in clinical practice may differ. For dose and injection point adjustments before treatment, physicians should carefully study the patient's facial anatomy at maximum frown as well as at rest. Physicians should also inquire about patient's preference between a more natural look and drastic changes. Special attention should be paid on any pre-treatment facial asymmetry. Gender, age and race were reported to have an impact on treatment efficacy.[12,14,15] When all patients received a fixed-dose of 50 s.U, the responder rate on Day 30 was higher for women, younger patients and non-Caucasians, although all subgroups demonstrated significantly higher efficacy than placebo ($P < 0.003$; Table 4).[14]

The efficacy and safety of variable dose treatment with BoNT-A (s.U) were evaluated in a randomized and double-blind study.[15] Depending on the muscle mass of their procerus and corrugator, women received injection of 50–70 s.U and men received injection of 60–80 s.U. All tested doses were well-tolerated. The investigator-evaluated responder rate at maximum frown on Day 30 was significantly greater in the BoNT-A group than in the placebo group ($P < 0.001$; 85% vs. 3%).

Patient satisfaction

It is important to gauge patient's satisfaction level which, to a large extent, depends on patients' expectation before treatment.[24] Patient education and counselling are crucial and should be integral parts of the treatment process. Before treatment, physicians should inform the patients about the expected onset of action and duration of response. Physicians should also explain about potential treatment-emergent adverse events, and if necessary, the treatments for correcting the unwanted effects.

Patient satisfaction was significantly higher in the 50 s.U BoNT-A group than the placebo group until at least 4 months after injec-

Table 4 The investigator-assessed responder rate with botulinum neurotoxin type A (Speywood Unit) on Day 30 post-injection reported in subgroup analysis studies[15,23]

	Gender		Age		Race	
	Female	Male	≤50 years	>50 years	Caucasian	Non-Caucasian
50 s.U (Brandt[23])	93% (*n* = 88)	67% (*n* = 15)	97% (*n* = 86)	53% (*n* = 17)	85% (*n* = 52)	94% (*n* = 51)
Variable dose (Kane[15])	87% (*n* = 475)	65% (*n* = 62)	88% (*n* = 304)	80% (*n* = 233)	84% (*n* = 358)	87% (*n* = 179)

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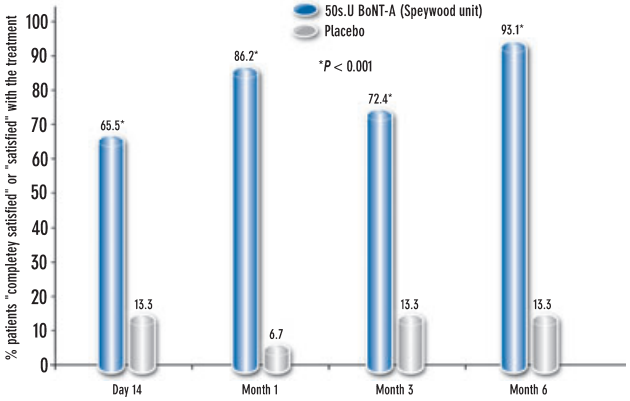

Figure 2 Percentage of patients reported to be 'completely satisfied' or 'satisfied' at various time points post-injection.[10] Patients rated their satisfaction level on a 4-point scale (completely satisfied, satisfied, somewhat satisfied or not satisfied).

tion.[10,16] The proportion of patients who reported to be 'completely satisfied' or 'satisfied' with the 50 s.U treatment was 86.2% at Month 1 and 93.1% at Month 6 ($P < 0.001$ vs. placebo; Fig. 2).[25] Similar high patient satisfaction level was reported in another study after the first and the second injections (Table 5),[16] suggesting that the high satisfaction level observed after initial injection did not decrease after repeated treatments.

The overall satisfaction level was examined in a retrospective study, in which up to five cycles of injection was administered in a total of 945 patients.[20] The satisfaction level was consistently high among all treatment cycles, with 96–99% of patients and 88–94% of physicians satisfied with treatment effect (Fig. 3).[27] The high patient satisfaction reported from those 3 studies involving more than 1000 patients is in contrast to a small case report showing low patient satisfaction rate with BoNT-A (s.U) at Week 16 post-injection.[28] The case report, which compared global satisfaction level with one BoNT-A to the satisfaction level at a specific time point after another BoNT-A treatment, was neither blinded nor randomized and therefore did not provide sufficient evidence for its conclusion to be validated.[29]

Summary

- The method of glabellar lines treatment with BoNT-A (Speywood Unit) has been validated in 10 international well-powered clinical studies.
- Azzalure and Dysport are quantified in unique 'Speywood Unit' and have distinct properties compared to other commercial preparations of BoNT-A.
- Proper methods of reconstitution and injection can be acquired through training programmes and are crucial for ensuring treatment efficacy and safety.
- Azzalure (125 s.U) should be reconstituted with 0.63 mL of saline for a final concentration of 200 s.U/mL (or 10 s.U/0.05 mL). Correspondingly, Dysport (300 s.U) and Dysport (500 s.U) should be reconstituted with 1.5 and 2.5 mL of saline, respectively.
- A five-point injection should be performed in the glabella region, with one point targeting the procerus and two points for each corrugator. The optimal dose for glabellar lines treatment is 50 s.U, with 10 s.U per injection point.
- Patient education is crucial. Consistent high patient satisfaction can be achieved when proper treatment procedures are adopted.

How efficient is the treatment with Azzalure?

