New Uses of AbobotulinumtoxinA in Aesthetics

Joel Schlessinger, MD; Erin Gilbert, MD, PhD; Joel L. Cohen, MD; and Joely Kaufman, MD

Abstract

BotulinumtoxinA (BoNT-A) is now widely established for the main approved indication of reducing glabellar lines, and is also widely used off-label to improve the appearance of wrinkles and lines in other parts of the face. The number of aesthetic procedures continues to increase as the patient population becomes more diverse, in particular with increasing numbers of people of color and men. Further developments in treatment may continue to expand the audience for BoNT-A by making procedures more comfortable and by delivering a more natural, less static appearance. These may be achieved through use of combinations of BoNT-A with other aesthetic procedures, tailoring the dose of toxin to the patient’s muscle mass or by using novel injection and application techniques. Beyond amelioration of facial lines, encouraging results have been seen with the use of BoNT-A to improve the appearance of hypertrophic and keloid scars and even to prevent them. Studies have been conducted with scars in various parts of the body and further research is ongoing. Dermatological and other medical uses for BoNT-A are also active areas of research. Injections of BoNT-A have been shown to reduce signs and symptoms of acne, rosacea, and psoriasis, to reduce neuromuscular pain, and to bring about significant improvements in a number of rare diseases that are caused or exacerbated by hyperhidrosis. This paper reviews these new uses for BoNT-A, looking at the rationale for their use and discussing the results of published case studies and clinical trials. These areas have shown great promise to date, but more and larger clinical studies will be required before these treatments become a clinical reality. To this end details are also provided of clinical trials currently listed in the main clinical trials database to highlight research areas of particular interest.

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Details of the mechanism of action of BoNT-A are discussed in detail elsewhere in this supplement. The main mechanism important to aesthetic uses involves neuromuscular paralysis through a process of chemical denervation. Following injection into a muscle, BoNT-A travels to the neuromuscular junction (NMJ), binding first to a high-affinity presynaptic receptor, which permits entry into the presynaptic nerve terminal through receptor-mediated endocytosis into an endosome. The component heavy and light chains dissociate by disulfide bond breakage, and the zinc-dependent endoprotease—the light chain—is released into the nerve ending. In the nerve terminus, this cleaves synaptosomal-associated protein 25 (SNAP-25), which plays a key role in acetylcholine release during nerve stimulation. This cleavage blocks the release of acetylcholine into the synaptic cleft, preventing the stimulus from reaching the muscle until the function of the NMJ is restored (3-6 months).

The aesthetic indications of ABO and other forms of BoNT-A will continue to expand owing to the increased diversity of individuals requesting aesthetic procedures, in particular people of color and men. Research into new techniques and formulations, and the effects of using botulinum toxin in combination with fillers and/or other aesthetic procedures, has uncovered the potential for further improvements in patient comfort, skin quality, and overall satisfaction.

As physicians develop a deeper understanding of how BoNT-A exerts various activities—not just those relating to blocking acetylcholine release—research will dictate additional medical uses for BoNT-A formulations. A number of research groups are investigating the efficacy of BoNT-A in inflammatory skin diseases, including common conditions, such as acne, and also some rare diseases that currently have few treatment options. Pain relief is another area of extensive research, with a number of ongoing clinical studies. Use of BoNT-A for improving the appearance of surgical wounds is an emerging area of research. Early results indicate promise in this area but larger trials are required before firm recommendations can be made.

This paper will consider the potential new uses of BoNT-A under 2 broad headings: aesthetic developments and medical developments. Where appropriate, listings of current and imminent clinical studies registered within the main clinical trials database, ClinicalTrials.gov, are included.

**AESTHETIC DEVELOPMENTS**

**Wound Healing**

Wound healing is a complex and dynamic process that is dependent on the coordinated activities of multiple cell types in the epithelium, connective tissue, and vasculature. The process consists of 3 overlapping phases. At the start is the inflammatory (or migration) phase, which lasts just a few days, during which an array of potent cytokines and growth factors recruit inflammatory cells such as macrophages, neutrophils, and fibroblasts for use in the second phase. The proliferative (or mitotic) second phase typically lasts for weeks and is characterized by the formation of granulation tissue. Recruited fibroblasts synthesize a scaffold of extracellular matrix (ECM), which builds a structural framework on which to bridge the wound and allow vascular ingrowth. Myofibroblasts help to initiate wound contraction. The final phase in the process is maturation, which starts once the wound is closed and typically lasts for 7 months. During this phase, the scar begins to shrink and swelling diminishes. The inflammatory cells gradually diminish in number, ECM is degraded, angiogenesis and fibroplasia cease, and immature type III collagen is modified into mature type I collagen.

Wound healing is frequently an imperfect process, leaving patients with blemishes, scars, and potential disfigurement. This may be influenced by the location of the wound, prolonged inflammation, wound infections, and delayed epithelialization (longer than 10 days). The muscles that facilitate eating, smiling, speaking, and blinking, etc., do so by altering the tension in adjacent skin (rather than via attachment into bone, as at a joint). Tension exerted perpendicular to a wound during the healing process can result in ongoing microtrauma to the lesion, which exacerbates inflammation, leading to overproduction of collagen and glycosaminoglycans, and delayed healing. This results in the formation of raised and often hyperpigmented scars, which frequently are in prominent positions on the face.

