Supplement Article

New Uses of AbobotulinumtoxinA in Aesthetics

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

Aesthetic Surgery Journal
2017, Vol 37(S1) S45–S58

DOI: 10.1093/asj/sjx005
www.aestheticsurgeryjournal.com
OXFORD UNIVERSITY PRESS

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.

Editorial Decision date: January 4, 2017.

Since the US Food and Drug Administration approval of therapeutic BotulinumtoxinA (BoNT-A) for aesthetic uses in 2002, the products have become well established in this field and are widely approved for correcting wrinkles in the glabella and eye areas.[1,2] The 3 currently approved commercial BoNT-A formulations—AbobotulinumtoxinA (ABO), incobotulinumtoxinA (INCO), and onabotulinumtoxinA (ONA)—are also used extensively off-label for a number of other purposes.[2]

Dr Schlessinger is a dermatologist and cosmetic surgeon in private practice in Omaha, NE. Dr Gilbert is a dermatologist in private practice in Brooklyn, NY. Dr Cohen is a dermatologist in private practice in Greenwood Village, CO. Dr Kaufman is dermatologist in private practice in Miami, FL.

Corresponding Author:
Dr Joel Schlessinger, Skin Specialists, PC, 2802 Oak View Drive, Suite 100, Omaha NE 68144, USA.
E-mail: skindoc@lovelyskin.com

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).[1]

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.[2-4] 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.[1,3]

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.[5-7]

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.[8,9] 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.[8,9]

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).[8,10,11] 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).[5] 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.[5,8,10] 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%.[5] 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.[9] These may represent successive stages of a fibroproliferative skin disorder differentiated by varying degrees of inflammation.[12] Facial HS and keloids are disfiguring and are often associated with clinical symptoms such as itching, pain, and restricted range of motion and contracture.[8]

The precise mechanisms underlying the formation of HS and keloids are not fully understood, and this makes their management difficult.[9] Facial scarring can have profound psychological effects on patients.[13,14] In one US study, 91% of patients said that they would value even small improvements in scarring.[15] Conventional options for the management of HS and keloids include intralesional corticosteroid injections, surgery, cryotherapy, pressure therapy, radiotherapy, laser therapy, and application of silicone gel sheeting.[9,16]

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.[8]

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.[11,16-18] These phenomena were not observed in fibroblasts isolated from normal skin.[18] Further investigation indicated that, as a result of the inhibition of fibroblast proliferation, production of the inflammatory cytokine transforming growth factor (TGF)-β1 and connective tissue growth factor were also diminished.[11,19] 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.[12]

Vascular endothelial growth factor (VEGF) has been shown to promote angiogenesis in wound healing.[20] 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.[21]

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.[22] 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.[23-28]

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.

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	ONA gave narrower scars with improved appearance, but height, color, vascularity, and pliability were not changed
5	Adults undergoing cheiloplasty revision	Six intraoperative injections of ONA or saline into the orbicularis oris	Compared with control, VSS scores were significantly lower ($P = 0.023$) for ONA-treated wounds and VAS scores were significantly higher ($P < 0.001$)
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 7.08 to 9.58, and VAS scores for saline control ranged from 4.08 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.

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 I/II 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.

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]

BoNT-A Plus Dermal Fillers

Several studies have indicated that a combination of BoNT-A and dermal fillers may achieve greater durability of effect.[33,36,39] One explanation for this is that fillers have a greater duration of action than neurotoxin, meaning that the return to baseline is more gradual. In addition, the relaxation effect of BoNT-A may assist the dermal filler to achieve better wrinkle smoothing effects. In a split-face study comparing ABO plus HA with ABO alone, there was a suggestion that the filler and toxin act synergistically to improve overall cosmesis when they are both at peak effectiveness.[39] In this study, blinded assessors found that the combination gave statistically significantly greater improvement in both dynamic and static lines in the glabella, and dynamic lines on the forehead, at 24 weeks after treatment. There was a nominal (but not statistically significant) improvement at 2, 6, and 12 weeks.[39]

BoNT-A Plus Laser/Light Fillers

Laser resurfacing is used to address epidermal and superficial dermal problems through epidermal ablation, collagen

shrinkage, stimulation of neocollagenesis, dermal remodeling, and regeneration of cellular organelles and intercellular attachments.[37,40]