Efficacy of BoNT-A treatment can be assessed by different evaluators using various methods. In clinical practice, physicians may document the treatment area with photographs and/or videos before and after injection, to compare treatment effect (Fig. 4). In clinical studies, efficacy can be evaluated in a real life setting by blinded investigators who assess the severity of wrinkles and the amount of effort the patient makes in attempt to frown. In addition, evaluators may notice details which are difficult to be captured on photographs. Alternatively, standardized digital photographs can be taken during the study and evaluated by an independent committee at the end of the study. As both the treatment and the

Table 5 Percentage of patients reported to be 'completely satisfied' or 'satisfied' at various time points post-injection[10,16]

	Ascher[10]		Ascher[16]		
	1st injection		1st injection		2nd injection
	50 s.U (n = 29)	Placebo (n = 15)	50 s.U (n = 50)	Placebo (n = 50)	50 s.U (n = 50)
Month 1 after injection (%)	86.2	6.7	78.0	10.0	85.4
Month 3 after injection (%)	72.4	13.3	73.5	14.2	85.1

Patients rated their satisfaction level on a 4-point scale (completely satisfied, satisfied, somewhat satisfied or not satisfied).
The satisfaction levels with botulinum neurotoxin type A were significantly higher than those with placebo in both studies and at both time points ($P < 0.001$).

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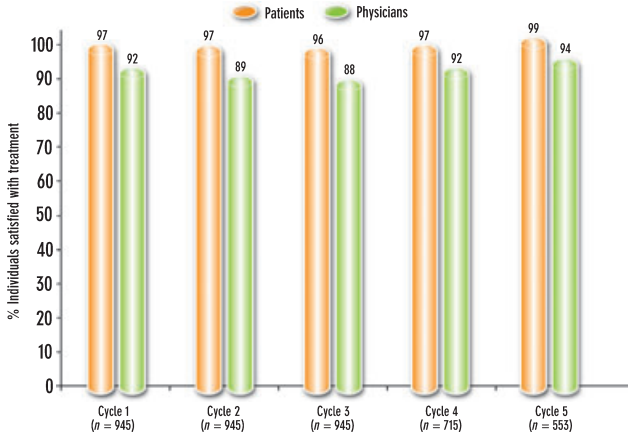

Figure 3 Percentage of patients or physicians reporting the overall effectiveness as 'satisfactory' for each treatment cycle with botulinum neurotoxin type A (Speywood Unit) on a 3-point scale (satisfactory, not satisfactory or unknown).[27]

time point are unknown to committee members, this approach leaves little room for subjective interpretation. However, organizing the committee requires substantial amount of effort. Furthermore, the independent committee cannot provide immediate estimation. Finally, blinded patients can also provide assessments on the severity of wrinkles at maximum frown and at rest.

The severity of glabellar lines at maximum frown and at rest is usually assessed using a standardized 4-point GLSS (0, none; 1, mild; 2, moderate; 3, severe). Good inter- and intra-observer reproducibility was achieved using this scale.[30]

The efficacy of glabellar lines treatment was demonstrated in 10 large-scale international clinical studies including about 4000 patients.[10–18,20] Injection with a fixed-dose of 50 s.U BoNT-A was used in five of those studies.[13,14,16–18] In all but one study,[11] the five injection points targeted the procerus (one injection point) and the corrugator (two injection points per side). All of the studies except one[17] were randomized, double-blind and placebo-controlled. Therefore, these study results provide high level of evidence regarding the overall treatment efficacy of BoNT-A (s.U).

Responder rate on Day 30

The responder rate 30 days after injection is a crucial criterion in evaluating the treatment efficacy of BoNT-A, as it provides an estimation on the 'success' rate of the treatment. In all but one study,[11] a 'responder' was defined as a patient having a GLSS of none (0) or mild (1) wrinkles after treatment. Since all patients had a GLSS of moderate (2) or severe (3) at study baseline, becoming a 'responder' corresponds to a 1–3 grade improvement on the severity of glabellar lines after treatment.

The proportion of patients responding to the treatment (responder rate) on Day 30 was assessed as a primary efficacy outcome in seven prospective studies (Table 6).[10,12–15,17,18] Assessments of wrinkle severity at maximum frown and/or at rest were provided by blinded investigators, patients and/or an independent committee. The Rzany *et al.*[11] study was not included because the injection points, definition of 'responder' and the grading scale were different form those of other studies.

The responder rate on Day 30 post-injection was significantly higher in the treatment group with 50 s.U BoNT-A than in the placebo group ($P < 0.001$) in all studies, regardless of the evaluator and the condition of assessment (at maximum frown or at rest), suggesting that BoNT-A (s.U) is significantly more efficacious than placebo in improving the severity of glabellar lines. Furthermore, the responder rate by investigator at maximum frown was consistently high across seven studies (76–96%), suggesting that both the method of assessment and the treatment efficacy are very reliable.

Similar responder rates had been reported previously in two multi-centre, randomized, double-blind and controlled studies on the efficacy of another commercial preparation of BoNT-A (Vistabel®/Botox®, Allergan).[31,32] In those studies, the investigator-assessed responder rate was 84% and 77% at maximum frown, and 80% and 70% at rest, 30 days after injection (Fig. 5). Four additional randomized and controlled studies with Vistabel/Botox were identified but not included for comparison, because the enrolled patients of those studies were either of a specific gender (female or male),[33,34] ethnic origin (Japanese)[35] or skin photoype (V and VI).[36] In addition, the injection points used in two studies