Chang et al found that, in contrast to other facial wounds, the incidence of hypertrophic scars (HS) affecting the upper lip is high, ranging between 12% and 27%. Raised scars that stay within the bounds of the original wound are known as HS, whereas those that continue to expand beyond the boundaries of the original wound as a result of ongoing fibroblast activity are known as keloids. These may represent successive stages of a fibroproliferative skin disorder differentiated by varying degrees of inflammation. Facial HS and keloids are disfiguring and are often associated with clinical symptoms such as itching, pain, and restricted range of motion and contracture.

The precise mechanisms underlying the formation of HS and keloids are not fully understood, and this makes their management difficult. Facial scarring can have profound psychological effects on patients. In one US study, 91% of patients said that they would value even small improvements in scarring. Conventional options for the management of HS and keloids include intralional corticosteroid injections, surgery, cryotherapy, pressure therapy, radiotherapy, laser therapy, and application of silicone gel sheeting.
**BoNT-A for Wound Healing**

A number of animal studies have demonstrated that treating HS by injecting BoNT-A can significantly improve the cosmetic appearance of facial scars. In vivo studies in animals and humans have demonstrated that, in addition to the known effects on acetylcholine at the NMJ, BoNT-A inhibits fibroblast proliferation (and hence collagen production) and downregulates expression of α-smooth muscle actin and myosin II proteins, which are found in fibroblasts, all in a dose-dependent fashion. These phenomena were not observed in fibroblasts isolated from normal skin. By contrast, collagen production and organization were significantly greater with intralesional ONA than with saline in a rat model of burn healing. This was associated with faster vascularization and reepithelialization of the wound and, ultimately, a smaller scar.

Vascular endothelial growth factor (VEGF) has been shown to promote angiogenesis in wound healing. BoNT-A may increase expression of VEGF, although the exact mechanism for this is not known. Results from studies investigating the effect of BoNT-A on the expression of VEGF in keloid scar healing are inconsistent: some appear to demonstrate benefit, but others show no effect.

A recent meta-analysis of randomized controlled trials (RCTs) evaluating BoNT-A in HS in the face and neck areas found that patients who received BoNT-A had better outcomes than those who did not receive BoNT-A. The analysis found that scars were significantly narrower \( P = 0.006 \) and visual analog scale (VAS) scores were significantly higher, indicating that patients receiving BoNT-A were more satisfied with the results than those who received saline. However, the number of studies eligible for the analysis was small (9) and only 3 were unbiased in all assessment domains. Clinical studies that have evaluated the efficacy of early injection of BoNT-A to ameliorate surgical HS are outlined in **Table 1**.

The positive effects of BoNT in wound healing have also been demonstrated in patients undergoing surgical reconstruction following Mohs micrographic surgery for skin cancer. It was suggested that the temporary muscle relaxation associated with BoNT enabled a more effective healing process through immobilization of the target area.

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**Table 1. Clinical Studies With Injection of Botulinum Toxin to Ameliorate Hypertrophic Surgical Scars**

| Study/studies | Study population | Injection site(s) | Findings |
|---------------|------------------|-------------------|----------|
| 23            | Infants undergoing cheiloplasty | Single, intraoperative injection of ONA into the orbicularis oris | Electromyographs demonstrated reduced tension in the orbicularis oris following treatment |
| 5,24          | Infants and adolescents undergoing primary and secondary cheiloplasty | Four postoperative injections of ONA into orbicularis oris | VAS scores were significantly lower \( P < 0.001 \) for ONA-treated wounds and VAS scores were significantly higher \( P = 0.001 \) |
| 5             | Adults undergoing cheiloplasty revision | Six intraoperative injections of ONA or saline into the orbicularis oris | RCT demonstrated significantly higher VAS scores for patients who received BoNT-A |
| 25            | Adults with recent thyroidectomy | Single postoperative injection of BoNT-A or saline into thyroidectomy scar | Split-scar study found significant improvements in scar width, height, color, and appearance with BoNT-A |
| 26            | Adults undergoing forehead flap nasal reconstruction | Multiple preoperative injections of ONA into frontalis | Split-scar study found significant improvements in VAS score with ONA |
| 27            | Adults with traumatic wounds to the forehead requiring wound closure | Postoperative injection into musculature adjacent to wound | RCT demonstrated significantly higher VAS scores for patients who received BoNT-A |
| 7             | Adults who had undergone facial trauma requiring wound closure | Single postoperative injection of ONA into muscle area of wound | In this RCT, VAS scores for ONA ranged from 3.08 to 9.58, and VAS scores for saline control ranged from 3.08 to 7.83 |
| 28            | Adults undergoing revision of ugly facial scars | BoNT-A injected in linear pattern along length of scar following revision surgery | Objective outcome, assessed using photography, considered highly satisfactory in 90% of patients. In subjective assessment, 75% of patients rated improvement as marked, 15% as significant, and 10% as unchanged. |

BoNT-A, Botulinum toxin-A; ONA, onabotulinumtoxinA; RCT, randomized controlled trial; VAS, visual analog scale; VSS, Vancouver Scar Scale.
BoNT-A and -B were shown to result in equally effective outcomes.29

**Ongoing Clinical Studies**

Three early phase clinical studies to investigate the effect of BoNT-A on HS were listed on ClinicalTrials.gov in Spring 2016 (Table 2). The first results should be available late 2016-early 2017.