Case studies demonstrate that treatment with, for example, a 595 nm pulsed dye laser in conjunction with BoNT-A injections (given immediately after the first laser treatment) can be safe and effective for correcting traumatic scars in the highly mobile chin area,[41] but this combination has not been investigated in formal clinical trials. A small split-face RCT demonstrated significantly greater improvement in crow's feet lines with ONA given 2 to 6 weeks before treatment with an Erbium:YAG laser as compared with laser treatment alone.[42]

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/cm^2.[43] 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.[44]

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.[45] 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.[46] 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.[45]

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,[38] while creams, serums, and gels containing HA can improve skin hydration and elasticity.[47] 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.[48]

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.[33,38]

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.[49] 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).[49] 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.[49]

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."[50,51] 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.[51] 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 microcannulae inserted at 4 points (2 in the temporomandibular area and 2 in the jugular area), although this is less accurate. The present authors have found the Aquagold Fine Touch microneedling device (Aquavit Pharmaceuticals, New York, NY), which can also be used to deliver combinations 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 formulations 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.

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.

Advances in Formulations

As a result of the large molecular size, BoNT-A has negligible 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 expectation 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 CO_2 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 significant 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 significantly 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 treatment 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 displayed 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 endpoints. 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]

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.[58] 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.[58] The differences in efficacy are readily explained by the fact that the potency units of botulinum products are specific to each product family and are not interchangeable.[1] 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.[59] 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.[59]

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.[60-62] This strategy has the potential to optimize the outcome for the individual, and to reduce dosages and numbers of injections.[61-63] 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.[60] 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.[60] 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.[63]

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.[62] 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).[62]

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

Table 4. Tailored Botulinum Toxin Type A Injection Protocol,[a] Republished with permission of Plastic and Reconstructive Surgery[62]; permission conveyed through Copyright Clearance Center, Inc.

Thickness (mm)	Bulging type					Total BoNT-A Dosage (units)
	I	II	III	IV	V	
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. [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.

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 I/II, randomized, double-blind, placebo-controlled study to evaluate BoNT-A for the relief of pelvic pain in adults with endometriosis	Recruiting
Pain	ONA	NCT02618603	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.

Photographic evidence confirmed these findings. Again, the only adverse event reported was mild pain from the injection.[65]

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.[65,66,72] 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.[73] 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.[74]

Ward et al demonstrated that ABO could induce remission of psoriasis in the keratinocyte-Tie2 (KC-Tie2) transgenic mouse model of psoriasis.[74] 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.[75] 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.[64,74] 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,[76-78] 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).[76] 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.[79] 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 intradermal injections of ONA.[79] 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.[79]

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.[78,80-85]

Bullous dermatoses are a cluster of rare autoimmune blistering diseases that can be debilitating and potentially fatal.[86] 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.[87] 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.[87]

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.[77,88] 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.[77,88]

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.[89] 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.[90-92]

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.

Acknowledgment

The authors would like to acknowledge the support of Jane Tricker, freelance writer, who assisted in the writing of this paper, and MedSense, Ltd. (High Wycombe, UK) for editorial support, both funded by Galderma.

Disclosures

Dr Schlessinger has participated on advisory boards for Allergan Pharmaceuticals, Alphaeon Corporation, Valeant Pharmaceuticals, Galderma, Novartis Pharmaceuticals, Ortho Dermatological, Johnson & Johnson, Excel Cosmeceuticals, Health and Wellness Council of America, MJD Communications, Omaha Symphony, and Film Streams; and has worked as a researcher for 3M Pharma, Abbvie Pharma, Actavis, Allergan, Amgen, Anacor, Bayer, Celgene, Dermik, Dermira, Eli Lilly, Galderma, Genentech, Glaxo/Stiefel, Kythera, MEDICIS, Mentor, Merck, Merz, Novartis, Ortho Pharma. (Johnson & Johnson), Perrigo, Pfizer, Quinnova, Revance, Sandoz, Schering Plough, Tolmar, Valeant, Zydus Cadila, Ulthera, Inc., and Viamet Pharma. Dr Cohen has served as a clinical investigator for Leo, Syneron-Candela, Neothetics, MELA Sciences, Evolus/Alpheon, Suneva, and Croma; and has received honoraria as a clinical investigator/consultant or speaker from Allergan, Valeant, Merz/Ulthera/Cellfina, Galderma, Sciton, and Thermi/Almirall. Dr Kaufman has received research funding from Allergan, grants and research funding from Merz, personal fees and honoraria from Galderma, and research funding from Revance. Dr Gilbert declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

Funding

This publication was supported by Galderma Laboratories, LP (Fort Worth, TX), who funded the development of this supplement. Editorial assistance was funded by Galderma Laboratories, LP, and provided by Jane Tricker and MedSense, Ltd. (High Wycombe, UK). The authors did not receive compensation for writing the manuscripts.