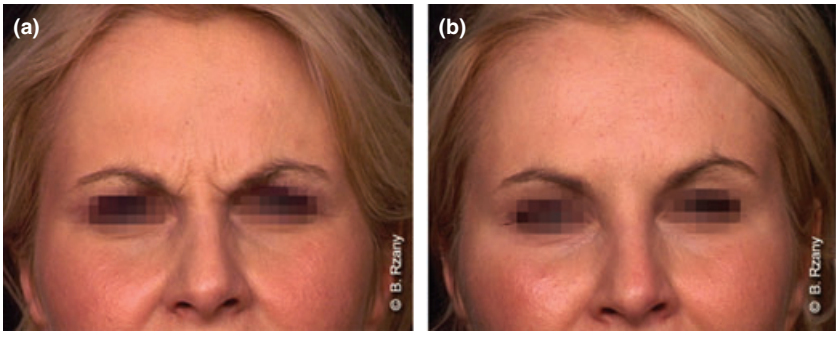

Figure 4 Representative photographs of a patient at maximum frown at baseline (a) and 14 days after treatment with 50 s.U Azzalure (b) in the glabella region

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Table 6 Responder rate with 50 s.U botulinum neurotoxin type A (BoNT-A; Speywood Unit) on Day 30 post-injection, assessed by independent committee, investigators or patients

		Ascher[10]	Monheit[22]	Study 718[13]	Brandt[23]	Kane[15*]	Moy[17†]	Rubin[18‡]
No. patients receiving 50 s.U BoNT-A[§]		34	93	200	105	22	1200	311
Committee	At maximum frown (%)	–	–	96	89	–	–	–
	At rest (%)	45	–	–	–	–	–	–
Investigator	At maximum frown (%)	76	77	90	90	96	84	82
	At rest (%)	93	68	73	–	–	–	–
Patient	At maximum frown (%)	–	–	86	76	96	75	71

*Variable dose was adopted in the study. Briefly, according to the muscle mass, women received 50, 60 or 70 s.U of BoNT-A (Speywood Unit); while men received 60, 70 or 80 s.U.

†Responder rate after the first injection of this study, which included five injection cycles.

‡Responder rate after the first injection of this study, which included one or two open-label cycle and two randomized and blind cycles.

[§]The actual sizes of the studies were larger, except those of Moy[17] and Rubin[18].

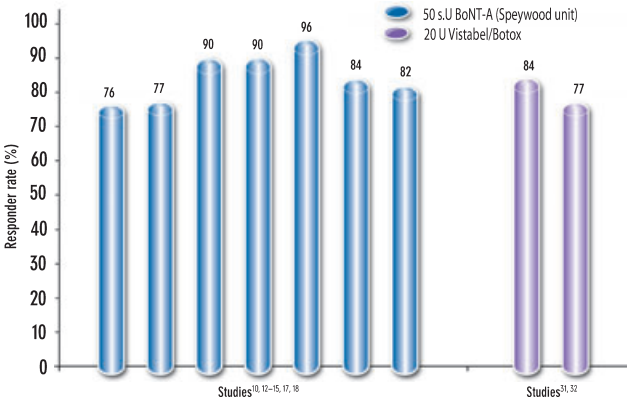

Figure 5 Investigator-assessed responder rates with botulinum neurotoxin type A at maximum frown on Day 30 post-injection

were different from the rest of studies.[33,34] Taken together, treatment of glabellar lines with approved quantity of BoNT-A products [50 s.U for BoNT-A (s.U) and 20U for Vistabel/Botox] leads to similar responder rates 30 days after injection.

Onset of response

Onset of response was assessed in five studies based on patient diary cards. It was defined as the first day a patient responded 'yes' to the question, 'Since being injected, have you noticed any effect on the appearance of your glabellar lines?' The median time to onset was determined to be 2–4 days in three single-treatment, double-blind studies[13–15] and two repeated-treatment studies[17,18] (Table 7). In each study, response as early as 24 hours after treatment was reported. In Brandt *et al.* the median time to onset was determined to be 3 days for BoNT-A (s.U) and 15 days for placebo ($P < 0.001$; Fig. 6).[23] The responder rate was 15% on Day 1 and 35% on Day 2. On Day 14, the cumulative responder rate reached 88%, similar to that observed on Day 30 (90%). Similar results were observed in study 718, in which 33% of total patients noticed treatment effect within 24 h after injection.[13] Taken together, the median time to onset of response for BoNT-A (s.U) treatment is 2–4 days. No such data are currently available for other commercial preparations of BoNT-A products.

Table 7 Median time to onset of response as reported by patient diary card

	Study 718[13]	Brandt[14]	Kane[15*]	Moy[17†]	Rubin[18‡]
Median time to onset (days)	2	3	4	3	3

*Variable dose was adopted in the study.

†,‡Median time to onset for all cycles in the studies.

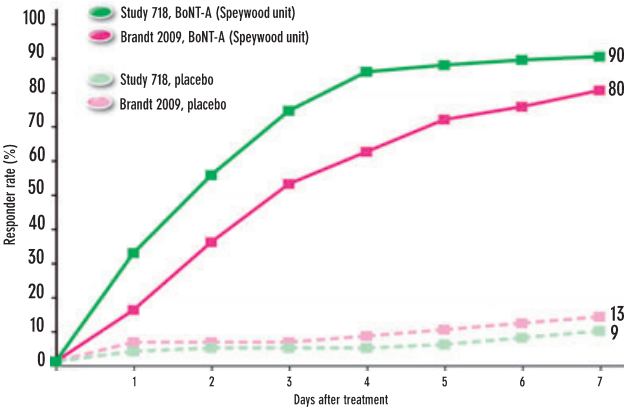

Figure 6 Cumulative responder rates as reported in patient diary cards[13,23]