**Keloids**

The evidence for the activity of BoNT-A in existing keloids is not so conclusive. In a cohort of 12 patients, repeated injections of BoNT-A (20-100 units at each session, depending on the size and location of the scar) were used to treat long-term keloids that had failed to respond to conventional treatments.30 On average, patients were treated for 11 months. The authors reported good success, with only 2 patients experiencing recurrences. In another study, patients with long-term keloids were randomized to receive a course of intralesional BoNT-A injections or intralesional steroid injections. Both treatments contributed to significant improvements in the keloids, but the authors judged that treatment with BoNT-A was marginally more effective.31 However, another group has reported that a 6-month course of BoNT-A injections failed to improve keloid appearance.10 In vitro investigations with fibroblasts isolated from keloids have not found evidence of reduced proliferation or cytokine production.21 In all these studies, no dose-ranging investigations have yet been carried out on an individual basis.

In summary, the evidence for using BoNT-A to ameliorate facial HS is promising, although large, randomized trials are required before firm recommendations can be made. The evidence for efficacy in the treatment of already formed keloids is much less convincing and more investigative work is required. Perhaps the time to intervene is prior to formation of the keloid, but future studies will need to be done to confirm this.

**Combination Therapies**

The aging face is characterized by a loss of firmness, smoothness, complexion radiance, and changes in skin color and homogeneity, which must all be addressed to achieve natural-looking rejuvenation of the whole face.32 Today, tailored combinations of BoNT-A to correct dynamic wrinkles, fillers (such as hyaluronic acid [HA], calcium hydroxylapatite, poly-L-lactic acid, and autologous fat tissue) for volume augmentation, and ablative lasers, collagen-stimulating treatments (such as platelet rich plasma, microneedling, and radiofrequency/ultrasound treatments), and skin peeling to reverse skin sagging and dullness, can achieve the same (or better) results than an individual treatment approach, potentially in a shorter timeframe and at considerably lower cost and inconvenience to the patient.32-38 This type of full face treatment also results in very high (>90%) patient satisfaction scores.36,38

**Table 2. Clinical Trials With Botulinum Toxin for Prevention and Amelioration of Scarring**

| Agent | ClinicalTrials.gov ID | Details | Status |
|-------|-----------------------|---------|--------|
| Not specified | NCT02168634 | Randomized, placebo-controlled trial to evaluate relief of itching in adults with hypertrophic scars due to laparotomy or thyroidectomy | Recruiting (Taiwan) |
| ONA | NCT02623829 | Phase II, randomized, double-blind, placebo-controlled study to evaluate BoNT-A for the prophylaxis of postexcisional scarring in adults undergoing surgical removal of cancerous tumors from the forehead | Recruiting (USA) |
| ONA | NCT02247193 | Phase III randomized, double-blind, placebo-controlled study to evaluate BoNT-A during surgical repair of cleft lip in infants | Recruiting (USA) |

BoNT-A, BotulinumtoxinA; ONA, onabotulinumtoxinA.
HA can improve skin hydration and elasticity. Other matrix metalloproteinases (MMPs), which break down blasts and, at the same time, increases the secretion of Photoaging reduces the production of collagen in fibroblasts and, at the same time, increases the secretion of MMPs. BoNT-A Plus Skincare

BoNT-A treatment has been shown to have a complementary, and possibly synergistic, effect when given after intense pulsed light (IPL) with fluence of 24 to 28 J/cm². Carruthers and Carruthers suggest that the effect of BoNT-A on the presynaptic network and the vascular system (ie, through inhibition of the release of vasodilating neuropeptides) and other autonomic systems may explain the enhanced effect on signs of aging—such as fine lines, wrinkles, telangiectasia, and erythema—seen with BoNT-A and IPL.

BoNT-A Plus Skincare

Photoaging reduces the production of collagen in fibroblasts and, at the same time, increases the secretion of matrix metalloproteinases (MMPs), which break down existing collagen leading to loss of volume and elasticity. Bonaparte and Ellis used a Cutometer (Courage + Khazaka electronic GmbH, Cologne, Germany) instrument to measure skin pliability and elastic recoil—measures of elasticity—before and after treatment with ONA at the glabellar, supraorbital, and lateral orbital sites. They found that in addition to the neuromuscular effects of BoNT-A, there is clear evidence of biomechanical changes in the skin. The authors hypothesized that BoNT-A may result in increased organization of the dermal network of collagen, and that this—along with increased production of procollagen, collagen, and elastin—effectively reverses the loss of elastic recoil caused by aging. Collagen synthesis can be increased simply by pricking the skin, but Permatasari et al have confirmed that BoNT-A promotes the synthesis of collagen and inhibits the secretion of MMPs.

Skincare regimens designed to rejuvenate the skin can enhance the effects of BoNT-A used alone. These regimens frequently contain a retinoid and/or HA. Used daily, topical retinoids reverse photoaging by preventing destruction of the dermal matrix and promoting collagen formation, while creams, serums, and gels containing HA can improve skin hydration and elasticity. Other components of skincare regimens used in combination with BoNT-A include hydroquinone for skin lightening, and adenosine, which has been proposed to act by a number of mechanisms to reduce wrinkles and improve skin tone and texture.