REFERENCES

1. Carruthers J, Fournier N, Kerscher M, Ruiz-Avila J, Trindade de Almeida AR, Kaeuper G. The convergence of medicine and neurotoxins: a focus on botulinum toxin type A and its application in aesthetic medicine—a global, evidence-based botulinum toxin consensus education initiative: part II: incorporating botulinum toxin into aesthetic clinical practice. *Dermatol Surg*. 2013;39(3 Pt 2):510-525.
2. Erickson BP, Lee WW, Cohen J, Grunebaum LD. The role of neurotoxins in the periorbital and midfacial areas. *Facial Plast Surg Clin North Am*. 2015;23(2):243-255.
3. Flynn TC. Advances in the use of botulinum neurotoxins in facial esthetics. *J Cosmet Dermatol*. 2012;11(1):42-50.
4. Davis EC, Callender VD. Aesthetic dermatology for aging ethnic skin. *Dermatol Surg*. 2011;37(7):901-917.
5. Chang CS, Wallace CG, Hsiao YC, Chang CJ, Chen PK. Botulinum toxin to improve results in cleft lip repair: a double-blinded, randomized, vehicle-controlled clinical trial. *PLoS One*. 2014;9(12):e115690.
6. Prodromidou A, Frountzas M, Vlachos DE, et al. Botulinum toxin for the prevention and healing of wound scars: a systematic review of the literature. *Plast Surg (Oakv)*. 2015;23(4):260-264.
7. Ziade M, Domergue S, Batifol D, et al. Use of botulinum toxin type A to improve treatment of facial wounds: a prospective randomised study. *J Plast Reconstr Aesthet Surg*. 2013;66(2):209-214.
8. Jablonka EM, Sherris DA, Gassner HG. Botulinum toxin to minimize facial scarring. *Facial Plast Surg*. 2012;28(5):525-535.
9. Gauglitz GG. Management of keloids and hypertrophic scars: current and emerging options. *Clin Cosmet Investig Dermatol*. 2013;6:103-114.
10. Gauglitz GG, Bureik D, Dombrowski Y, Pavicic T, Ruzicka T, Schauber J. Botulinum toxin A for the treatment of keloids. *Skin Pharmacol Physiol*. 2012;25(6):313-318.