Duration of response

Duration of response is another crucial indicator of efficacy, as it determines the necessary treatment frequency in clinical practice. The responder rate usually peaked at about 2–4 weeks after treatment and gradually decreased afterwards. As shown in Fig. 7, the responder rate at maximum frown was similar in three randomized, double-blind and placebo-controlled studies, where monthly responder rates after treatment with BoNT-A (s.U) were

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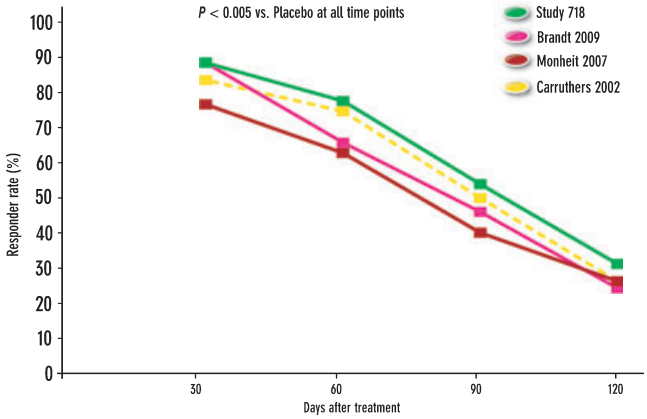

Figure 7 Investigator-assessed responder rates with botulinum neurotoxin type A (BoNT-A) at maximum frown reported in four randomized, double-blind and placebo-controlled studies.[13,22,23,31] Solid lines, BoNT-A (Speywood Unit); dash line, Vistabel/Botox

available.[13,22,23] At Month 4, the responder rate in the treatment groups was 24–31%, remained to be significantly higher than the responder rate in respective placebo groups ($P < 0.005$). In study 718, significantly higher responder rate with BoNT-A (s.U) compared to placebo was also reported at Month 5 (17% vs. 1%; $P < 0.001$).[13] Taken together, the responder rate for BoNT-A (s.U) was significantly higher than that of placebo for at least 4 months after treatment.

The responder rates at maximum frown at 1–4 months after treatment with Vistabel/Botox were assessed in Carruthers *et al.*[31] and the reported responder rates were included in Fig. 7. Significantly higher responder rate at Month 4 was also reported with Vistabel/Botox compared to placebo (26% vs. 0%; $P < 0.001$). Similar responder rates were reported for the two different commercial preparations of BoNT-A at 1–4 months post-injection. Taken together, both BoNT-A (s.U) and Vistabel/Botox demonstrated significantly greater efficacy compared to placebo for up to 4 months.

Similar to responder rate assessed at maximum frown, responder rate assessed at rest also increased after injection, peaked on Day 30 and gradually decreased (Fig. 8).[10] Significantly higher responder rate with treatment compared with placebo was reported for 3 months after injection ($P < 0.05$). At Month 6, responder rate at rest remained as high as 31%, although it was no longer significant compared with the placebo group.

Duration of response can be directly calculated using Kaplan-Meier (or survival) analysis. In Ascher *et al.*, duration, defined as the time between the randomized first injection and the open-label second injection, was decided consensually between the investigator and the patient.[16] In other studies, duration was defined as the time from the recorded onset of response until investigator-assessed GLSS returned to 2 or 3.[13–15,17,18] The duration of response with BoNT-A (s.U) was assessed in six studies, including three single-treatment studies[13–15] and three repeated-treatment studies (Table 8).[16–18] In each study, durations of response estimated by investigators and patients were highly consistent. Similar duration of response was observed in the six studies, with the median duration between 85 and 117 days (about 3–4 months).

Although the median duration was estimated to be 3–4 months, in a repeated-treatment study, a small proportion of patients (2–7%) had responses that persisted up to 336 days based on investigator assessment.[17] In subgroup analysis, African American patients had a slightly longer duration of response, with a median time of 117 days and 129 days based on assessments by investigators and patients respectively compared to 109 days and 107 days for the entire population.[15]

In a retrospective study including up to five treatment cycles, the median interval between two cycles was determined to be 5.9–6.5 months.[20] The between-treatment interval was longer than the duration of response reported in randomized and controlled studies,[13–16,18] possibly because patients had to cover their own treatment expense during the retrospective study. Furthermore, the majority of patients in Rzany *et al.* received concomitant aesthetic treatments, which could contribute to the long-lasting treatment

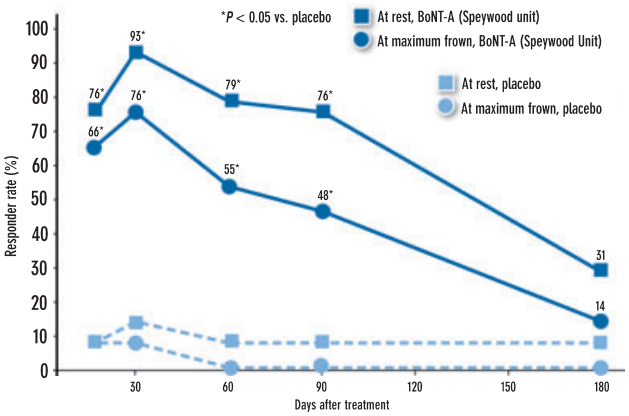

Figure 8 Investigator-assessed responder rates at rest and at maximum frown reported in Ascher *et al.*[10] study

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Table 8 Median duration (day) of response after treatments with botulinum neurotoxin type A (Speywood Unit) assessed by investigators and patients

	Study 718[13]	Brandt[14]	Kane[15] *	Ascher[16]	Moy[17] †	Rubin[18]‡
Investigator	117	85	109	98	88	88
Patient	117	85	107	98	84	88

*Variable dose was adopted in the study.
†Overall median duration of response for the first three cycles. (Since the study was up to 13 months, many patients had truncated cycles 4 and 5.)
‡Median duration of response for 1 or 2 open-label cycles and 1 randomized and blind cycle.

