OnabotulinumtoxinA and Hyaluronic Acid in Facial Wrinkles and Folds: A Prospective, Open-Label Comparison

Aesthetic Surgery Journal
2019, Vol 39(2) 187–200

DOI: 10.1093/asj/sjy116
www.aestheticsurgeryjournal.com
OXFORD
UNIVERSITY PRESS

Joel L. Cohen, MD; Arthur Swift, MD; Nowell Solish, MD; Steve Fagien, MD; and Dee Anna Glaser, MD

Abstract

Background: OnabotulinumtoxinA and hyaluronic acid are effective in improving moderate to severe facial wrinkles and folds, with treatment selection traditionally based upon facial area.

Objectives: This prospective, multicenter, open-label, crossover study evaluated physician-rated efficacy and patient-rated outcomes following moderate to severe facial wrinkles and folds treatment with onabotulinumtoxinA and hyaluronic acid.

Methods: 152 subjects (25-65 years) were randomized (1:1) to a treatment-sequence of onabotulinumtoxinA/hyaluronic acid or hyaluronic acid/onabotulinumtoxinA, with initial treatment administered on day 1 and 6 additional visits: week 2 (touch-up); week 4 (crossover); week 6 (touch-up); and weeks 8, 12, and 24 (follow-up).

Results: Between 92% and 100% of subjects in each treatment-sequence group exhibited at least some improvement from baseline at each study visit in the Physician Aesthetic Improvement Scale and the Objective Observer and Patient Global Assessments of Improvement, with no significant between-sequence differences. Subjects reported looking 3 to 6 years younger at each visit, with significant improvements in glabellar, lateral canthal, and horizontal forehead lines, and nasolabial folds. Treatments were well tolerated.

Conclusions: OnabotulinumtoxinA and hyaluronic acid provide clinically meaningful improvements as rated by physicians, objective observers, and subjects, with clinical synergy in aesthetic effects and duration of response regardless of treatment administration order in subjects seeking improvement in moderate to severe facial wrinkles and folds.

Level of Evidence: 2

Editorial Decision date: April 25, 2018; online publish-ahead-of-print May 11, 2018.

Glabellar frown lines (GFLs) result from prolonged overactivity of the corrugator and procerus muscles.[1,2] GFLs and hyper-functional facial lines such as crow's feet, horizontal forehead lines as well as moderate to severe nasolabial folds (at rest or at maximal facial contraction) are a cause of aesthetic concern to many adults.[1-3] Aesthetic options currently available to temporarily improve the appearance of these facial wrinkles and folds include skin resurfacing (laser and chemical peels), tightening devices such as radiofrequency, surgical interventions, and injectables with filler/botulinum toxins.[3,4]

Dr Cohen is an Associate Clinical Professor of Dermatology, University of Colorado; and an Assistant Clinical Professor of Dermatology, University of California, Irvine, CA. Dr Swift is a plastic surgeon in private practice in Montreal, Quebec, Canada. Dr Solish is an Assistant Professor, Division of Dermatology, University of Toronto, Ontario, Canada. Dr Fagien is an oculoplastic surgeon in private practice in Boca Raton, FL. Dr Glaser is a Professor, Department of Dermatology, St. Louis University, St Louis, MO.

Corresponding Author:
Dr Joel L. Cohen, 5340 South Quebec Street, Suite 300, Greenwood Village, CO 80111, USA.
E-mail: jcohenderm@yahoo.com

OnabotulinumtoxinA is indicated for the temporary improvement in the appearance of moderate to severe GFLs associated with corrugator and/or procerus muscle activity in adults 65 years of age and younger, but also for improvement of lateral canthal rhytids. It is marketed as BOTOX® Cosmetic in the United States (US) (Allergan, Inc., Irvine, CA) and as VISTABEL® in the European Union (EU), and is used at a recommended dose of 20 units (U) for the GFL indication and 24 U for the lateral canthal indication.

Cross-linked hyaluronic acid plus 0.3% lidocaine (hyaluronic acid or HA) is available in a wide variety of formulations including JUVÉDERM® Ultra XC and JUVÉDERM® Ultra Plus XC injectable gels that are marketed in the US and Canada. It is marketed as JUVÉDERM® Ultra 2 and JUVÉDERM® Ultra 3 in the EU. Hyaluronic acid gel products are injected into the mid- to deep dermis as a temporary tissue filler for facial wrinkles and folds including nasolabial folds. JUVÉDERM® Ultra XC and JUVÉDERM® Ultra 2 are indicated for the improvement of moderate facial lines or folds, while JUVÉDERM® Ultra Plus XC and JUVÉDERM® Ultra 3 are indicated for severe facial lines or folds.

Patients with aesthetic concerns often have issues with multiple facial areas and features, with clinicians taking a more global approach to facial rejuvenation and treatments.[5-12] A few studies have examined the global rejuvenation approach using individual products, generally administered in 1 or 2 facial areas.[12-17] Combination therapy using botulinum toxin and dermal filler was found to provide synergistic benefit in lower facial rejuvenation.[18] In addition, data from the recently published prospective, multicenter, rater-blinded, 4-month HARMONY study indicate that a comprehensive, minimally invasive, multimodal aesthetic treatment approach combining onabotulinumtoxinA, hyaluronic acid fillers, and bimatoprost results in substantial improvements in patient satisfaction with their facial appearance and the perception of a younger facial appearance.[8,12,19]

The current study was designed to assess physician- and objective-observer-rated efficacy and subject-rated perceptions of outcomes following facial treatments with onabotulinumtoxinA in combination with hyaluronic acid, and to examine differences based upon the order of their administration sequence

METHODS

Study Design

This was a prospective, multicenter (6 investigative sites in US and Canada), randomized, open-label, unblinded, crossover study (https://clinicaltrials.gov/ct2/show/NCT01269801). Eligible subjects were randomized (1:1) to 1 of 2 groups: group 1: onabotulinumtoxinA (day 1), then hyaluronic acid (week 4) or group 2: hyaluronic acid (day 1), then onabotulinumtoxinA (week 4). The initial assigned treatment was administered at day 1 (screening/randomization/initial treatment), followed by 6 additional study visits: weeks 2 (potential touch-up visit), 4 (crossover visit), 6 (potential touch-up visit), 8, 12, and 24. Week 4 was selected as the evaluation/crossover visit, as in our experience this amount of time allows the response to treatment to develop, any swelling to subside, and for patients to appreciate the impact of the treatment. Patients and investigators completed several assessment parameters at this treatment crossover visit, as well as at subsequent timepoints. The study was conducted between February 2011 and January 2012.

The randomization schedule was generated using SAS (SAS Institute, Cary, NC) prior to study initiation. The study was conducted in compliance with Good Clinical Practice and the ethical guidelines outlined in the Declaration of Helsinki. Before subject enrollment, a centralized institutional review board reviewed and approved the study protocol (Institutional Review Board Services, Aurora, ON). Written informed consent was obtained from all participants before study-related activities.

Subjects

Adult females and males between 25 and 65 years of age seeking treatment with onabotulinumtoxinA and hyaluronic acid and having 1 or more moderate to severe hyperfunctional facial lines of the upper face (ie, GFLs, crow's feet, or horizontal forehead lines) and/or moderate to severe nasolabial folds at rest or maximal facial contraction (with these defined as a physician observer rating scale score of at least 2 at rest or maximal facial contraction) and who met all other inclusion criteria and none of the exclusion criteria were eligible for study participation. Females were to be of non-childbearing potential (ie, surgically sterilized or post-menopausal) with those of childbearing potential having a negative urine pregnancy test at the day 1 (screening/randomization/treatment) visit.

The main exclusion criteria were prior cosmetic procedures or visible scars that may affect evaluation of a response and/or quality of photography and previous botulinum toxin treatment within the prior 12 months. Subjects who had received dermal filler treatments within defined periods as follows were also excluded: bovine collagen, 6 months; porcine or human collagen, or hydroxylapatite, 12 months; and hyaluronic acid, 18 months. Subjects who had ever received treatment with autologous fat, polymethylmethacrylate or other acrylates, polyacrylamide, polyethylene oxide, polylactic acid, liquid silicone, or other permanent implant material were also excluded. Other exclusion criteria included a history of

facial nerve palsy; any severe or uncontrolled systemic disease or medical condition; any disease or use of agents that may interfere with neuromuscular function; and/or known hypersensitivity to any of the investigational products or their components.