Use of such a skin care regimen by individuals who have had BoNT-A injections has been demonstrated to have a beneficial effect on the mean volume and depth of facial lines, hyperpigmentation, smoothness of skin, and evenness of skin tone and color compared with BoNT-A injections alone. A “microbotox” method of BoNT-A application has recently been developed. The microbotox method (also known as meso-Botox) developed by Wu in 2000, aimed to provide more natural-looking effects for patients. The method involves the systematic injection of multiple tiny blebs of highly diluted BoNT-A (mainly ONA) at 0.8 to 1.0 cm intervals into the dermis or the interface between the dermis and the superficial surface of the facial muscles. It is proposed that the microinjections smooth and tighten the skin by inducing bulk atrophy of the sweat and sebaceous glands, and by weakening the superficial muscle fibers that insert into the skin, thus reducing the pulling and tethering effects of the facial muscles that result in fine lines and wrinkles. Using microdroplets of dilute BoNT-A thus prevents diffusion into deeper muscles, which can lead to a “frozen” appearance. An additional advantage is that decreased sweat and sebaceous gland activity improves the appearance (quality) of the skin (especially the forehead). Practitioners must receive training in how to deliver the microdroplet technique consistently and superficially in order to avoid inadvertently paralyzing deeper muscle groups in the treated area. The effects of treatment typically last for 3 to 4 months but may last for up to 6 months. Although originally developed for ONA, the technique has also been successfully used with ABO. The author reports results with ABO have been less reliable, possibly due to the lack of a fixed dose ratio between ABO and ONA and the need to perform dose-ranging studies with the technique.

Patients who have experienced substantial photoaging and loss of volume may develop sets of fine parallel lines, which may also be deep, stretching from the orbital frame up to the temples and neck—a condition variously described as “scratched face” or “accordion lines.” Hypertrophy of the orbicularis and the zygomaticus major are responsible for periorbital and para-commissural wrinkles, but wrinkles may also appear at some distance from these muscles.

Treatment is challenging, due to the shallowness and length of the lines. The technique developed by Môle et al employs superficial injections (25-30) of highly diluted BoNT-A and noncross-linked HA. While the effects of the toxin on the muscles are clearly expected, the effects on the midcheek areas are surprising. The effects may be explained by diffusion of toxin into dermal cells (facilitated by the high dilution) where the BoNT-A can alter...
rates of biosynthesis of collagen and production of inflammatory cytokines.50

Only a limited number of patients have undergone the procedure to date. The results for patients treated with ABO and HA indicate that the correct dose is critical, with low doses not achieving the desired effect and some patients experiencing “frozen” features due to overdosing. The investigator recommends a maximum of 40 units of ABO per side. Successful treatment not only corrected the scratched face appearance, but also improved “gummy smile” and the appearance and texture of the skin.50,51 The effects lasted for 4 to 6 months. Some patients (and physicians) may find the large number of required injections unacceptable. This may be overcome by use of microcan- nulae inserted at 4 points (2 in the temporomandibular area and 2 in the jugular area), although this is less accu- rate. The present authors have found the Aquagold Fine Touch microneedling device (Aquavit Pharmaceuticals, New York, NY), which can also be used to deliver com-binations of BoNT-A and fillers, to be effective.

**Ongoing Clinical Studies**

Two Phase IV studies investigating ABO in combination with fillers were registered on ClinicalTrials.gov in spring 2016 (Table 3). The first results should be available late 2016-early 2017.

**New Formulations/Delivery Methods**

Injections of ABO and other forms of BoNT-A may be associated with undesirable effects such as pain, bruising, ptosis, and immunogenicity.52 Moreover, some patients are quite simply frightened of needles.53 Formulations and delivery mechanisms that avoid these problems are therefore desirable. There is also scope for improved convenience for physicians with new for- mulations that avoid the need to reconstitute the toxin from the powder form—all of the main toxin products

that are currently available worldwide are provided as either freeze-dried or vacuum-dried powders that require reconstitution before use.

**Advances in Formulations**

As a result of the large molecular size, BoNT-A has neg- ligible cutaneous bioavailability—simply applying solutions of toxin to intact skin is not effective.54 Two studies have used a fractional ablative laser to create microscopic columns in the epidermis and dermis with the expecta-tion that this would permit molecules of ABO to reach the superficial orbicularis oculi in the lower dermis.55,56

In one split-face study, crow’s feet on each side of the face were first treated with a fractional ablative CO2 laser set to allow penetration of the dermis of no more than 50 mm to avoid scarring. ABO (100 U) in saline solution was then applied to one side of the face, and saline solution only to the other side. Whereas no signifi-cant difference was identified in the appearance of static wrinkles from baseline to 1 month on either side of the face, dynamic wrinkles exhibited a statistically significant reduction (P = 0.016) from baseline to 1 month on the treated side. Wrinkles on the treated side were also signifi-cantly reduced (P = 0.039) compared with the control side. There were no differences in pain and swelling on either side, but patient satisfaction was much greater with BoNT-A treatment. Although the combination treat-ment was successful, the investigators point out that the procedure is more complicated and more expensive than injections.56