Table 9 Total dose (s. U) in each cycle for treatment of the upper third face with botulinum neurotoxin type A (Speywood Unit) reported in Rzany *et al.*[27]

	Cycle1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
Median	100	100	100	100	100
25%, 75%	70, 120	70, 130	70, 130	70, 130	70, 130

Table 10 Median between-cycle interval (month) for treatment of the upper third face with botulinum neurotoxin type A (Speywood unit) reported in Rzany *et al.*[20]

	Cycle 1–2	Cycle 2–3	Cycle 3–4	Cycle 4–5
Median	5.9	6.2	6.2	6.5
25%, 75%	4.4, 7.9	4.8, 8.4	4.7, 8.2	5.1, 8.9

effect and high level of patient satisfaction.[20] Thus, a treatment interval of about 6 months reflects the treatment frequency one might expect in clinical practice.

Consistency of efficacy with repeated treatments

It is important to determine whether treatment efficacy is consistent in repeated treatments. As the activity of BoNT-A as a muscle relaxant is not permanent, repeated treatments are necessary in clinical practice for long-term control of facial wrinkles. The treatment consistency with BoNT-A (s.U) was examined in a retrospective study including a total of 945 patients.[20] In the study, the inclusion criterion was very simple [patients receiving a minimum of three consecutive treatment cycles with BoNT-A (s.U) in the upper third face including glabellar, frontalis and lateral periorbital regions], ensuring that the study population was representative and unbiased. Moreover, although the most frequent treatment area was glabella (93.9%), a majority of patients (81.5%) received treatments in more than one facial area. The total dose, treatment interval and patient satisfaction were all very consistent among treatment cycles (Tables 9 and 10 and Fig. 3), suggesting that there was no secondary non-response or tachyphylaxis when BoNT-A (s.U) was administered repeatedly.

Consistency of treatment efficacy was examined in two prospective clinical studies, in which more than two cycles of treatment were administered.[17,18] In Moy *et al.*, patients underwent up to five consecutive open-label treatment cycles.[17] In Rubin *et al.* 2009, patients received three or four treatment cycles, two of which were randomized and placebo-controlled.[18] Response rate on Day 30, median time to onset and median duration of effect were all similar among treatment cycles in both studies, further supporting the conclusion that the efficacy with BoNT-A (s.U) was consistent upon repeated treatments (Figs 9–11). The investigator-assessed responder rate at maximum frown on Day 30 was 80–91% and 82–88% in the two studies respectively. The median time to onset was 3 days for all treatment cycles of both studies. Taken together, treatment efficacy with BoNT-A (s.U) was highly consistent among multiple treatment cycles, with no obvious loss of efficacy upon repeated injections.

Summary

- The multi-centre, randomized, double-blind and controlled clinical studies provide high level of evidence regarding the efficacy of BoNT-A (Speywood Unit) treatment.
- High responder rate: investigator-assessed responder rate was 76–90% at maximum frown on Day 30 after treatment with BoNT-A (Speywood Unit). Significantly higher responder rate was observed with BoNT-A (Speywood Unit) than with placebo for at least 4 months post-injection ($P < 0.005$).
- Rapid onset of action: median onset of response was reported to be 2–4 days after receiving BoNT-A (Speywood Unit) treatment. Onset of response as soon as 24 h post-injection was reported in all studies.
- Long-lasting effect: Median duration of response was estimated to be 85–117 days. Treatment interval observed in a retrospective study was 5.9–6.5 months.
- High consistency between repeated treatments: no loss of efficacy or tachyphylaxis was reported in two repeated-treatment studies and one retrospective study. In all studies, the responder rate on Day 30, median time to onset and median duration of response were similar between all treatment cycles.

How safe is the treatment with Azzalure?

The BoNT-A (s.U) (or abobotulinumtoxinA in the U.S.) has a product consistency of 20 years and a good safety profile in both therapeutic and aesthetic applications. When ingested at a very high dose, BoNT-A can lead to an acute paralytic condition called botulism. However, only a small quantity of Dysport is used for

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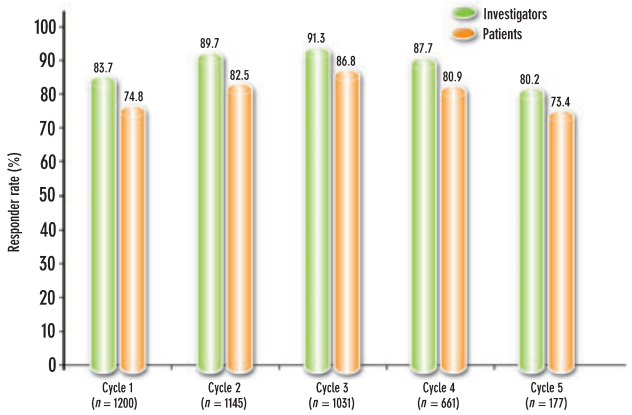

Figure 9 Investigator- and patient-assessed responder rates at maximum frown on Day 30 post-injection for each treatment cycle reported in Moy *et al.*[17]