11. Xiao Z, Zhang M, Liu Y, Ren L. Botulinum toxin type a inhibits connective tissue growth factor expression in fibroblasts derived from hypertrophic scar. *Aesthetic Plast Surg*. 2011;35(5):802-807.
12. Huang C, Akaishi S, Hyakusoku H, Ogawa R. Are keloid and hypertrophic scar different forms of the same disorder? A fibroproliferative skin disorder hypothesis based on keloid findings. *Int Wound J*. 2014;11(5):517-522.
13. Marshall GN. Screening for psychiatric problems in the orofacial trauma setting. *Oral Maxillofac Surg Clin North Am*. 2010;22(2):225-229.
14. Tebble NJ, Adams R, Thomas DW, Price P. Anxiety and self-consciousness in patients with facial lacerations one week and six months later. *Br J Oral Maxillofac Surg*. 2006;44(6):520-525.
15. Young VL, Hutchison J. Insights into patient and clinician concerns about scar appearance: semiquantitative structured surveys. *Plast Reconstr Surg*. 2009;124(1):256-265.
16. Chen M, Yan T, Ma K, et al. Botulinum toxin type A inhibits its α-smooth muscle actin and myosin II expression in fibroblasts derived from scar contracture. *Ann Plast Surg*. 2016;77(3):e46-e49.
17. Xiao Z, Qu G. Effects of botulinum toxin type a on collagen deposition in hypertrophic scars. *Molecules*. 2012;17(2):2169-2177.
18. Jeong HS, Lee BH, Sung HM, et al. Effect of botulinum toxin type A on differentiation of fibroblasts derived from scar tissue. *Plast Reconstr Surg*. 2015;136(2): 171e-178e.
19. Xiao Z, Zhang F, Lin W, Zhang M, Liu Y. Effect of botulinum toxin type A on transforming growth factor beta1 in fibroblasts derived from hypertrophic scar: a preliminary report. *Aesthetic Plast Surg*. 2010;34(4):424-427.
20. Wilgus TA, Ferreira AM, Oberyszyn TM, Bergdall VK, Dipietro LA. Regulation of scar formation by vascular endothelial growth factor. *Lab Invest*. 2008;88(6):579-590.
21. Haubner F, Leyh M, Ohmann E, Sadick H, Gassner HG. Effects of botulinum toxin A on patient-specific keloid fibroblasts in vitro. *Laryngoscope*. 2014;124(6):1344-1351.
22. Zhang DZ, Liu XY, Xiao WL, Xu YX. Botulinum toxin type A and the prevention of hypertrophic scars on the maxillofacial area and neck: a meta-analysis of randomized controlled trials. *PLoS One*. 2016;11(3):e0151627.
23. Galárraga IM. Use of botulinum toxin in cheiloplasty: A new method to decrease tension. *Can J Plast Surg*. 2009;17(3):e1-e2.
24. Chang CS, Wallace CG, Hsiao YC, Chang CJ, Chen PK. Botulinum toxin to improve results in cleft lip repair. *Plast Reconstr Surg*. 2014;134(3):511-516.
25. Kim YS, Lee HJ, Cho SH, Lee JD, Kim HS. Early postoperative treatment of thyroidectomy scars using botulinum toxin: a split-scar, double-blind randomized controlled trial. *Wound Repair Regen*. 2014;22(5):605-612.
26. Zelken J, Yang S-Y, Chang C-S, et al. Donor site aesthetic enhancement with preoperative botulinum toxin in forehead flap nasal reconstruction. *Ann Plast Surg*. 2016;77(5):535-538.