A novel BoNT-A, daxibotulinumtoxinA (RT001), was being developed as a topical gel for the treatment of hyperhidrosis and crow’s feet.52 In this formulation, BoNT-A is complexed with a novel peptide-based drug delivery system (TransMTS; Revance, Newark, CA). By varying the length of the carrier peptides, the toxin can be delivered to a defined depth or target site. In a small (n = 45), double-blind, placebo-controlled study, patients were randomized to receive 1 application of daxibotulinumtoxinA or placebo to crow’s feet wrinkles. After 4 weeks, 89% of patients in the active arm dis-played a clinically significant reduction in the severity of their wrinkles compared with baseline (investigator and patient assessment), with 44% meeting the primary efficacy endpoint of a 2-point improvement. No patient in the placebo arm met this endpoint.52 However, initial results from the Phase III REALISE 1 placebo-controlled study in 450 patients with moderate-to-severe crow’s feet (NCT02580370) did not achieve the primary or other end-points. In view of these results, Revance Therapeutics, Inc. (Newark, CA) have disclosed that they do not plan to continue development of RT001 topical for crow’s feet, or to pursue the current clinical development plan for RT001 in axillary hyperhidrosis.57

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**Table 3. Clinical Trials With Botulinum Toxin Combined With Fillers for Aesthetic Procedures**

| Agent   | ClinicalTrials.gov ID | Details                                                                 | Status  |
|---------|-----------------------|-------------------------------------------------------------------------|---------|
| ABO     | NCT02297503           | Phase IV, randomized, open-label, investigator-blinded, active-controlled study comparing BoNT-A plus filler with filler or BoNT-A alone in patients undergoing facial aesthetic treatments | Ongoing |
| ABO     | NCT02297516           | Phase IV, randomized, open-label, investigator-blinded, active-controlled study comparing BoNT-A plus filler with filler or BoNT-A alone in patients undergoing facial aesthetic treatments | Ongoing |

BoNT-A, Botulinum toxin-A; ABO, AbobotulinumtoxinA.
A ready-to-use sterile liquid formulation of a novel BoNT-A, MT10109L, developed by MedyTox, Inc. (Cheongju, South Korea), does not require dilution before use and contains no albumin or animal-derived proteins. The molecular weight and diffusion capacity of MT10109L are reported to be similar to those of ONA.

In a study to compare MT10109L with ONA in the treatment of glabellar lines, participants were randomized to receive 20 U of BoNT-A–4 units at 5 injection points. Both groups showed significant improvement of glabellar lines from baseline to week 4 (investigator rating), which persisted until week 16. However, the percentage of responders at maximum frown by live assessment at week 16 was significantly lower with ONA than with MT10109L (P = 0.0064). Self-assessment of improvement of glabellar lines and satisfaction yielded comparable results for the 2 groups. Similarly, rates of adverse events were similar in each group. The differences in efficacy are readily explained by the fact that the potency units of botulinum toxins are specific to each product family and are not interchangeable. Therefore, even at apparently “equal” doses, these differences may manifest as different clinical responses.

**Advances in Delivery Methods**

Many patients experience notable pain when receiving BoNT-A injections. The treatment protocol includes application of topical analgesics (such as ice packs, anesthetic ointments, and/or vapocoolant sprays) to reduce patient discomfort, but these are not always effective and may be associated with adverse events such as contact dermatitis and pigmentary changes. Evidence from dentistry and therapeutic dermatology procedures suggests that vibration anesthesia may help to reduce injection pain. It is hypothesized that vibration anesthesia works by dampening pain sensation by costimulation of the nerve fibers with waves of vibrations.

**Tailored Treatments**

Each patient who presents for aesthetic treatment is different in terms of physical attributes, the way that they feel about their appearance, and what they would like to change. In recent years, a number of groups have proposed that fixed injection patterns should be replaced by protocols that tailor the number of injections and dose of toxin to individual patient characteristics. Two groups have developed protocols based on the classification of muscles, while a third has developed an assessment protocol that should be used before a patient is injected in order to determine the size of the dose and the number of injections.

Kane et al classified the fan pattern of crow’s feet wrinkles in more than 2500 photographs and then analyzed them by patient age, sex, and severity. They found that an upper fan pattern was uncommon (found in only 5% of patients), lower fan patterns were more common in males, and the frequency of full fan patterns increased with age. Full fan and lower fan patterns were also associated with more severe wrinkles. The authors therefore suggest that, rather than follow a “one size fits all” guideline for injecting crow’s feet, injection patterns could be guided by the fan pattern. Kane’s group has also demonstrated that classifying the muscles of the forehead by mass and increasing the dose of ABO for larger muscles, as well as increasing the dose of ABO for men compared with women in the same class, can be beneficial in terms of onset and duration of effect.

Xie et al used a combination of clinical palpation and ultrasound examination to classify masseter muscles in healthy volunteers and prospective patients according to bulging type on clenching and muscle thickness. They used this information, in addition to an understanding of masseter anatomy from dissection studies, to develop a tailored treatment protocol for masseter hypertrophy (an unlicensed indication). Palpation identified 5 classes of bulging type in masseter muscles: minimal, mono-bulging, double-bulging, triple-bulging, and excessive bulging. Ultrasound examination gave rise to 3 classes based on muscle thickness, ranging from mild (<10 mm), to moderate (10-13.9 mm), to severe (>14 mm). In their treatment protocol, the dose of BoNT-A was determined by masseter thickness ranging from ONA 20 U for mild hypertrophy to 40 U for severe hypertrophy. Bulging type was used to determine the number of injections and the injection sites (Table 4).