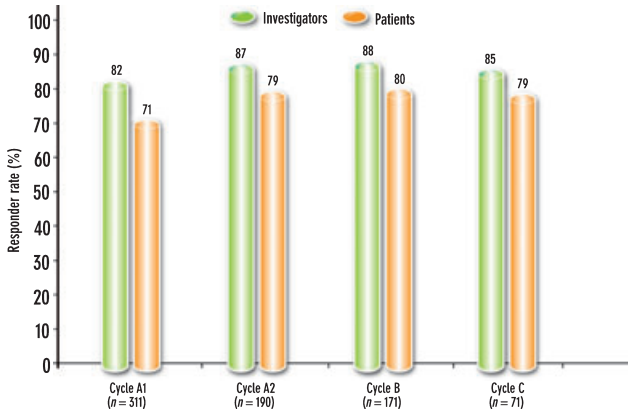

Figure 10 Investigator- and patient-assessed responder rates at maximum frown on Day 30 post-injection for each treatment cycle reported in Rubin *et al.*[18]

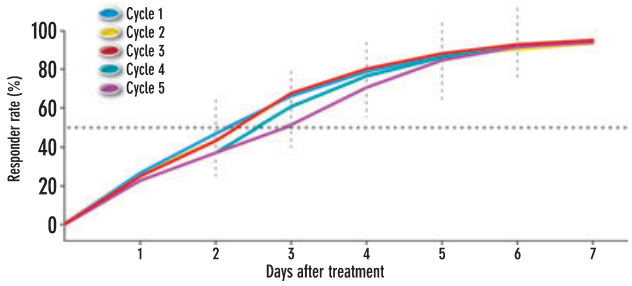

Figure 11 Onset of response for each treatment cycle reported in Moy *et al.*[17]

therapeutic applications, with a total dose of up to 1000 s.U for the treatment of focal spasticity or spasmodic torticollis and a recommended dose of 120 s.U per eye for the treatment of blepharospasm or hemifacial spasm.[37] Long-term (up to 10 years) studies demonstrated that Dysport was well tolerated even when a relatively high dose (mean dose 800 s.U per session for the treatment of cervical dystonia) was administered, with no toxicity reported.[38,39]

For the treatment of glabellar lines, the approved dose of BoNT-A (s.U) is 50 s.U, much less than the dose for therapeutic applications. Safety of the treatment was evaluated by monitoring AE in six single-treatment studies, four repeated-treatment studies and one retrospective study. These studies involved a total of more than 4000 patients and 12 000 treatments with BoNT-A (s.U).

Common treatment-emergent adverse events

The overall safety results of single treatments with 50 s.U BoNT-A (s.U) are summarized in Table 11. In all fixed-dose single-treatment studies, a majority of treatment-emergent adverse events (TEAE) were either unlikely or not related to treatment.[13,14,16–19] Most related adverse events (AE) were mild or moderate in intensity and were reported by Day 14 post-injection. The profile of related AE was similar between the treatment and the placebo groups, with the exception of eye disorder, which was predominantly observed in the group receiving BoNT-A (s.U). The most common related AE in the BoNT-A (s.U) group were headache and injection site reaction.[40] The injection site reaction could be pain, haemorrhage, discomfort, anaesthesia, swelling, bruising, irritation, stinging, erythema or pruritus experienced by patients during the study period. The incidence and profile of AE reported in these studies were similar to those reported in two randomized,

Table 11 Frequency of treatment-emergent adverse events (TEAE) with single injections of 50 s.U botulinum neurotoxin type A (Speywood Unit)[9]

Eye disorders	Common Asthenopia, ptosis, eyelid oedema, lacrimation increase, dry eye, muscle twitching around eyes Uncommon Visual disturbances, vision blurred, diplopia, eye movement disorder
General disorders and administration site conditions	Very common Injection site reactions
Immune system disorders	Uncommon Hypersensitivity
Nervous system disorders	Very common Headache Common Facial paresis
Skin and subcutaneous tissue disorders	Uncommon Pruritus, rash Rare Urticaria

The frequency of TEAE is classified as following: very common (≥1/10), common (≥1/100 to <1/10), uncommon (≥1/1000 to <1/100), rare (≥1/10000 to <1/1000) and very rare (≤1/10000).

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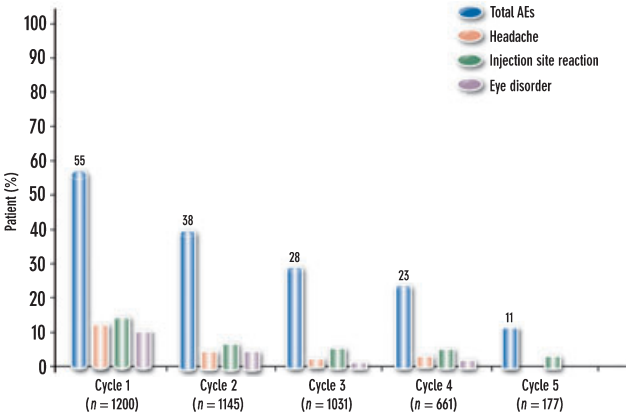

Figure 12 Percentage of patients who experienced treatment-emergent adverse events as reported in Moy *et al.*[17]

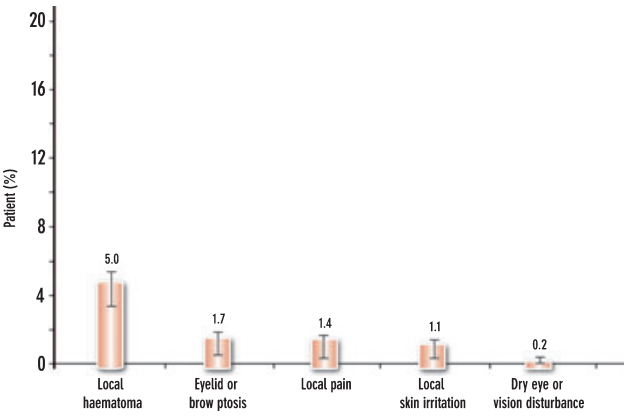

Figure 13 Percentage of patients who reported adverse events during the entire study (average 4.3 cycles) as reported in the Rzany *et al.*[20] Error bar, 95% confidence interval

blind and controlled studies with Vistabel/Botox, another commercial preparation of BoNT-A.[31,32]

In 3 studies where patients were treated for more than two cycles, the percentage of patients who reported TEAE was the highest during the first treatment cycle and decreased in subsequent cycles (Fig. 12).[17–19] Similar to single-treatment studies, the two most common related AEs were headache and injection site reaction. The percentage of patients who experienced headache or injection site reactions also decreased in later treatment cycles. Thus, repeated treatments with BoNT-A (s.U) for up to 23 months and up to six cycles were well tolerated and did not demonstrate cumulative safety issue.