27. Gassner HG, Brissett AE, Otley CC, et al. Botulinum toxin to improve facial wound healing: a prospective, blinded, placebo-controlled study. *Mayo Clin Proc*. 2006;81(8):1023-1028.
28. Wilson AM. Use of botulinum toxin type A to prevent widening of facial scars. *Plast Reconstr Surg*. 2006;117(6):1758-1766; discussion 1767.
29. Flynn TC. Use of intraoperative botulinum toxin in facial reconstruction. *Dermatol Surg*. 2009;35(2):182-188.
30. Robinson AJ, Khadim MF, Khan K. Keloid scars and treatment with botulinum toxin type A: the Belfast experience. *J Plast Reconstr Aesthet Surg*. 2013;66(3):439-440.
31. Shaarawy E, Hegazy RA, Abdel Hay RM. Intralesional botulinum toxin type A equally effective and better tolerated than intralesional steroid in the treatment of keloids: a randomized controlled trial. *J Cosmet Dermatol*. 2015;14(2):161-166.
32. Pavicic T, Few JW, Huber-Vorländer J. A novel, multistep, combination facial rejuvenation procedure for treatment of the whole face with incobotulinumtoxinA, and two dermal fillers- calcium hydroxylapatite and a monophasic, polydensified hyaluronic acid filler. *J Drugs Dermatol*. 2013;12(9):978-984.
33. Ascher B, Fanchon C, Kanoun-Copy L, Bouloc A, Benech F. A skincare containing retinol adenosine and hyaluronic acid optimises the benefits from a type A botulinum toxin injection. *J Cosmet Laser Ther*. 2012;14(5):234-238.
34. Beer KR, Julius H, Dunn M, Wilson F. Remodeling of periorbital, temporal, glabellar, and crow's feet areas with hyaluronic acid and botulinum toxin. *J Cosmet Dermatol*. 2014;13(2):143-150.
35. Lorenc ZP, Daro-Kaftan E. Optimizing facial rejuvenation outcomes by combining poly-L-lactic acid, hyaluronic acid, calcium hydroxylapatite, and neurotoxins: two case studies. *J Drugs Dermatol*. 2014;13(2):191-195.
36. Molina B, David M, Jain R, et al. Patient satisfaction and efficacy of full-facial rejuvenation using a combination of botulinum toxin type A and hyaluronic acid filler. *Dermatol Surg*. 2015;41(Suppl 1):S325-S332.
37. Rubin MG, Cox SE, Kaminer MS, Solish N. Correcting age-related changes in the face by use of injectable fillers and neurotoxins. *Semin Cutan Med Surg*. 2014;33(4 Suppl):S81-S84.
38. Schlessinger J, Kenkel J, Werschler P. Further enhancement of facial appearance with a hydroquinone skin care system plus tretinoin in patients previously treated with botulinum toxin Type A. *Aesthet Surg J*. 2011;31(5):529-539.
39. Dubina M, Tung R, Bolotin D, et al. Treatment of forehead/glabellar rhytide complex with combination botulinum toxin a and hyaluronic acid versus botulinum toxin A injection alone: a split-face, rater-blinded, randomized control trial. *J Cosmet Dermatol*. 2013;12(4):261-266.
40. Ganceviciene R, Liakou AI, Theodoridis A, Makrantonaki E, Zouboulis CC. Skin anti-aging strategies. *Dermatoendocrinol*. 2012;4(3):308-319.
41. Lee SJ, Jeong SY, No YA, Park KY, Kim BJ. Combined treatment with botulinum toxin and 595-nm pulsed dye laser for traumatic scarring. *Ann Dermatol*. 2015;27(6):756-758.