Treatments

OnabotulinumtoxinA (BOTOX® Cosmetic for injection, Allergan, Inc.), and the hyaluronic acid formulations of JUVÉDERM® Ultra XC and JUVÉDERM® Ultra Plus XC Injectable Gel (Allergan, Inc.) were used in the study. Doses for injection were prepared in an unblinded, open-label manner (by a pharmacist, research nurse/assistant, or physician) according to the randomization schedule. Treatment was consistent with the current North American and EU labeling of onabotulinumtoxinA and hyaluronic acid. Dosing of each product was based on the ranges of the Facial Aesthetics Consensus Group-Facial Rejuvenation 2008 Consensus Recommendations[5] and at the discretion of the investigator. Investigators were allowed to administer a maximum onabotulinumtoxinA dosage of 200 U, and a maximum of 6.4 mL (ie, up to 8 syringes that individually contained 0.8 mL each) of hyaluronic acid per subject. As per the protocol (NCT01269801), participants who were treated received injections into affected facial regions.

Pre-injection application of a topical anesthetic cream or other appropriate anesthetic procedures was allowed at the investigator's discretion, with use of these to be documented in the case report form. Injections of onabotulinumtoxinA and hyaluronic acid were performed by the investigative physician (eg, a dermatologist, cosmetic physician) with the subject being in an upright position. Injection into the vermillion border or other perioral tissues for the purpose of correcting changes associated with aging were allowed, but direct lip augmentation (mucosal injection) was to be avoided.

Study Procedures

At visit 1, day 1 [baseline]), subjects underwent a complete medical history, medication history, and an abbreviated physician examination, including a complete baseline evaluation of all facial wrinkles or folds. Eligible subjects were randomized to group 1 or group 2, had baseline standardized photographs of their affected facial regions, and received their initial treatment. Subjects randomized to group 1 received onabotulinumtoxinA injections to the affected facial regions. Subjects randomized to group 2 received injections with hyaluronic acid formulations based upon the severity of their facial lines or folds. The hyaluronic acid formulation of JUVÉDERM® Ultra XC was used for moderate facial lines or folds and that of JUVÉDERM® Ultra Plus XC was used for severe facial lines or folds. In both groups, post-injection standardized photographs were taken of the affected facial regions at visit 1.

Subjects attended 6 additional study visits consisting of an abbreviated follow-up evaluation and potential touch-up treatment (determined by the investigator) at visit 2 (week 2); a combined evaluation and crossover treatment administration visit at visit 3 (week 4); an abbreviated follow-up evaluation and potential touch-up treatment (determined by the investigator) at visit 3 (week 6); and follow-up evaluation visits at weeks 8 (visit 4), 12 (visit 5), and 24 (visit 6).

At visits involving treatment injections, subjects remained at the investigative site for at least 30 minutes post-treatment for monitoring of any treatment-emergent adverse events. Following treatment injections, subjects were instructed to avoid heavy exercise, lifting, bending, or lying flat on their back for a minimum of 4 hours; not to massage or rub the injection site for 24 hours; not to apply make-up for 4 hours; and to expect minimal redness, swelling, or bruising at the injection site. Subjects were instructed to report moderate to severe swelling or difficulty swallowing immediately.

Assessments and Outcomes

Photography

Pre-injection (before use of any anesthetic, if applicable) and post-injection standardized photographs were obtained at week 1 (initial treatment visit) and week 4 (crossover treatment visit), and at weeks 2 and 6 in those who received touch-up treatments. Standardized photographs were also obtained at weeks 8, 12, and 24 follow-up visits in all subjects. Photographs were taken by the same photographer when possible (or a limited number of trained photographers), using the same resolution settings, general lighting, and extent of subject grimacing to maximize the consistency of the photographs. Subjects were photographed consistently in the rest and/or maximal contraction state at all visits using a digital camera (eg, Canfield Scientific Inc. equipment) in a well-lighted area of the research facility having a solid, light-colored background (eg, a wall or a cubicle panel). Photographs of the affected areas of the face were taken prior to and immediately after the injections at a distance of approximate 4 feet and in 3 positions—directly in front of the subject's face and at approximately 30° degree angles to the left and to the right of the subject.

Primary Efficacy Assessments

At the week 4, 8, 12, and 24 visits, investigators (eg, dermatologist, cosmetic physician) completed 2 primary efficacy assessment measures: the Investigator-rated Physician Global Aesthetic Improvement Scale (GAIS) and the Objective Observer Global Assessment of Improvement. The

GAIS was used to assess the subject's condition relative to day 1 with ratings of: very much improved, much improved, improved, no change, or worse. The Objective Observer Global Assessment of Improvement also assessed the subject's condition relative to day 1 with a 9-point scale on which +4 = complete improvement, 0 = no change, and -4 = very marked worsening. Both assessments were performed using the subject's day 1 photograph for reference.

Secondary Efficacy Assessments

At the week 4, 8, 12, and 24 visits, patients and the investigators completed secondary efficacy assessment measures.

Patient-rated efficacy assessments were the Patient Global Assessment of Improvement (PGAI), the Self-Perception of Age (SPA) measurement, and the Facial Line Outcomes (FLO-11) questionnaire. The PGAI assessed subject satisfaction relative to the day 1 visit (using the subject's day 1 photograph for reference) and rated on a 9-point scale on which +4 = complete improvement, 0 = no change, and -4 = very marked worsening. The SPA (©Allergan Inc., 2009) is a single-item scale on which the subject assesses how old he/she looks on the day of the measurement (ie, my current age, __ years younger, or __ years older). The FLO-11 (©Allergan Inc., 2009) comprises an 11-item validated instrument that assesses subjects' perceptions about specific aspects of their facial lines for the previous 7 days and has been used in other trials.[4,20,21] These items include how good they feel about their facial appearance, and the extent to which they consider their facial lines to bother them, make them look older than they would like to look, detract from their facial appearance, keep them from looking younger, attractive, or rested, prevent them from having a smooth facial appearance, and make them look tired, stressed, or angry when this is not how they feel. Each item is rated on a 10-point Likert-type scale (from "not at all" to "very much"), and a total mean FLO score representing improvement can be calculated as "total mean reversed FLO score = 10 x [100 – (sum of questions 1 – 10) + question 11] + 11.

Physician-rated secondary efficacy assessments were the Glabellar, the Lateral Canthal, and Horizontal Forehead Line Scales. Each was measured on a 0 to 3 scale (0 = non-detectable [none], 1 = minimal [mild], 2 = moderate, and 3 = severe). Physicians assessed wrinkles with the 0 to 4 Nasolabial Fold Scale (wrinkle assessment scale) on which 0 = none (no wrinkle), 1 = mild (shallow, just perceptible wrinkle), 2 = moderate (moderately deep wrinkle), 3 = severe (deep wrinkle, well-defined edges but not overlapping), and 4 = extreme (very deep wrinkle, redundant fold with overlapping skin). At the investigator's discretion, marionette lines could be injected, with assessments made using the 0 to 4 Marionette Lines Grading Scale on which 0 = no wrinkles, 1 = wrinkles present at rest but fine lines with facial expression, 2 = fine lines at rest and deep lines with facial expression, 3 = fine lines present at rest and deep lines with facial expression, and 4 = deeper wrinkles at rest and deeper furrows with facial expression.

Safety

Safety assessments including treatment-emergent adverse events (TEAEs) were determined at each study visit, rated as to their severity (mild, moderate, or severe) and causality, and documented in the case report form by the investigative site personnel. Any event considered serious or unexpected was reported to the sponsor within 24 hours.