Treatment with up to 80 s.U BoNT-A (s.U) was well-tolerated, as demonstrated in four variable-dose studies.[10–12,15] In a dose-finding study, the percentage of patients who experienced at least one TEAE was comparable between the placebo group and the three treatment groups, receiving 20, 50 or 75 s.U of BoNT-A (s.U) respectively.[12] In a variable dose study, the dose ranged from 50 to 70 s.U in female patients according to their muscle mass. In that study, the frequency of TEAE was not dose-proportional, with the overall incidence of TEAE in BoNT-A groups similar to that in placebo group.[15] Although more patients receiving BoNT-A (s.U) than placebo reported eye disorder, the incidence of this related AE did not increase with the dose of administered BoNT-A. The frequency of patients experiencing injection site reactions was the same for all treatment groups and the placebo group, suggesting that this related AE occured because of the treatment procedure (insertion of a needle) rather than the injected product [BoNT-A (s.U)].

The safety of treatment with BoNT-A (s.U) was also examined in a large-scale retrospective study including 4103 treatments and up to five treatment cycles.[20] Because of the retrospective nature of the study, safety was assessed with questionnaires about the occurrence of AE possibly related to the treatment. Of the total 945 patients, 856 (90.6%) did not experience any related AE after any treatment cycle. Consistent with the results of repeated-treatment studies, the frequency of AE was the highest in Cycle 1 (4.1%) and decreased in later cycles (2.0% in Cycle 5). The profile of the most commonly reported AE, including local haematoma, local pain, brow or eyelid ptosis and local skin irritation, was also similar to reports form other studies (Fig. 13). In the retrospective study, a majority of patients (81.5%) received injection of BoNT-A (s.U) in more than one facial area, and most patients (57.5%) received concomitant aesthetic procedures during the injection session. Overall, BoNT-A (s.U) treatment was safe and well-tolerated as demonstrated in this retrospective study, the study design and administered treatments of which were similar to those of clinical practice.

Eyelid ptosis

Eyelid ptosis is one of the least desired AEs during the treatment of glabellar lines with BoNT-A. The injected BoNT-A may diffuse through the orbital septum, weaken the upper eyelid levator muscle and cause eyelid ptosis. The incidence of eyelid ptosis is usually considered technique-dependent, and can be reduced by adopting proper methods of reconstitution and injection.

The rate of eyelid ptosis reported in large-scale clinical studies was low in general (Fig. 14).[10–20,31,32] In six randomized, double-blind and controlled studies, <3% of patients receiving a single injection of 20–80 s.U BoNT-A (s.U) reported eyelid ptosis and the incidence was experienced by <2% of patients in four out of the six studies. In five repeated-treatment studies, the percentage of patients reporting eyelid ptosis was <4% during each study period, which included up to six consecutive treatments during up to 23 months. Similar to TEAE, the frequency of eyelid ptosis was also the highest during the first cycle and then decreased in subsequent cycles. A majority of them were mild in intensity, reported before Day 14 after injection and resolved without additional treatment. Therefore, eyelid ptosis occurred only in low frequency during treatment with BoNT-A (s.U).

It was falsely suggested that compared to Vistabel/Botox, BoNT-A (s.U) has a higher risk of spreading to adjacent muscles.[41]

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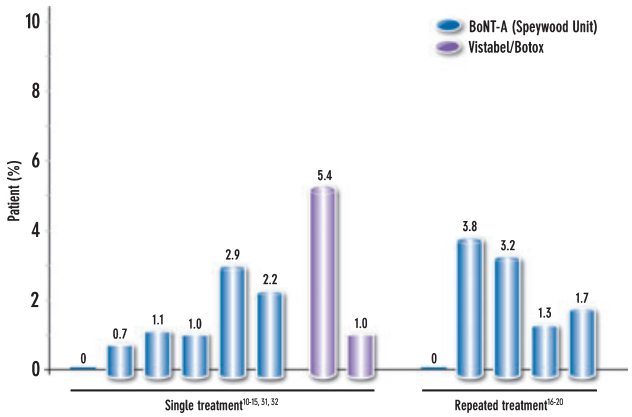

Figure 14 Percentage of patients experiencing eyelid ptosis in 11 studies with botulinum neurotoxin type A (Speywood Unit)[10–20] and two studies with Vistabel/Botox.[31,32] In the Rzany *et al.* study,[20] ptosis included both eyelid and brow ptosis. The Vistabel/Botox study[31] with higher rate of eyelid ptosis was conducted earlier.