42. Yamauchi PS, Lask G, Lowe NJ. Botulinum toxin type A gives adjunctive benefit to periorbital laser resurfacing. *J Cosmet Laser Ther*. 2004;6(3):145-148.
43. Khoury JG, Saluja R, Goldman MP. The effect of botulinum toxin type A on full-face intense pulsed light treatment: a randomized, double-blind, split-face study. *Dermatol Surg*. 2008;34(8):1062-1069.
44. Carruthers J, Carruthers A. The effect of full-face broadband light treatments alone and in combination with bilateral crow's feet Botulinum toxin type A chemodenervation. *Dermatol Surg*. 2004;30(3):355-366; discussion 366.
45. Permatasari F, Hu YY, Zhang JA, Zhou BR, Luo D. Anti-photoaging potential of Botulinum toxin type A in UVB-induced premature senescence of human dermal fibroblasts in vitro through decreasing senescence-related proteins. *J Photochem Photobiol B*. 2014;133:115-123.
46. Bonaparte JP, Ellis D. Alterations in the elasticity, pliability, and viscoelastic properties of facial skin after injection of onabotulinum toxin A. *JAMA Facial Plast Surg*. 2015;17(4):256-263.
47. Pavicic T, Gauglitz GG, Lersch P, et al. Efficacy of cream-based novel formulations of hyaluronic acid of different molecular weights in anti-wrinkle treatment. *J Drugs Dermatol*. 2011;10(9):990-1000.
48. Abella ML. Evaluation of anti-wrinkle efficacy of adenosine-containing products using the FOITS technique. *Int J Cosmet Sci*. 2006;28(6):447-451.
49. Wu WTL. Microbotox of the lower face and neck: evolution of a personal technique and its clinical effects. *Plast Reconstr Surg*. 2015;136(5 suppl):92S-100S.
50. Môle B. Accordion wrinkle treatment through the targeted use of botulinum toxin injections. *Aesthetic Plast Surg*. 2014;38(2):419-428.
51. Môle B. Griffose faciale: traitement des rides dynamiques du visage par injections dermiques simultanées de toxine botulique A et d'acide hyaluronique. *Ann Chir Plast Esthet*. 2012;57(3):194-201.
52. Glogau R, Blitzer A, Brandt F, Kane M, Monheit GD, Waugh JM. Results of a randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of a botulinum toxin type A topical gel for the treatment of moderate-to-severe lateral canthal lines. *J Drugs Dermatol*. 2012;11(1):38-45.
53. Lim EC, Seet RC. Botulinum toxin: description of injection techniques and examination of controversies surrounding toxin diffusion. *Acta Neurol Scand*. 2008;117(2):73-84.
54. Mahmoud BH, Ozog D, Burnett C, Cohen JL. Prospective randomized split-face comparative study between topical botulinum toxin A surface application and local injection for Crow's feet. *Dermatol Surg*. 2016;42(4):554-556.
55. Zhu J, Ji X, Li M, et al. The efficacy and safety of fractional CO_2 laser combined with topical type A botulinum toxin for facial rejuvenation: a randomized controlled split-face study. *Biomed Res Int*. 2016;2016:3853754.
56. Mahmoud BH, Burnett C, Ozog D. Prospective randomized controlled study to determine the effect of topical application of botulinum toxin A for crow's feet after treatment with ablative fractional CO2 laser. *Dermatol Surg*. 2015;41(Suppl 1):S75-S81.
57. Revance Therapeutics. Revance Reports Results for RT001 Topical Phase 3 Trial for Lateral Canthal Lines. GlobeNewswire News Room. 2016. Available from: https://globenewswire.com/news-release/2016/06/13/848193/0/en/Revance-Reports-Results-for-RT001-Topical-Phase-3-Trial-for-Lateral-Canthal-Lines.html. Accessed 25 July 2016.
58. Kim JE, Song EJ, Choi GS, Lew BL, Sim WY, Kang H. The efficacy and safety of liquid-type botulinum toxin type A for the management of moderate to severe glabellar frown lines. *Plast Reconstr Surg*. 2015;135(3):732-741.
59. Sharma P, Czyz CN, Wulc AE. Investigating the efficacy of vibration anesthesia to reduce pain from cosmetic botulinum toxin injections. *Aesthet Surg J*. 2011;31(8):966-971.
60. Kane MA. Nonsurgical periorbital and brow rejuvenation. *Plast Reconstr Surg*. 2015;135(1):63-71.
61. Guerrrerosantos J, Carlos Eduardo PG, Mateos Arriola J, et al. Effectiveness of botulinum toxin (type-A) administered by the fixed-site dosing approach versus the muscle area identification. *Aesthetic Plast Surg*. 2015;39(2):243-251.
62. Xie Y, Zhou J, Li H, Cheng C, Herrler T, Li Q. Classification of masseter hypertrophy for tailored botulinum toxin type A treatment. *Plast Reconstr Surg*. 2014;134(2):209e-218e.
63. Kane MA, Brandt F, Rohrich RJ, Narins RS, Monheit GD, Huber MB; Reloxin Investigational Group. Evaluation of variable-dose treatment with a new U.S. Botulinum Toxin Type A (Dysport) for correction of moderate to severe glabellar lines: results from a phase III, randomized, double-blind, placebo-controlled study. *Plast Reconstr Surg*. 2009;124(5):1619-1629.
64. Wang TS, Tsai TF. Intralesional therapy for psoriasis. *J Dermatolog Treat*. 2013;24(5):340-347.
65. Bloom BS, Payongayong L, Mourin A, Goldberg DJ. Impact of intradermal abobotulinumtoxinA on facial erythema of rosacea. *Dermatol Surg*. 2015;41(Suppl 1):S9-16.
66. Park KY, Hyun MY, Jeong SY, Kim BJ, Kim MN, Hong CK. Botulinum toxin for the treatment of refractory erythema and flushing of rosacea. *Dermatology*. 2015;230(4):299-301.
67. Zouboulis CC. Acne and sebaceous gland function. *Clin Dermatol*. 2004;22(5):360-366.
68. Li ZJ, Park SB, Sohn KC, et al. Regulation of lipid production by acetylcholine signalling in human sebaceous glands. *J Dermatol Sci*. 2013;72(2):116-122.
69. Shah AR. Use of intradermal botulinum toxin to reduce sebum production and facial pore size. *J Drugs Dermatol*. 2008;7(9):847-850.
70. Rose AE, Goldberg DJ. Safety and efficacy of intradermal injection of botulinum toxin for the treatment of oily skin. *Dermatol Surg*. 2013;39(3 Pt 1):443-448.
71. Tugnoli V, Marchese Ragona R, Eleopra R, et al. The role of gustatory flushing in Frey's syndrome and its treatment with botulinum toxin type A. *Clin Auton Res*. 2002;12(3):174-178.
72. Dayan SH, Pritzker RN, Arkins JP. A new treatment regimen for rosacea: onabotulinumtoxinA. *J Drugs Dermatol*. 2012;11(12):e76-e79.