The investigators trialed the protocol in 220 patients and achieved significant reductions (P < 0.05 to P < 0.01) in masseter hypertrophy, which persisted for 4 months in 40 cases. Unfortunately, the study results do not make it clear whether masseter tailoring resulted in an even reduction across all classes; however, 96% of all patients (n = 211) reported that they were satisfied or very satisfied with

| Thickness (mm) | Bulging type | Total BoNT-A Dosage (units) |
|---------------|--------------|-----------------------------|
| Mild (<10)    | 1 1 2 3 —    | 20-25                       |
| Moderate (10-13.9) | 2 1 2 3 —    | 25-30                       |
| Severe (>14)  | 3 1-2 2-3 3 3 | 30-40                       |

BoNT-A, Botulinumtoxin-A. *The total botulinum toxin type A dosage and the number of injection sites per masseter are determined by the respective masseter type and thickness.
the result. The investigators report that more studies are planned to refine the protocol.62

The objective-muscle-identification technique (OMIT) requires the surface area of the target muscle to be mapped before treatment. Mapping the surface area of the muscle to be injected allows an individual dose to be calculated and the injection sites to be pinpointed. Careful targeting of the injection point reduces the risk of puncturing the supraorbital nerve and blood vessels, and delivers the toxin to the medial portion of the muscle, from where it can be encouraged to diffuse through to the internal and lateral portion of the muscle by applying gentle finger pressure to the wheal created by the injection. This individualized approach is particularly helpful in patients with asymmetric muscle hypertrophy.61

The OMIT technique has been compared directly with a fixed point technique (based on a general consensus) for treating the glabellar area.61 A total of 31 patients in the fixed point arm received 4 injections of ONA 4 U at distances of 0.5 cm apart into the corrugator muscle on each side of the face. They received a total dose of 16 U in ~0.5 mL saline solution in each muscle. The majority of the 31 patients in the OMIT arm received only 2 injections into each corrugator muscle, with a mean total dose of approximately 12 U (in approximately 0.3 mL saline) per muscle—significantly lower than in the fixed site arm ($P = 0.0001$). All patients in the fixed pattern arm required top-up injections compared with no patients in the OMIT arm ($P = 0.001$). Duration of activity was also significantly longer in the OMIT arm ($P = 0.0001$), and patient satisfaction scores were higher. Further studies are necessary to confirm that OMIT may be generalized to other types of BoNT-A.61

**MEDICAL DEVELOPMENTS**

Intradermal uses for botulinum toxin represent a new and exciting area of research with BoNT-A. The inhibition of inflammatory processes observed by researchers investigating the mechanism of action of BoNT-A in wound healing (as described above and in Wang and Tsai$^{64}$) may also be exploited in the treatment of these conditions.

The mode of action of BoNT-A in inflammatory diseases remains to be fully elucidated and, although there is some evidence to suggest efficacy, results in clinical studies conducted to date have not been conclusive.$^{64-66}$ However, research is at a very early stage and more clinical studies are ongoing (Table 5).

Interestingly, BoNT-A has demonstrated efficacy in a number of rare skin diseases for which there are few therapeutic options.

**Acne and Rosacea**

No single factor is entirely responsible for the development of acne, but changes in sebaceous gland functions, including increased production of sebum, are important.$^{67}$ BoNT-A has been shown to decrease sebum production and reduce pore size in individuals with oily skin, probably by blocking the activity of acetylcholine in sebocytes.$^{68}$ BoNT may also exert its effects via its action on the sebaceous gland.$^{69,70}$

Inhibition of acetylcholine signaling has also been implicated in the prevention of erythema and flushing—key symptoms of rosacea—and has been demonstrated in patients with Frey’s syndrome.$^{65,66}$ Frey’s syndrome is a neurological sequel to parotid surgery that causes sweating and flushing whenever saliva production is triggered.$^{71}$ These effects may be due to the inhibition by BoNT-A of acetylcholine and vasoactive intestinal polypeptide, and BoNT-A might therefore be an effective treatment for acne and rosacea.$^{65,66,72}$

BoNT-A has been investigated in several case studies of patients with recalcitrant rosacea. Two Caucasian patients received microdroplet intradermal injections of ONA into the glabella and/or cheeks at intervals of 0.5 cm. The total dose was 10 to 11 units. Patients reported an improvement in symptoms within 2 weeks of treatment, and the effects lasted for up to 4 months.$^{72}$ Two Korean patients underwent 2 treatment sessions with intradermal ONA at 1-week intervals. The total dose of ONA was 50 units across the 2 sessions for the first patient and 40 units across 2 sessions for the second. The cheeks, chin, and supra-eyebrow were injected at each session.$^{66}$ Improvements in rosacea flushing were evident 1 week after the second treatment and lasted for 3 months.$^{66,72}$ The only side-effect reported was mild pain during injection.$^{66}$

A proof-of-concept study investigated ABO in 15 patients with facial erythema of erythematotelangiectatic rosacea.$^{65}$ Initially patients received intradermal injections to the nasal tip, nasal bridge, and nasal alae, but the protocol was altered to also include the cheeks, forehead, and chin. The mean dose was 25 units (range 15-45 units). In this study, ABO was preferred to ONA because of apparently “greater diffusion and migration,” which the authors felt was more desirable when treating large areas of the face. There is no evidence to support greater diffusion of ABO from any study to date, only that the doses of ABO used in comparative studies are generally higher than those for other products, giving a larger ABO field of effect. Compared with baseline, erythema scores were significantly improved at 1 ($P < 0.05$), 2 ($P < 0.001$), and 3 months ($P < 0.05$) after treatment.
Photographic evidence confirmed these findings. Again, the only adverse event reported was mild pain from the injection.