Statistical Analysis

Demographic data (ie, age, gender, Fitzpatrick skin type, baseline facial wrinkle and fold severity) and treatment injection dosages were summarized using descriptive statistics. Baseline demographic and facial line characteristics were assessed for between-treatment sequence similarities with a t-test for continuous variables and Fisher's Exact test or Chi-square test for categorical variables.

Efficacy analyses were performed on an efficacy evaluable basis for all subjects who received open-label treatments and completed the day 1 and at least the week 4 visit. Global improvement measures completed by the physician (GAIS, Objective Observer Global Assessment of Improvement) and the subject (PGAI) were summarized descriptively by category for each visit with *P*-value comparisons at each visit made using Fisher's Exact test. Other efficacy assessments (ie, FLO-11, SPA, line and fold scale measures, and Marionette Lines Grading Scale) were summarized descriptively for each visit and for change from baseline at each visit, with between-group *P*-value comparisons made using the Mann-Whitney test. Within group *P*-value comparisons of pre- and post-treatment scores were made using the Wilcoxon signed-rank test; between-group *P*-value comparisons for change from baseline were made using the Kruskal-Wallis test.

Demographic and efficacy analyses tests were 2-tailed with significance at $\alpha = 0.05$. Parametric statistical tests were used for normally distributed data, and nonparametric tests were used for data that were not normally distributed.

Safety analyses were performed on an intent-to-treat (ITT) basis for all subjects who received treatment. TEAEs were summarized descriptively.

All analyses were performed using SAS version 9.3 (Cary, NC).

Sample Size

Assuming a 20% dropout rate, 150 subjects were to be enrolled, such that approximately 120 subjects would be randomized and complete the study. This sample size is expected to allow for the evaluation of the incremental treatment effects of onabotulinumtoxinA and hyaluronic acid treatment

Table 1. Baseline Demographics and Characteristics of Enrolled and Evaluable Subjects

Parameter	Enrolled (N = 156)	Received treatment (N =154)	Treatment sequence	
			Ona/HA (n = 80)	HA/Ona (n = 74)
Gender, n (%)				
Female	143 (91.7%)	142 (92.2%)	76 (95.0%)	66 (89.2%)
Male	13 (8.3%)	12 (7.8%)	4 (5.0%)	8 (10.8%)
			$P = 0.233$[a]	
Age, years				
Mean (SD)	50.54 (7.61)	50.54 (7.60)	49.83 (7.42)	51.12 (7.78)
Median (range)	51 (33-64)	50.5 (33-64)	50.0 (33-64)	51.0 (35-64)
			$P = 0.292$[b]	
Race, n (%)				
White	155 (99.4%)	153 (99.4%)	80 (100.0%)	73 (98.6%)
Asian	1 (0.6%)	1 (0.6%)	0 (0.0%)	1 (1.4%)
			$P = 0.481$[a]	
Fitzpatrick skin type, n (%)				
Type 1		3 (1.9%)	1 (1.3%)	2 (2.7%)
Type 2		49 (31.8%)	20 (25.0%)	29 (39.2%)
Type 3		71 (46.1%)	44 (55.0%)	27 (36.5%)
Type 4		28 (18.2%)	13 (16.3%)	15 (20.3%)
Type 5		3 (1.9%)	2 (2.5%)	1 (1.4%)
			$P = 0.138$[a]	
SPA, mean (SD) [range]			0.13 (4.09) [-8, 10]	0.62 (4.15) [-8, 10]
FLO-11, mean (SD) [range]			30.29 (17.45) [0.0, 84.55]	27.00 (16.28) [0.0, 70.91]

SD, standard deviation; SPA, patient self-perception of age; [a]Fisher's Exact test; [b]Two-tailed t-test.

injections with a reasonable precision of at least 80% power at the significance level of $\alpha = 0.05$, rather than demonstrate superiority with respect to the primary endpoint.

RESULTS

Participants

A total of 156 subjects were enrolled; 154 received at least 1 treatment dose (safety population), and 2 were screen failures and were not dosed. The efficacy evaluable population consisted of 152 subjects (80 enrolled in group 1 [onabotulinumtoxinA (Ona) followed by hyaluronic acid (HA)] and 74 enrolled in group 2 [HA then Ona]) who were treated and completed at least the week 4 visit (efficacy analysis population). Overall, 151 subjects completed the study and attended the week 24 (±7 day) visit with 3 subjects discontinuing due to an adverse event.

Baseline demographic and subject characteristics were similar in the treatment-sequence groups and were reflective of the patient population commonly seeking treatment for facial wrinkles and folds in clinical practice (Table 1).

The doses of Ona and HA administered over the 4 study visits and the percentages of subjects who received touch-up treatments at visit 2 and 6 are summarized in Table 2.

Primary Efficacy Measures

Physician-Rated GAIS

At all post-injection treatment visits, 100% of subjects in each sequence-treatment group were rated as being very much improved, much improved, or improved with the exception of week 4 in the Ona/HA sequence group (96% were rated as very much, much, or improved; Figure 1). There were no significant differences based upon treatment sequence ($P \geq 0.265$).

Table 2. Summary on Ona and HA Dosing

Visit	Parameter	Ona dose, in units	HA dose, in mL
Visit 1, day 1	N	80	72
	Mean (SD)	77.46 (26.20)	2.55 (1.24)
	Median	70	2.4
	Range	42-200	0.4-5.6
Visit 2, week 2 touch-up visit	Touch-up injection?		
	Yes	43 (53.8%)	30 (40.5%)
	No	37 (46.2%)	44 (59.5%)
	N	43	24
	Mean (SD)	13.36 (13.48)	1.20 (0.62)
	Median	9	0.8
	Range	1-60	0.2-2.5
Visit 3, week 4	N	74	77
	Mean (SD)	81.30 (33.30)	2.23 (1.28)
	Median	71	2.1
	Range	44-206	0.6-5.1
Visit 4, week 6 touch-up visit	Touch-up injection?		
	Yes	26 (35.1%)	29 (37.2%)
	No	48 (64.9%)	49 (62.8%)
	N	26	6
	Mean (SD)	16.90 (16.41)	0.97 (0.55)
	Median	10.50	0.9
	Range	1-70	0.3-1.6
Visit 1 and 3 combined	N	154	149
	Mean (SD)	79.31 (29.78)	2.38 (1.27)
	Median	70	2.3
	Range	42-206	0.4-5.6
Visit 2 and 4 combined	Touch-up injection?		
	Yes	69 (44.8%)	59 (38.8%)
	No	85 (55.2%)	93 (61.1%)
	N	69	49
	Mean (SD)	14.70 (14.64)	1.06 (0.58)
	Median	10	0.8
	Range	1-70	0.2-2.5

Objective Observer Global Assessment of Improvement

At least 92% or more of subjects in each treatment-sequence group had Objective Observer Global Assessment of Improvement ratings of +1 or greater (ie, some, definite, substantial, or complete improvement), and at least 72% had ratings of +2 or greater (ie, definite,

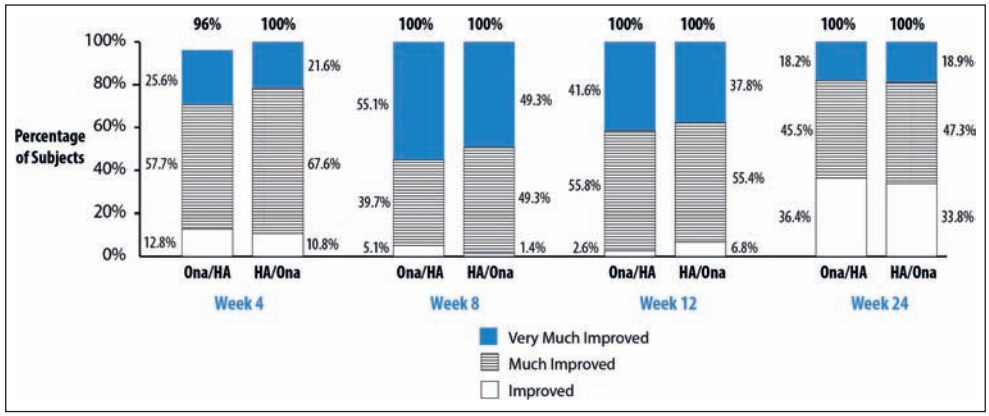

Figure 1. Percentages of subjects rated as being improved, much improved, and very much improved on the physician-rated GAIS, by Ona/HA and HA/Ona treatment-sequence group. Using the physician-rated Global Aesthetic Improvement Scale (GAIS) that utilizes terms of no change, improved, much improved, and very much improved, between 96% and 100% of all subjects were considered to be improved to very much improved, with no significant difference in outcome between the two Ona/HA or HA/Ona treatment-sequence groups ($P \geq 0.265$). HA, hyaluronic acid; Ona, onabotulinumtoxinA.