73. Zanchi M, Favot F, Bizzarini M, Piai M, Donini M, Sedona P. Botulinum toxin type-A for the treatment of inverse psoriasis. *J Eur Acad Dermatol Venereol*. 2008;22(4):431-436.
74. Ward NL, Kavlick KD, Diaconu D, Dawes SM, Michaels KA, Gilbert E. Botulinum neurotoxin A decreases infiltrating cutaneous lymphocytes and improves acanthosis in the KC-Tie2 mouse model. *J Invest Dermatol*. 2012;132(7):1927-1930.
75. Gilbert E, Ward NL. Efficacy of botulinum neurotoxin type A for treating recalcitrant plaque psoriasis. *J Drugs Dermatol*. 2014;13(11):1407-1408.
76. Alikhan A, Gorouhi F, Zargari O. Fox-Fordyce disease exacerbated by hyperhidrosis. *Pediatr Dermatol*. 2010;27(2):162-165.
77. Swartling C, Karlqvist M, Hymnelius K, Weis J, Vahlquist A. Botulinum toxin in the treatment of sweat-worsened foot problems in patients with epidermolysis bullosa simplex and pachyonychia congenita. *Br J Dermatol*. 2010;163(5):1072-1076.
78. Lapiere JC, Hirsh A, Gordon KB, Cook B, Montalvo A. Botulinum toxin type A for the treatment of axillary Hailey-Hailey disease. *Dermatol Surg*. 2000;26(4):371-374.
79. González-Ramos J, Alonso-Pacheco ML, Goiburú-Chenú B, Mayor-Ibarguren A, Herranz-Pinto P. Successful treatment of refractory pruritic Fox-Fordyce disease with botulinum toxin type A. *Br J Dermatol*. 2016;174(2):458-459.
80. Bedi M, Taylor AL. Recalcitrant Hailey-Hailey disease responds to oral tacrolimus and botulinum toxin type A. *Cutis*. 2015;96(6):E14-E16.
81. Bessa GR, Grazziotin TC, Manzoni AP, Weber MB, Bonamigo RR. Hailey-Hailey disease treatment with Botulinum toxin type A. *An Bras Dermatol*. 2010;85(5):717-722.
82. Ho D, Jagdeo J. Successful botulinum toxin (onabotulinumtoxinA) treatment of Hailey-Hailey disease. *J Drugs Dermatol*. 2015;14(1):68-70.
83. López-Ferrer A, Alomar A. [Botulinum toxin A for the treatment of familial benign pemphigus]. *Actas Dermosifiliogr*. 2012;103(6):532-535.
84. White F, Shvartsbeyn M, Meehan SA, Urbanek RW. Benign familial pemphigus (Hailey-Hailey disease). *Dermatol Online J*. 2015;21(12): pii: 13030/qt6z35r538.
85. Koeyers WJ, Van Der Geer S, Krekels G. Botulinum toxin type A as an adjuvant treatment modality for extensive Hailey-Hailey disease. *J Dermatolog Treat*. 2008;19(4):251-254.
86. Bickle K, Roark TR, Hsu S. Autoimmune bullous dermatoses: a review. *Am Fam Physician*. 2002;65 (9):1861-1870.
87. Legendre L, Maza A, Almalki A, Bulai-Livideanu C, Paul C, Mazereeuw-Hautier J. Botulinum toxin A: an effective treatment for linear immunoglobulin A bullous dermatosis located in the Axillae. *Acta Derm Venereol*. 2016;96(1):122-123.
88. Swartling C, Vahlquist A. Treatment of pachyonychia congenita with plantar injections of botulinum toxin. *Br J Dermatol*. 2006;154(4):763-765.
89. Guo BL, Zheng CX, Sui BD, Li YQ, Wang YY, Yang YL. A closer look to botulinum neurotoxin type A-induced analgesia. *Toxicon*. 2013;71:134-139.
90. Pickett A. Re-engineering clostridial neurotoxins for the treatment of chronic pain: current status and future prospects. *BioDrugs*. 2010;24(3):173-182.
91. Patil S, Willett O, Thompkins T, et al. Botulinum toxin: pharmacology and therapeutic roles in pain states. *Curr Pain Headache Rep*. 2016;20(3):15.
92. Ney JP, Joseph KR. Neurologic uses of botulinum neurotoxin type A. *Neuropsychiatr Dis Treat*. 2007;3(6):785-798.
93. Purkiss J, Welch M, Doward S, Foster K. Capsaicin-stimulated release of substance P from cultured dorsal root ganglion neurons: involvement of two distinct mechanisms. *Biochem Pharmacol*. 2000;59(11):1403-1406.