The results of these small, but important, pilot studies suggest that intradermal BoNT-A injections are safe and efficacious for reducing erythema and flushing in rosacea. Larger, controlled, randomized studies are warranted to determine optimum dosing and duration of activity. More research is also required to elucidate the mechanism of action of BoNT-A in rosacea. The studies ongoing or due to report (Table 5) are, unfortunately, still quite small.

**Psoriasis**

Inverse (or intertriginous) psoriasis results from sweating and friction in skin folds. In 2008, Zanchi et al used ONA (50-100 units, depending on the extent and severity of the psoriasis) to reduce sweating and inflammation in 15 patients with inverse psoriasis. Photographic evidence and subjective patient assessment of pain and itch indicated that treatment had been successful in 87% of cases.

Other researchers have provided evidence that supports a role for the nervous system in psoriasis pathogenesis.

| Table 5. Clinical Trials With Botulinum Toxin in New Therapeutic Areas |
|----------------|----------------|----------------|----------------|----------------|
| Condition      | Agent          | ClinicalTrials.gov ID | Details                                                                 | Status                        |
| Bruxism        | ONA            | NCT02202070            | Phase I, randomized, double-blind, placebo-controlled, cross-over study to evaluate BoNT-A in adults with bruxism | Not yet recruiting            |
| Psoriasis vulgaris | Intradermal ONA | NCT00816517          | Phase I open-label study in adults with psoriasis vulgaris involving at least 1 area intolerant or recalcitrant to ≥2 topical or systemic treatments | Completed                     |
| Psoriasis vulgaris | ONA            | NCT02577185           | Phase I, randomized, single-blind internal-controlled study vs placebo in adults with chronic stable disease with lesions on arms and/or legs and/or trunk | Completed                     |
| Pain           | N/a            | NCT01553201           | Phase III, randomized, double-blind, placebo-controlled study to evaluate BoNT-A for the relief of pelvic pain in adults with endometriosis | Recruiting                    |
| Pain           | ONA            | NCT02616603           | Phase IV, randomized, double-blind, active controlled study to compare efficacy of ultrasound-guided ONA injections with triamcinolone acetonide for reducing pain in adults with shoulder pain following a stroke | Not yet recruiting            |
| Pain           | ONA            | NCT01905137           | Randomized, placebo-controlled study in women with confirmed myofascial pain, persistent pelvic pain rating at least 6 on a 10-point VAS | Recruiting                    |
| Pain           | ONA            | NCT02044302           | Phase II, prospective, randomized, double-blind, active controlled study to evaluate BoNT-A for relief of postoperative pain in adults undergoing breast reconstruction | Recruiting                    |
| Pain           | ONA            | NCT01591746           | Phase III, prospective, randomized, placebo-controlled study to evaluate BoNT-A for relief of pain in adults undergoing tissue expander breast reconstruction | Recruiting                    |
| Pain           | Not specified  | NCT02460107           | Randomized, placebo controlled study to evaluate BoNT-A for the relief of pain in diabetic peripheral polyneuropathy | Recruiting                    |
| Pain           | ONA            | NCT02173405           | Phase I, randomized, double-blind, placebo-controlled cross-over study in women with severe myofascial pelvic pain | Recruiting                    |
| Pain           | ONA            | NCT02058836           | Phase II, randomized, double-blind, placebo controlled trial in men with chronic testicular pain | Recruiting                    |
| Pain           | ONA            | NCT02512536           | Phase II, open-label, feasibility study to evaluate ultrasound-guided BoNT-A injections in patients with rotator cuff tear arthropathy | Not yet recruiting            |
| Rosacea        | INCO           | NCT01614743           | Phase II, randomized, double-blind, placebo-controlled, pilot cross-over study in adults with rosacea | Ongoing                       |

BoNT-A, Botulinum toxin-A; ONA, onabotulinumtoxinA; INCO, incobotulinumtoxinA; VAS, visual analog scale.
Ward et al demonstrated that ABO could induce remission of psoriasis in the keratinocyte-Tie2 (KC-Tie2) transgenic mouse model of psoriasis. One intradermal injection of ABO significantly reduced infiltration of CD11c+ dendritic cells ($P < 0.0001$) and CD4+ T cells ($P < 0.002$) at 6 weeks compared with saline-treated skin, and this was associated with a significant reduction in acanthosis ($P = 0.011$). Recently, the same group demonstrated local clearance of plaque psoriasis following an off-label intradermal injection of ABO in a single patient with recalcitrant disease. The duration of effect, showing complete clearance of plaques, was 7 months, with recurrence starting by month 8.

Based on current findings, BoNT-A may not be a practical approach for patients with extensive psoriasis. This is due to the number of injections needed and thus the treatment cost, as well as the risk of inducing muscle weakness with extensive dosing. However, BoNT-A may be a useful and long-lasting treatment for patients with focal lesions that are recalcitrant to standard therapy. Ongoing (Table 5) and future clinical trials are needed to provide additional evidence to confirm or refute this.