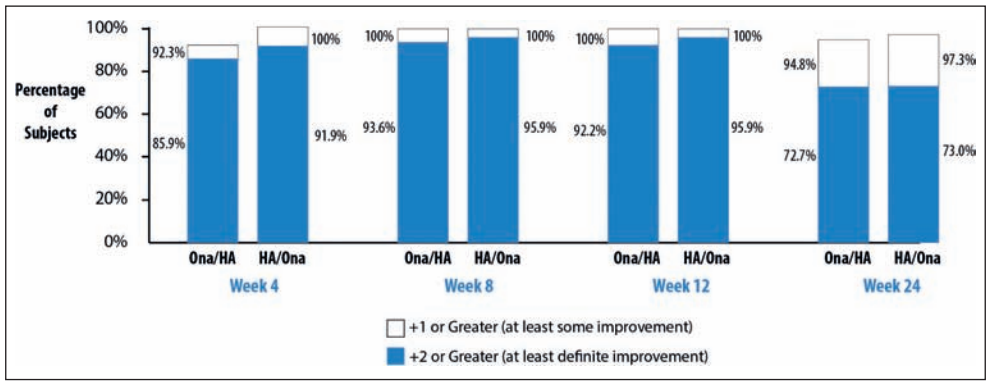

Figure 2. Percentages of subjects rated as having at least some and at least definite improvement on the Objective Observer Global Assessment of Improvement Scale, by Ona/HA and HA/Ona treatment-sequence group. Using the Objective Observer Global Assessment of Improvement instrument that is rated on a scale of 0 to +4 [0 = unchanged, +1 = some (25%) improvement, +2 = definite (50%) improvement, +3 = substantial (75%) improvement, and +4 = complete (100%) improvement], at least 92% of all subjects in each Ona/HA and HA/Ona treatment-sequence group had an improvement of at least +1 with at least 72% having an improvement of at least +2. There were no significant differences in scores between the sequence groups ($P \geq 0.098$). HA, hyaluronic acid; Ona, onabotulinumtoxinA.

substantial, or complete improvement) at each post-injection treatment visit (Figure 2). There were no significant differences based upon treatment sequence ($P \geq 0.098$).

Secondary Efficacy Measures

Patient Global Assessment of Improvement

At least 93% or more of subjects in each treatment-sequence group rated their PGAI outcome as +1 or greater (ie, some, definite, substantial, or complete improvement) at each visit (Figure 3) with at least 74% rating their outcome as +2 or greater (ie, definite, substantial, or complete improvement). There were no significant differences based upon treatment sequence ($P \geq 0.123$).

FLO-11 Questionnaire

Facial lines and their impact were rated similarly at baseline in the 2 treatment-sequence groups (mean scores of 30.29 and 27.00 in the Ona/HA and HA/Ona sequence groups, respectively; $P = 0.240$; Table 1). The total mean FLO score improved significantly ($P < 0.0001$) from baseline at all visits in both treatment groups (Figure 4), with no differences between the groups ($P > 0.148$ at each visit).

Patient Self-Perception of Age

At the visit 1 (day 1, baseline visit prior to any treatments), subjects in both treatment-sequence groups rated themselves as being older than their current age (ie, 0.13 years older in the Ona/HA group and 0.62 years older in the HA/Ona group; $P = 0.353$; Table 1). At the week 4, 8, 12, and

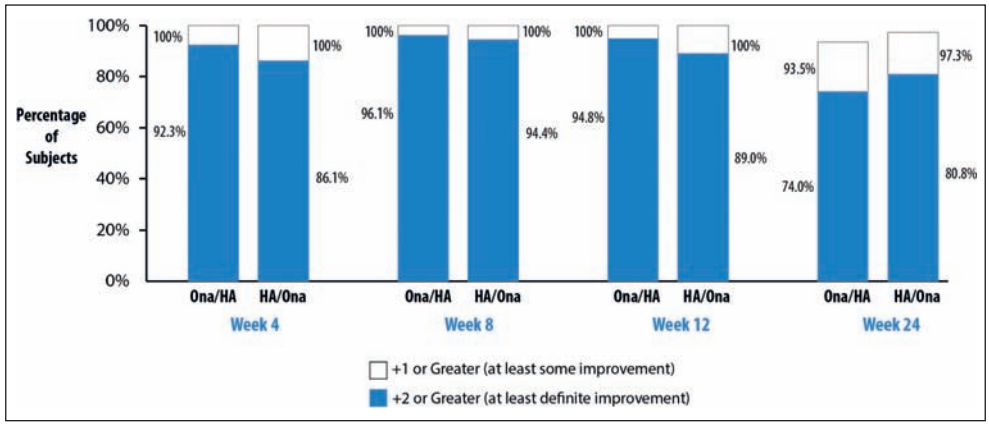

Figure 3. Percentages of subjects rating their treatment outcome as at least some improvement and at least definite improvement on the Patient Global Assessment of Improvement, by Ona/HA and HA/Ona treatment-sequence group. Using the Patient Global Assessment of Improvement instrument that is rated on a 0 to +4 scale (0 = unchanged, +1 = some improvement, +2 = definite improvement, +3 = substantial improvement, +4 = complete improvement), at least 93% of subjects rated their outcome as +1 or better, and at least 74% rated their outcome as +2 or better. There were no significant differences in scores between the sequence groups ($P \geq 0.123$). HA, hyaluronic acid; Ona, onabotulinumtoxinA.

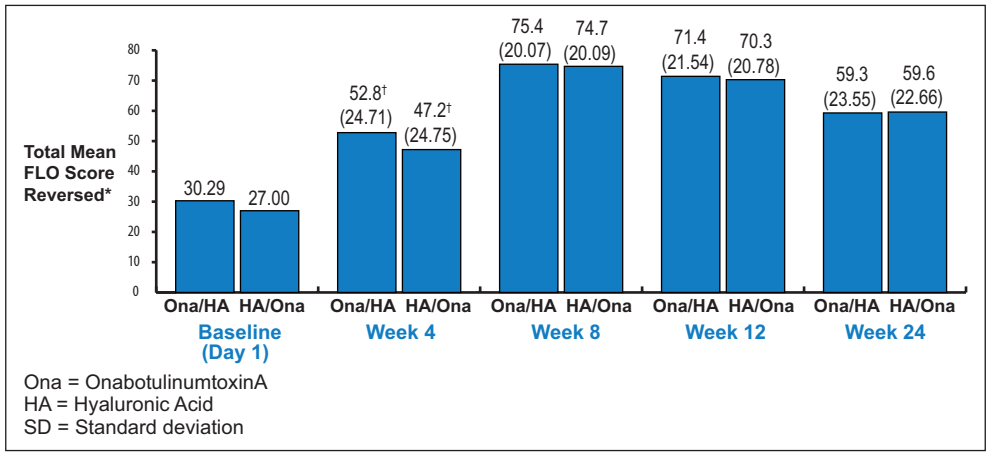

Figure 4. Subject's facial line outcomes (FLO-11) total score mean (SD) changes from baseline at each visit, by Ona/HA and HA/Ona treatment-sequence group. The total mean FLO score improved significantly ($P < 0.0001$) from baseline at all visits in both treatment-sequence groups, with no differences between the groups at any visit ($P > 0.148$). HA, hyaluronic acid; Ona, onabotulinumtoxinA; SD, standard deviation. * Higher mean total FLO scores indicate improvement. The score is calculated as the total mean reversed FLO score = 10 x [100 – (sum of questions 1 – 10) + question 11] + 11. FLO = Facial Line Outcomes. †At week 4 there was a significant ($P < 0.0001$) within-group comparison of change from baseline determined by Kruskal-Wallis test.