If this were the case, we would expect higher incidence of eyelid ptosis associated with BoNT-A (s.U) than with Vistabel/Botox. The rate of eyelid ptosis reported from glabellar lines treatment with Vistabel/Botox was 5.4% in the first randomized, double-blind and placebo-controlled study[31] and decreased to 1.0% in a subsequent study (Fig. 14).[32] This decrease of eyelid ptosis frequency possibly reflected improvement in injection technique. Taken together, eyelid ptosis occurred in similarly low frequency when either BoNT-A (s.U) or Vistabel/Botox was administered. Indeed, the field of effect of BoNT-A is related to the dose, volume and injection technique. A recent report demonstrated the same field of effect of BoNT-A (s.U) and Vistabel/Botox when they were injected at a dose equivalence of 2.5 : 1 under strictly identical conditions (same volume, injection depth and injection technique).[42] In addition, the field of effect of BoNT-A is unrelated to the size of the toxin complex, in contrast to the claim by de Almeida *et al.*,[41] as the toxin complex dissociates almost instantaneously under physiological condition to release the 150kDa neurotoxin molecule, which is the same for all preparations of BoNT-A products.[43,44]

Neutralizing antibody

When injected into the human body, BoNT-A, as a foreign protein, may induce the formation of neutralizing antibodies. Such antibodies can render the next injection of BoNT-A ineffective and thus reduce the overall treatment efficacy. In practice, only injection of a large quantity of protein may raise antibodies. In a long-term study of Dysport in the treatment of cervical dystonia, the mean dose was as high as 800 s.U per treatment session and the cumulative dose was higher than 20 000 s.U over an average of 26.8 treatment cycles. Only three out of 90 patients in that study reported secondary non-response after 10 years of repeated injections with such high doses.[38] In comparison, the dose for glabellar line treatment is only 50 s.U, much smaller than those for common therapeutic applications, and therefore has much less risk of inducing the formation of neutralizing antibodies.

Secondary non-response or tachyphylaxis was not observed in any of the repeated-treatment studies or the retrospective study.[16–20] Responder rate, onset of action and duration of response were reported to be similar among treatment cycles in all fixed-dose studies (Figs 9–11). In the retrospective study, the average dose for the upper third face treatments (including glabella, frontalis and lateral periorbital regions) was the same for each of the five treatment cycles (Table 10), suggesting that similar treatment efficacy was achieved after repeated injections with BoNT-A (s.U). Furthermore, in a subgroup analysis, the responder rate on Day 30 after injection was not different between the patients who were naive to BoNT-A treatment and those who were already injected before the study (85% vs. 85%).[15]

The presence of neutralizing antibodies was directly tested in three single-treatment studies and an open-label repeated-treatment study, involving a total of more than 1700 patients.[12–14,17] No neutralizing antibodies were detected in any patient either at baseline or at the last study visit.[45] This finding suggested that repeated injections of BoNT-A (s.U) with the recommended doses did not induce the formation of neutralizing antibodies under the study settings, and further confirmed the limited risk of secondary non-response.

Summary

- Treatment safety with BoNT-A (Speywood Unit) was examined extensively in 11 clinical studies, which involve a total of more than 4000 patients and 12 000 treatments.
- Dysport has demonstrated its good safety in various therapeutic applications, in which the dosage administered per treatment session were up to 1000 s.U, 20 times higher than that used for the treatment of glabellar lines.
- Most of treatment-emergent adverse events (TEAE) during studies were unrelated to the treatment. The most frequent related AE included headache and injection site reaction. A majority of related AE were mild in intensity and resolved without additional treatment.
- In repeated-treatment studies, the incidence of TEAE was the highest in the first cycle and decreased in subsequent treatment cycles.
- Eyelid ptosis occurred rather rarely after the treatment with BoNT-A (Speywood Unit). The percentage of patients reporting eyelid ptosis was low, <3% in all single-treatment studies and <4% in all repeated-treatment studies.
- Neutralizing antibodies were not detected in a total of more than 1700 patients after more than 4800 treatments with BoNT-A (Speywood Unit).

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Capsule summary

- Azzalure and other BoNT-As (Speywood Unit) (or abobotulinumtoxinA in the U.S.) possess unique chemical and biological properties compared to BoNT-As of other commercial preparations.
- To ensure optimal safety and efficacy for the treatment of glabellar lines, it is crucial to adopt standard procedures of Azzalure reconstitution and injection, which can be acquired through proper trainings.
- Azzalure [125 Speywood Unit (s.U)] should be reconstituted with 0.63 mL of saline for a final concentration of 200 s.U/mL. For the treatment of glabellar lines, a five-point injection with 10 s.U per point should be administered to target the procerus and the corrugator muscles.
- The unique efficacy and safety profiles of BoNT-A (Speywood Unit) were examined extensively and demonstrated in 11 clinical studies, which involved more than 4000 patients and 12 000 treatments.
- Single or repeated treatments with BoNT-A (Speywood Unit) led to high responder rate (~76–90% of patients with no or mild wrinkles on Day 30 evaluated at maximum frown), rapid onset of action (2–4 days) and long duration of response (median duration of 85–117 days).
- Treatment efficacy was consistent among multiple cycles of repeated-treatment studies, with no loss of efficacy or secondary non-response observed.
- Most of treatment-emergent adverse events (AE) were unlikely or not related to treatment. A majority of related AE were mild in intensity and resolved without additional treatment. The most frequent related AE were headache and injection site reactions. The frequency of eyelid ptosis was low in all clinical studies.
- During repeated treatments, the incidences of treatment-emergent AE and treatment-related AE were the highest in the first cycle and decreased in subsequent cycles.
- High level of patient satisfaction was reported for single and double treatments with BoNT-A (Speywood Unit). Very high patient satisfaction (>95%) was reported for all five treatment cycles in a retrospective study.
- Treatment of glabellar lines with BoNT-A (Speywood Unit) is both efficacious and safe, leading to high level of satisfaction among physicians and patients.

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