### Rare Skin Diseases

A number of rare skin diseases are caused by and/or have symptoms that are exacerbated by hyperhidrosis, a condition that can be treated successfully with BoNT-A. Fox-Fordyce disease (FFD) is a rare inflammatory disease of the apocrine glands characterized by the presence of discrete, dome-shaped, skin-colored, pruritic follicular papules in apocrine-rich areas of the skin (ie, the axillary, anogenital, and periareolar skin). The etiology of FFD has not been fully determined, but the condition is exacerbated by emotional, physical, and pharmacological stimulation that induces increased sweating.

The primary aim of therapy is to reduce pruritus. For this reason, the agents of choice are usually topical and intralesional corticosteroids; however, antiperspirants, calcineurin inhibitors, tretinoin, isotretinoin, clindamycin, and contraceptives have all shown some benefit in case studies. When pharmacotherapy fails, removal of the apocrine glands via liposuction-assisted curettage, electrocautery, or surgical excision is an option.

In a case study, a patient with refractory FFD was treated with refractory FFD was treated with intradermal injections of ONA. Both axillae were treated with a total of 50 injections of 2 units each, spaced 2 cm apart. Pruritus resolved after 15 days, and a marked reduction in the number and appearance of papules was also observed. The response was sustained for a period of at least 8 months.

The authors recommended that BoNT-A injections should be considered for patients with highly pruritic or recalcitrant FFD, but noted that clinical trials are needed to evaluate optimal treatment modalities and to further investigate how BoNT-A is exerting its effect in FFD.

Hailey–Hailey disease (familial benign chronic pemphigus) is a rare, inherited, bullous disease in which red scaly areas that can be itchy and sore can lead to superficial blisters and eroded areas of the skin folds of the groin, armpits, neck, and under the breasts. ONA and ABO, alone or in combination with other anti-inflammatory treatments, have demonstrated efficacy in patients with Hailey–Hailey disease in a range of case studies.

Bullous dermatoses are a cluster of rare autoimmune blistering diseases that can be debilitating and potentially fatal. Individual conditions affect different areas of the body, but in most cases the blistering can be extensive. Treatment typically includes corticosteroids and other immunosuppressive agents. One case study documented treatment of linear immunoglobulin A bullous dermatosis with BoNT-A. The disease was mostly controlled by immunosuppressive drugs, but sweating caused flares of symptoms. Multiple injections of BoNT-A into the axillae not only reduced blistering for up to 6 months, but also improved patient quality of life scores.

Pachyonychia congenita is a genodermatosis, which may be associated with painful hyperkeratotic lesions on the soles of the feet that blister when the feet become hot and start to sweat. The condition can make walking difficult. A series of 3 case studies using ABO and a small retrospective study with ONA have shown that BoNT-A given after local anesthetic reduced sweating and provided pain relief that lasted for 3 to 6 months. Importantly for this chronic condition, repeated treatments did not result in loss of efficacy.

### Pain Relief

A glance at Table 5 will reveal that the most active new therapeutic area for BoNT-A is pain relief. Relief of pain is an important aspect of some of the currently approved indications for ONA, notably in chronic migraine, chronic headache, and cervical dystonia. Clinical studies that focus on these conditions are not included in Table 5. Additional sources of pain in which BoNT-A has been shown to be useful are diabetic polyneuropathy, trigeminal neuralgia, chronic back and shoulder pain, myofascial pain, temporomandibular joint disorders, and pain due to arthritis and multiple sclerosis.

There is some speculation about the mechanism for pain relief with BoNT-A. Hypotheses involving both the peripheral and central nervous systems have been proposed. Clearly, the effect of BoNT-A on neurotransmission at the NMJ must play a role in relieving muscular pain, such as that associated with spasm and cramping. Some
chronic diseases cause the wide dynamic range neurons of the central nervous system (CNS) to perceive nonpainful stimuli as causing pain, and this sense of pain can increase as the disease state persists. BoNT-A attenuates this condition by diminishing nonnociceptive stimuli, altering postganglionic cholinergic nerve fibers with blood vessels, and interfering with peripheral glutamatergic pain modulation, hence effecting changes in neuroplastic mechanisms within the CNS that lead to a reduction in chronic pain.93 Inhibition of acetylcholine signaling in the NMJ is also known to inhibit the release of neurotransmitters such as substance P, glutamate, and calcitonin gene-related peptide, which also serves to dull the sensation of pain.90

There is accumulating evidence from animal models and completed clinical studies that BoNT-A shows promise for the relief of chronic pain.90 However, larger and better-designed studies are still required to confirm these important effects.

CONCLUSIONS

ABO is well established around the world for the treatment of wrinkles in the upper face, and is frequently used off-label to treat other regions of the face and neck. Ongoing research is identifying new ways of using BoNT-A (including ABO) to treat facial lines, which have the potential to increase its efficacy, make procedures more comfortable, and give an overall more pleasing effect for patients.

In addition, recent and ongoing research suggests that use of ABO could be extended to other aesthetic and medical uses—in particular intralesional and intradermal therapy for the treatment of HS and inflammatory diseases, respectively, and the relief of musculoskeletal and neuropathic pain. These areas have shown great promise to date, but more and larger clinical studies, beyond those already in progress or about to start, will be required before these treatments become a clinical reality.

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