24 visits, there were statistically significant ($P < 0.001$) improvements in patients' SPA from baseline in both treatment groups (ie, looking 3 to 6 years younger on average; Figure 5). There were no significant differences based on treatment sequence ($P \geq 0.257$).

Glabellar, Lateral Canthal, and Horizontal Forehead Lines; Nasolabial Folds; and Marionette Line Ratings

Mean scores were determined for the overall study population at rest and at maximal contraction at baseline and at each treatment visit. At each visit, there were statistically significant ($P < 0.0001$) improvements (score reductions) in the study population when assessed at rest as well as at maximum contraction. Table 3 depicts the glabellar, lateral canthal, and horizontal forehead lines, and nasolabial folds scores at baseline (day 1) and at each visit, at rest and at maximum contraction.

Marionette lines also improved significantly at each post-injection visit with mean (SD) values decreasing from 2.43 (1.30) at day 1 to 1.59 (1.26) at week 4, 0.75 (0.79) at week 8, 0.84 (0.81) at week 12, and 1.15 (0.82) at week 24.

Figures 6A and 7A depict 2 participants at rest prior to their treatments, with Figures 6B and 7B depicting facial areas following treatment. The participant in Figure 6 received HA

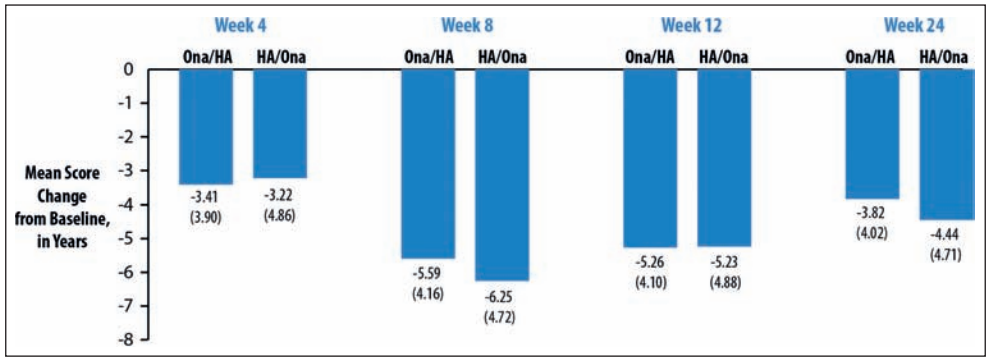

Figure 5. Subject's self-perception of mean (SD) age changes from baseline at each visit, by Ona/HA and HA/Ona treatment-sequence group. Subjects reported statistically significant improvements in their self-perception of age in each treatment-sequence group with no significant differences between the Ona/HA and HA/Ona groups ($P \geq 0.257$). Negative scores indicate the number of year(s) younger than current age. HA, hyaluronic acid; Ona, onabotulinumtoxinA; SD, standard deviation.

Table 3. Mean (SD) Facial Lines Rating Scores for All Dosed Subjects

At rest	Study visit				
	Visit 1, day 1 (baseline)	Week 4	Week 8	Week 12	Week 24
Glabellar lines, mean (SD)	1.48 (0.79)	1.01 (0.79)	0.49 (0.58)	0.54 (0.56)	0.89 (0.72)
Lateral canthal lines, mean (SD)	1.56 (0.76)	1.12 (0.91)	0.58 (0.59)	0.70 (0.59)	1.21 (0.70)
Horizontal forehead lines	1.56 (0.77)	0.97 (0.80)	0.48 (0.55)	0.69 (0.6)	1.03 (0.67)
Nasolabial folds	2.31 (0.67)	1.68 (0.92)	1.01 (0.63)	1.00 (0.65)	1.44 (0.68)
At maximum contraction	**Visit 1, day 1 (baseline)**	**Week 4**	**Week 8**	**Week 12**	**Week 24**
Glabellar lines, mean (SD)	2.47 (0.61)	1.59 (1.10)	0.50 (0.59)	0.86 (0.60)	1.80 (0.71)
Lateral canthal lines, mean (SD)	2.42 (0.62)	1.73 (1.00)	0.93 (0.58)	1.15 (0.69)	2.03. (0.73)
Horizontal forehead lines	2.44 (0.57)	1.73 (0.95)	0.95 (0.56)	1.27 (0.63)	1.94 (0.68)
Nasolabial folds	2.77 (0.69)	2.07 (0.97)	1.48 (0.82)	1.34 (0.70)	1.79 (0.74)

initially with 0.5 mL in each cheek, 0.8 mL in each nasolabial fold, 0.3 mL in the upper lip, 0.8 mL in the oral commissure, and 0.4 mL in each marionette line. At week 4, she received Ona at a dose of 31 U in each glabellar fold, 12 U in each side for crow's feet, 8 U in each side of "bunny" nasalia, 2 U in each levator labii superioris alaque nasi, 6 U in each depressor angularis oris, and 5 U in each mentalis. The participant in Figure 7 received HA initially with 0.2 mL in each infraorbital, 2 mL in each oral commissure, 0.6 mL in the right nasolabial fold, 0.8 mL in the left nasolabial fold, 0.2 mL in each vermillion (lips), 0.4 mL in the lips (mucosa upper), 0.75 mL in the right marionette line, and 0.7 mL in the left marionette line. At week 4, this participant received Ona at a dose of 31 U in each glabellar fold, 14 U in each side for crow's feet, 3 U in each side of "bunny" nasalia, 4 U in each depressor angularis oris, and 5 U in each mentalis. This participant received a touch-up at week 6 with Ona at a dose of 4 U on each side for lateral canthal lines/crow's feet.

Safety

The adverse events that led to study discontinuation in 3 subjects were considered unrelated to study treatment. One subject experienced severe endometriosis, one had severe sinusitis, and one died suddenly, with hypertensive cardiovascular disease listed as the contributory factor.

Treatment-emergent adverse events regardless of relationship and those considered treatment-related are listed in Table 4 by the last treatment received by the subject before the event onset. The percentage of subjects reporting a TEAE regardless of relationship was 27.6% in those who last received HA and 20.1% in those who last received Ona. Reported treatment-related adverse events were consistent with the product labeling, were mild in intensity, and also resolved during the study. The percentage of subjects reporting a TEAE that was considered by the physician to be treatment related was 15.1% in those who last received HA and 7.8% in those who last received Ona. The most

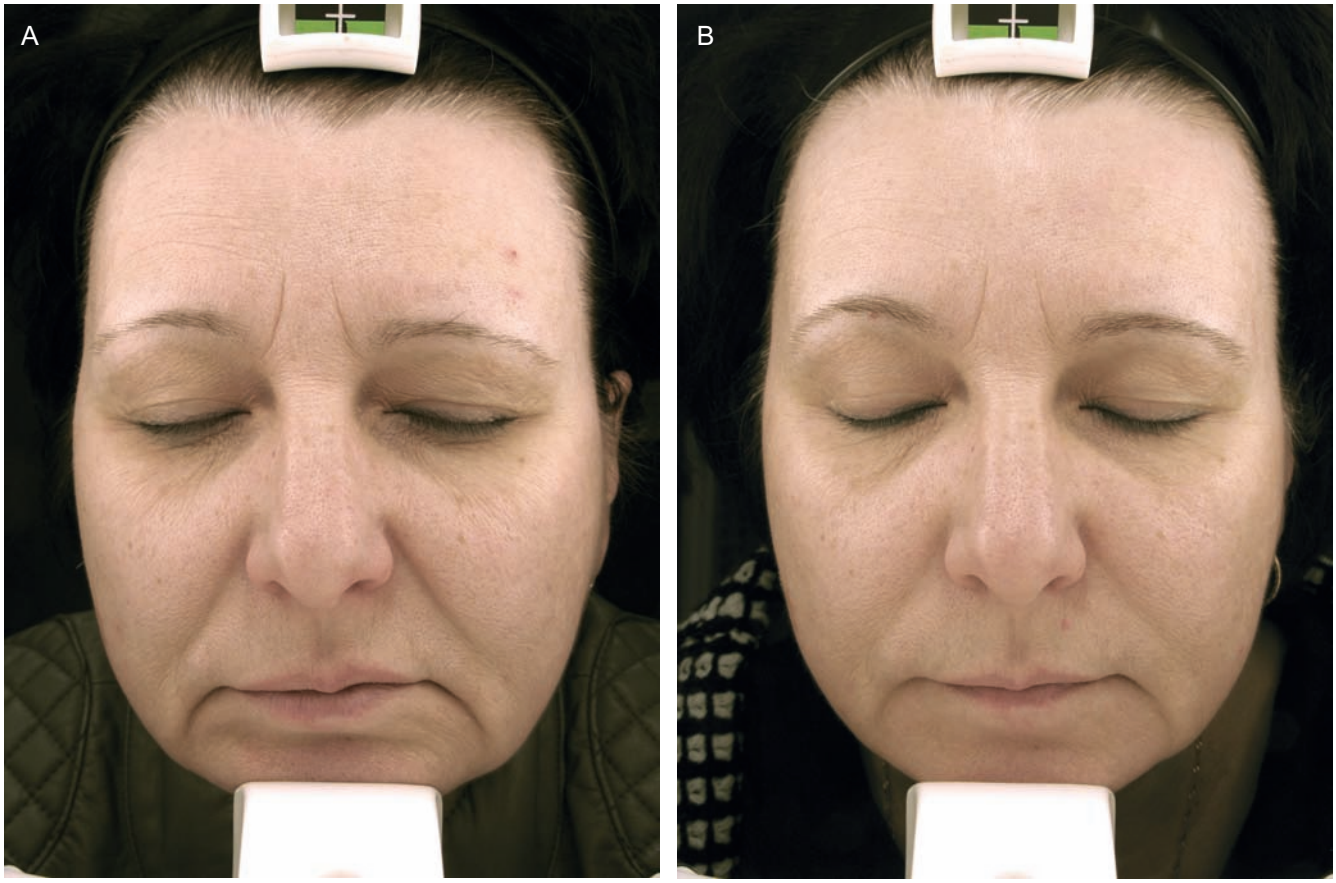

Figure 6. (A) Photographs before treatment and (B) at study completion of a 48-year-old woman who received initial treatment with HA 0.5 mL in each cheek, 0.8 mL in each nasolabial fold, 0.3 mL in the upper lip, 0.8 mL in the oral commissure, and 0.4 mL in each marionette line. At week 4, she received Ona at a dose of 31 U in each glabellar fold, 12 U in each side for crow's feet, 8 U in each side of "bunny" nasalia, 2 U in each levator labii superioris alaque nasi, 6 U in each depressor angularis oris, and 5 U in each mentalis.

commonly reported treatment-related TEAEs were facial paresis and eyelid ptosis following Ona treatment, and contusion (bruising) and tenderness at the site following HA injection.

DISCUSSION

The treatment of facial wrinkles and folds with botulinum toxin products and dermal fillers is characterized as a favorable benefit-risk profile that is supported by numerous studies of their duration of effect, high levels of patient satisfaction, long-term safety, and undiminished efficacy with repeated treatments. The overall goal of using botulinum toxin products and dermal fillers is to provide patients with a refreshed, best version of their face. Consensus guidelines indicate that there is "an evolving paradigm" in facial rejuvenation, in which a 3-dimensional approach to volume replacement that provides optimal clinical outcomes and a higher level of patient satisfaction has supplanted the traditional 2-dimensional approach.[5] This newer paradigm requires considerable knowledge and skill on the part of the physician. In general, we have divided the face into thirds and utilized different agents, doses, and techniques based on the location to be treated and through our understanding of the anatomy and physiology of the aging face, and the particular patient's characteristics.[22] The use of fillers in combination with botulinum toxin type A allows clinicians to address facial rejuvenation from this 3-dimensional approach and may provide a more pleasing, longer-lasting aesthetic outcome.[11] Our study examined the full facial rejuvenation potential with onabotulinumtoxinA in combination with hyaluronic acid (primary injections separated by 4 weeks with optional touch-up injections 2 weeks after initial treatments) and to characterize the effects, rated by physicians and subjects, by treatment administration order (ie, Ona/HA or HA/Ona).

As measured by a variety of physician and subject-rated outcome measures, onabotulinumtoxinA and hyaluronic acid treatments, regardless of the order administered, provided clinically meaningful and consistent improvements in measures of GAIS, Objective Observer Global Assessment of Improvement, PGAI, SPA, and the FLO-11

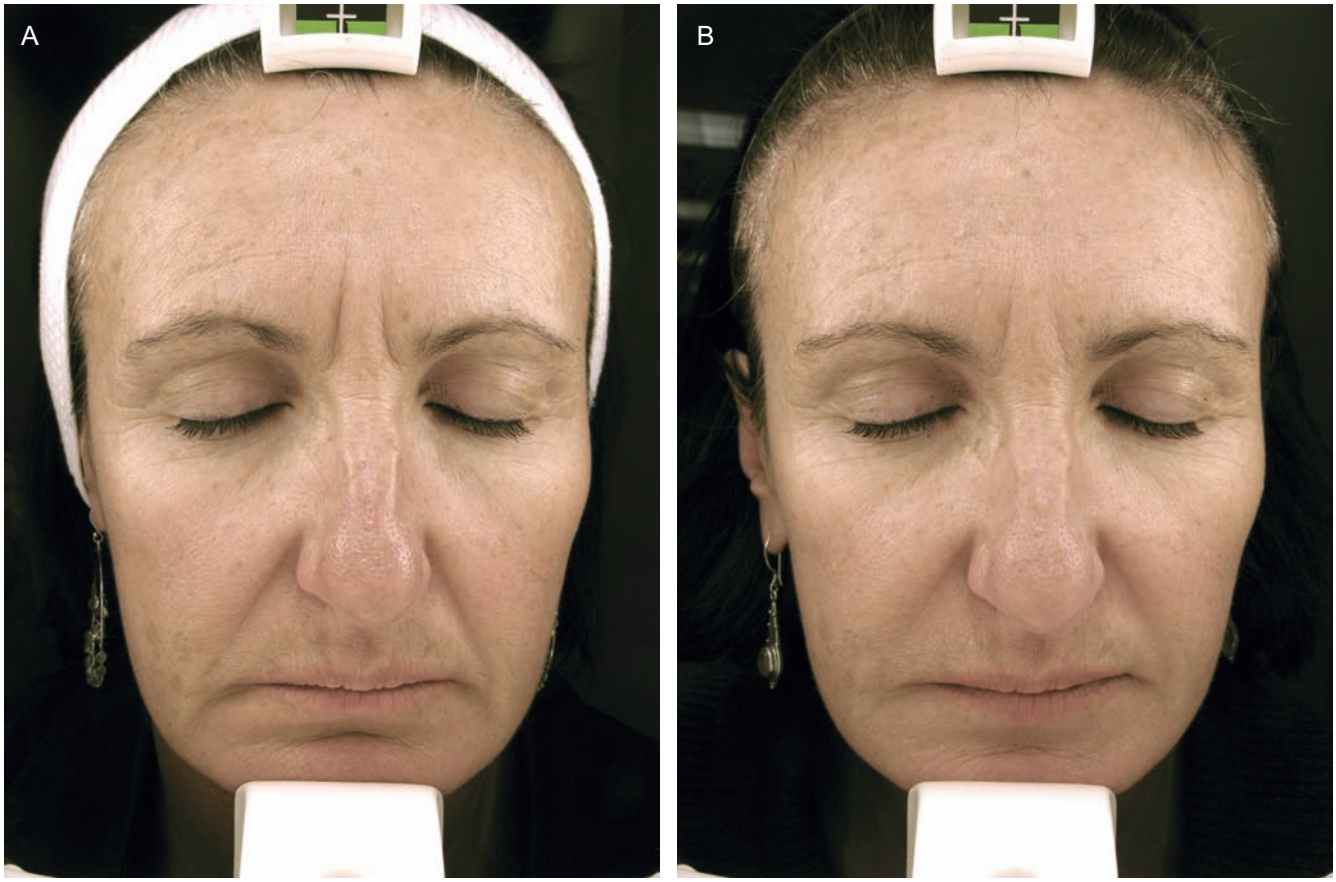

Figure 7. (A) Photographs before treatment and (B) at study completion of a 50-year-old woman who received initial treatment with HA at a dose of 0.2 mL in each infraorbital, 2 mL in each oral commissure, 0.6 mL in the right nasolabial fold, 0.8 mL in the left nasolabial fold, 0.2 mL in each vermillion (lips), 0.4 mL in the lips (mucosa upper), 0.75 mL in the right marionette line, and 0.7 mL in the left marionette line. At week 4, this participant received Ona at a dose of 31 U in each glabellar fold, 14 U in each side for crow's feet, 3 U in each side of "bunny" nasalia, 4 U in each depressor angularis oris, and 5 U in each mentalis. This participant received a touch-up at week 6 with Ona at a dose of 4 U on each side for lateral canthal lines/crow's feet.

questionnaire, as well as in individual facial line rating scales. In addition to these favorable outcomes being similar regardless of the onabotulinumtoxinA and hyaluronic acid treatment sequence, they also persisted for at least 6 months (24 weeks) following the initial injection in both groups.

Clinically meaningful findings included, across all of the study visits, high proportions of subjects (≥96%) were given ratings of improved or higher (ie, much improved or very much improved) when assessed using the physician-rated GAIS. Further, 93% or more of subjects were given GAIS ratings of much improved or very much improved at weeks 8 and 12. When assessed using the Objective Observer Global Assessment of Improvement scale, 86% or more of subjects were given ratings of at least definite improvement at the week 4, 8, 12, and 24 visits. In the patient-rated Global Assessment of Improvement, 93.5% to 100% reported at least some improvement at all study visits. Facial lines, nasolabial folds, and marionette lines improved significantly both at rest as well as at maximum contraction. Across the outcomes measured, the improvements persisted for at least 4 weeks following the initial treatment (subjects received crossover treatment at week 4) and for up to 20 weeks following the second treatment (last study visit was at week 24). Both treatments were well tolerated.

In the analysis of mean total FLO scores, which provide a barometer of subjects' perceptions about specific aspects of their facial lines, there was no difference in the amount of improvement between the groups, with significant improvements from baseline observed at each of the study assessment visits. By week 8, subjects reported at least a 2-fold increase in their FLO scores, which persisted at week 12. At week 4, subjects were rating themselves as appearing more than 3 years younger, with this further improving to an appearance of at least 5.5 years younger at week 8, with either treatment sequence. In the current study, the physician-rated outcome findings observed with

Table 4. Treatment-Emergent Adverse Events (TEAE) Occurring in ≥1% of Subjects or Considered Treatment Related, by Last Treatment before Adverse Event

	All dosed subjects	Last treatment before adverse event onset	
		Onabotulinumtoxin (N = 154)	Hyaluronic acid (N = 152)
Number (%) of subjects with any TEAE	57 (37.0%)	31 (20.1%)	42 (27.6%)
Number (%) of subjects with any treatment-related TEAE	26 (16.9%)	12 (7.8%)	23 (15.1%)
TEAEs (MedDRA preferred term), in alphabetical order[a]			
Acne	3 (1.9%)	0 (0%)	3 (2.0%)
Back pain	3 (1.9%)	2 (1.3%)	1 (0.7%)
Bronchitis	2 (1.3%)	1 (0.6%)	1 (0.7%)
Contusion (bruising)[a]	20 (13.0%)	1 (0.6%)	19 (12.5%)
Extraocular muscle paresis[a]	1 (0.6%)	1 (0.6%)	0 (0%)
Eyelid ptosis[a]	2 (1.3%)	2 (1.3%)	0 (0%)
Facial paresis[a]	8 (5.2%)	8 (5.2%)	0 (0%)
Headache[a]	4 (2.6%)	4 (2.6%)	0 (0%)
Injection site mass[a]	2 (1.3%)	0 (0%)	2 (1.3%)
Migraine	2 (1.3%)	2 (1.3%)	0 (0%)
Nasopharyngitis	3 (1.9%)	2 (1.3%)	1 (0.7%)
Oral herpes	5 (3.2%)	1 (0.6%)	4 (2.6%)
Sinusitis	3 (1.9%)	2 (1.3%)	1 (0.7%)
Swelling face[a]	2 (1.3%)	2 (1.3%)	0 (0%)
Tenderness[a]	7 (4.5%)	0 (0%)	7 (4.6%)
Urinary tract infection	2 (1.3%)	1 (0.6%)	1 (0.7%)

[a]Indicates those considered at least possibly related to treatment (treatment-related TEAE).

each treatment order are consistent with previous publications that have supported the labeling of each of these products as well as meta-analyses on the use of onabotulinumtoxinA[23] and a review of phase 2 and phase 3 trials of onabotulinumtoxinA in the treatment of moderate to severe crow's feet lines.[3] In the practice of aesthetics, many recognize that patient-rated outcomes, and especially that of how the wrinkles and folds make one feel and that of age appearance, are of major importance to patients. The significant improvements in the FLO questionnaire and patients' self-perception of age seen with the treatment order groups are similar to that reported in an earlier study of onabotulinumtoxinA,[4] with these investigators reporting that FLO-11 scores improved by nearly 40% by day 14 (total mean score improving from approximately 32 at baseline to 72 at day 14) and patients reported looking a mean of 3.1 years younger at day 14 as compared to baseline. In the HARMONY study, the combination of onabotulinumtoxinA, hyaluronic acid fillers, and bimatoprost injected into GFLs, crow's feet lines, or both was associated with a significant ($P < 0.0001$) increase in patients' satisfaction with a very large effect size (2.7) using the FACE-Q 10-item Satisfaction with Facial Appearance Overall Scale.[11] Self-perceived age decreased from 0.2 years older than actual age at baseline to 4.6 years younger at month 4, and nearly all patients (99%) rated themselves as improved or much improved on the Global Aesthetic Improvement Scale.[11]

The overall findings of this study support those of prior investigations of individual products as well as when onabotulinumtoxinA and hyaluronic acid are used in combination to treat the upper face.[5,7,24-27] In a prospective, randomized study of onabotulinumtoxinA with hyaluronic acid to treat the upper face, the investigators reported that the combination produced improved outcomes as compared to hyaluronic acid alone.[24] In this study, 19 females with deep resting glabellar rhytides were given hyaluronic acid alone or with onabotulinumtoxinA—with the order of 30 U onabotulinumtoxinA administered 1 week prior to hyaluronic acid treatment. The use of the onabotulinumtoxinA/hyaluronic acid combination nearly doubled the

median duration of response with hyaluronic acid alone, and a higher percentage of combination-treated subjects versus hyaluronic acid-alone subjects had aesthetic improvement at week 16 (95% vs 83%).

Based on these findings and those of others, including a facial aesthetics consensus group and findings from the HARMONY study,[5,8,11,18] there is clinical synergy with the use of onabotulinumtoxinA and hyaluronic acid fillers in combination. Specifically, in the consensus review document, the faculty agreed that, for the upper face, botulinumtoxinA remains the foundation of treatment but that hyaluronic acid augments the results in several ways, including the management of deep resting folds and lines that remain after botulinumtoxinA treatment. For the midface, hyaluronic acid serves a central role, with botulinumtoxinA serving as an important adjunct depending on the treatment plan. For the lower face, both botulinumtoxinA and hyaluronic acid are important because rejuvenation involves control of muscle movement as well as volume restoration.

Limitations to the interpretation of our current findings and the applicability of these to the general population include that our participants were primarily white females, which limits the generalizability of our findings to other racial groups and to males. In addition, investigators were allowed to administer onabotulinumtoxinA to a maximum dosage of 200 U, which is higher than typical dosages and may impart a higher financial burden on patients who are paying for treatment. It is also notable that the majority of participants had a Fitzpatrick skin type of 2, 3, or 4, with only a small percentage (< 2%) having type 1 or type 5, and no participants having a type 6.

CONCLUSION

The findings of our analysis support those of the consensus faculty in that the use of onabotulinumtoxinA and hyaluronic acid in facial rejuvenation provides clinical synergy in terms of aesthetic effects and duration of response. Further, this analysis provides clarity on the issue of which product should be administered first. The efficacy and safety of onabotulinumtoxinA and hyaluronic acid in these subjects with moderate to severe facial wrinkles and folds were similar, regardless of treatment administration order, with facial aesthetic improvements persisting for at least 24 weeks (6 months) following treatment.

Acknowledgements

The authors acknowledge the contributions of Susan Ruffalo, PharmD, in the editorial development and assistance with this manuscript.

Disclosures

Dr Cohen is a clinical investigator and consultant for Allergan. Dr Swift is a clinical investigator and consultant for Allergan, Croma, Galderma, and Merz. Dr Solish is a clinical investigator and consultant for Allergan, Galderma, Merz, and Revance; and is a speaker for Allergan and Galderma. Dr Fagien is a clinical investigator and consultant for Allergan, Galderma, Revance, and Alpheon. Dr Glaser is a clinical investigator and consultant for Allergan.

Funding

This study was sponsored by Allergan, Inc. All authors have been consultants and/or investigators for Allergan, Inc. Editorial assistance in the development of this manuscript was provided by Med Write, Inc. (Susan Ruffalo, PharmD), with funding support provided by the sponsor.

REFERENCES

1. Carruthers A, Carruthers J. Clinical indications and injection technique for the cosmetic use of botulinum A exotoxin. *Dermatol Surg.* 1998;24(11):1189-1194.
2. Carruthers A, Kiene K, Carruthers J. Botulinum A exotoxin use in clinical dermatology. *J Am Acad Dermatol.* 1996;34(5):788-797.
3. Carruthers A, Bruce S, Cox SE, et al. OnabotulinumtoxinA for treatment of moderate to severe crow's feet lines: a review. *Aesthet Surg J.* 2016;36(5):591-597.
4. Beer KR, Boyd C, Patel RK, et al. Rapid onset of response and patient-reported outcomes after onabotulinumtoxinA treatment of moderate-to-severe glabellar lines. *J Drugs Dermatol.* 2011;10(1):39-44.
5. Carruthers JD, Glogau RG, Blitzer A; Facial Aesthetics Consensus Group Faculty. Advances in facial rejuvenation: botulinum toxin type a, hyaluronic acid dermal fillers, and combination therapies–consensus recommendations. *Plast Reconstr Surg.* 2008;121(5):5S-30S; quiz 31S.
6. Carruthers A, Sadick N, Brandt F, et al. Evolution of facial aesthetic treatment over five or more years: a retrospective cross-sectional analysis of continuous onabotulinumtoxinA treatment. *Dermatol Surg.* 2015;41(6):693-701.
7. Coleman KR, Carruthers J. Combination therapy with BOTOX and fillers: the new rejuvnation paradigm. *Dermatol Ther.* 2006;19(3):177-188.
8. Narurkar VA, Cohen JL, Dayan S, et al. A comprehensive approach to multimodal facial aesthetic treatment: injection techniques and treatment characteristics from the HARMONY study. *Dermatol Surg.* 2016;42(Suppl 2):S177-S191.
9. Narurkar V, Shamban A, Sissins P, Stonehouse A, Gallagher C. Facial treatment preferences in aesthetically aware women. *Dermatol Surg.* 2015;41(Suppl 1):S153-S160.
10. 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.

11. Sadick NS, Manhas-Bhutani S, Krueger N. A novel approach to structural facial volume replacement. *Aesthetic Plast Surg.* 2013;37(2):266-276.
12. Weinkle SH, Werschler WP, Teller CF, et al. Impact of comprehensive, minimally invasive, multimodal aesthetic treatment on satisfaction with facial appearance: the HARMONY study. *Aesthet Surg J.* 2017. doi: 10.1093/asj/sjx179.
13. Baumann LS, Shamban AT, Lupo MP, et al.; JUVEDERM vs. ZYPLAST Nasolabial Fold Study Group. Comparison of smooth-gel hyaluronic acid dermal fillers with cross-linked bovine collagen: a multicenter, double-masked, randomized, within-subject study. *Dermatol Surg.* 2007;33(Suppl 2):S128-S135.
14. Carruthers A, Bruce S, de Coninck A, et al. Efficacy and safety of onabotulinumtoxinA for the treatment of crows feet lines: a multicenter, randomized, controlled trial. *Dermatol Surg.* 2014;40(11):1181-1190.
15. Brandt F, Bassichis B, Bassichis M, O'Connell C, Lin X. Safety and effectiveness of small and large gel-particle hyaluronic acid in the correction of perioral wrinkles. *J Drugs Dermatol.* 2011;10(9):982-987.
16. Jones D, Murphy DK. Volumizing hyaluronic acid filler for midface volume deficit: 2-year results from a pivotal single-blind randomized controlled study. *Dermatol Surg.* 2013;39(11):1602-1612.
17. Weinkle SH, Bank DE, Boyd CM, Gold MH, Thomas JA, Murphy DK. A multi-center, double-blind, randomized controlled study of the safety and effectiveness of Juvéderm injectable gel with and without lidocaine. *J Cosmet Dermatol.* 2009;8(3):205-210.
18. Carruthers A, Carruthers J, Monheit GD, Davis PG, Tardie G. Multicenter, randomized, parallel-group study of the safety and effectiveness of onabotulinumtoxinA and hyaluronic acid dermal fillers (24-mg/ml smooth, cohesive gel) alone and in combination for lower facial rejuvenation. *Dermatol Surg.* 2010;36(Suppl 4):2121-2134.
19. *Patient Satisfaction Study of Combined Facial Treatment With BOTOX® Cosmetic, JUVÉDERM® and LATISSE® (HARMONY Study)*. https://clinicaltrials.gov/ct2/show/NCT02176356?id=NCT02176356&rank=1. Accessed April 25, 2018.
20. Carruthers A, Carruthers J. A single-center dose-comparison study of botulinum neurotoxin type A in females with upper facial rhytids: assessing patients' perception of treatment outcomes. *J Drugs Dermatol.* 2009;8(10):924-929.
21. Shamban A. Customized approach to facial enhancement. *Facial Plast Surg Clin North Am.* 2015;23(4):471-477.
22. Guo Y, Lu Y, Liu T, et al. Efficacy and safety of botulinum toxin type A in the treatment of glabellar lines: a meta-analysis of randomized, placebo-controlled, double-blind trials. *Plast Reconstr Surg.* 2015;136(3):310e-318e.
23. Dayan S, Coleman WP 3rd, Dover JS, et al. Effects of onabotulinumtoxinA treatment for crow's feet lines on patient-reported outcomes. *Dermatol Surg.* 2015;41(Suppl 1):S67-S74.
24. Carruthers J, Carruthers A. A prospective, randomized, parallel group study analyzing the effect of BTX-A (Botox) and nonanimal sourced hyaluronic acid (NASHA, Restylane) in combination compared with NASHA (Restylane) alone in severe glabellar rhytides in adult female subjects: treatment of severe glabellar rhytides with a hyaluronic acid derivative compared with the derivative and BTX-A. *Dermatol Surg.* 2003;29(8):802-809.
25. Carruthers J, Carruthers A. Botulinum toxin type A treatment of multiple upper facial sites: patient-reported outcomes. *Dermatol Surg.* 2007;33(1):S10-S17.
26. de Maio M. The minimal approach: an innovation in facial cosmetic procedures. *Aesthetic Plast Surg.* 2004;28(5):295-300.
27. Maas CS. Botulinum neurotoxins and injectable fillers: minimally invasive management of the aging upper face. *Facial Plast Surg Clin North Am.* 2006;14(3):241-245.