

DaxibotulinumtoxinA for Injection for the Treatment of Glabellar Lines: Results from Each of Two Multicenter, Randomized, Double-Blind, Placebo-Controlled, Phase 3 Studies (SAKURA 1 and SAKURA 2)

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Background: DaxibotulinumtoxinA for Injection (DAXI) is a novel botulinum toxin type A formulation in clinical development. A phase 2 dose-ranging study identified an optimal dose and demonstrated efficacy with a median duration of 24 weeks.

Methods: In two phase 3, multicenter, randomized, double-blind, placebo-controlled studies (SAKURA 1 and SAKURA 2), subjects with moderate or severe glabellar lines at maximum frown were assigned randomly to receive placebo or 40 U of DAXI. Glabellar lines were evaluated at least every 4 weeks for at least 24 weeks until severity returned to baseline (≤ 36 weeks).

Results: Overall, 609 subjects were enrolled (DAXI, $n = 405$; placebo, $n = 204$). DAXI was significantly more effective than placebo in achieving the primary efficacy outcome (≥ 2 -point improvement in glabellar line severity at maximum frown at week 4 according to both investigator and subject ratings): 73.6 percent versus 0.0 percent (SAKURA 1), and 74.0 percent versus 1.0 percent (SAKURA 2) (both $p < 0.0001$). Composite investigator and subject ratings of maximum frown after DAXI treatment showed that glabellar line severity of none or mild was maintained for a median of 24.0 weeks (SAKURA 1) and 23.9 weeks (SAKURA 2), and glabellar line severity did not return to baseline levels for a median of 27.7 and 26.0 weeks, respectively. DAXI was generally well tolerated, with the most common adverse events related to DAXI treatment being headache (SAKURA 1, 7.0 percent; SAKURA 2, 5.9 percent) and injection-site pain (5.0 percent and 2.4 percent, respectively).

Conclusions: Results from both studies were highly consistent. DAXI may offer a prolonged duration of response (median, ≥ 24 weeks) and is generally well tolerated. (*Plast. Reconstr. Surg.* 145: 45, 2020.)

CLINICAL QUESTION/LEVEL OF EVIDENCE: Therapeutic, I.

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For many years, botulinum toxin type A has offered an effective and well-tolerated approach to treating glabellar lines. Botulinum toxin type A achieves its effects through the selective but temporary denervation of injected muscles, and repeated injections are required to maintain responses. Although the majority of patients are no longer responders within 3 to 4 months of treatment with currently available botulinum toxin type A products,¹⁻⁴ data indicate that in a real-world clinical setting, repeated treatments

are usually received approximately every 5 to 6 months,⁵ suggesting that patients spend a significant portion of the year without effective glabellar line effacement. A botulinum toxin type A with a longer duration of clinical benefit could provide a longer period of amelioration of glabellar lines while preserving the current average frequency of retreatment. This could also enhance patient satisfaction, a key measure of success for aesthetic treatments.

DaxibotulinumtoxinA for Injection (DAXI) is a novel botulinum toxin type A formulation in clinical development for the treatment of glabellar lines⁶ and a number of neurologic and musculoskeletal conditions. The formulation consists of highly purified daxibotulinumtoxinA (RTT150, a 150-kDa botulinum toxin type A) together with a proprietary stabilizing peptide (RTP004) that binds to the neurotoxin with high avidity and other excipients including polysorbate-20 (a surfactant), buffers, and a sugar. RTP004 is a peptide of 35 amino acids that is highly positively charged at physiologic pH and forms a strong electrostatic bond with daxibotulinumtoxinA. The peptide allows the product to be formulated without human serum albumin and to be stable at room temperature before reconstitution.

A phase 2, multicenter, randomized, double-blind, placebo-controlled, dose-ranging study in glabellar lines that evaluated DAXI 20, 40, and 60 U demonstrated that the greatest duration of clinical efficacy—a median of 24 weeks—and the most favorable risk-to-benefit profile were attained with the 40-U dose.⁶ Based on the results of that study, the 40-U dose of DAXI was selected for evaluation in two 36-week pivotal studies (SAKURA 1 and SAKURA 2) and an 84-week open-label safety study (which included repeated dosing and evaluated approximately 1600 additional subjects). This is the first publication reporting the results from the SAKURA 1 and SAKURA 2 studies.

PATIENTS AND METHODS

Study Design

SAKURA 1 and SAKURA 2 were multicenter, randomized, double-blind, parallel-group studies designed to assess the efficacy and safety of DAXI relative to placebo in the treatment of glabellar lines. Both studies followed the same protocol (NCT03014622 and NCT03014635 were approved by the relevant institutional review boards), and all subjects signed informed consent.

Disclosure: *This article discusses DaxibotulinumtoxinA for Injection, a novel botulinum toxin type A in clinical development. Studies and manuscript development were funded by Revance Therapeutics, Inc., Newark, Calif. Dr. Carruthers has been a consultant and researcher with Allergan, Alpheon, Bonti, Merz, and Revance; an editor and author with Elsevier; an assistant editor of Dermatologic Surgery; and a reviewer for Plastic and Reconstructive Surgery and Aesthetic Journal. Dr. Fagien has participated in research studies for Allergan, Galderma, Evolus, Revance, and MTF Biologics. He has also been a consultant and/or received honoraria from Allergan, Galderma, MTF Biologics, and Revance. Dr. Joseph has been a principal investigator and consultant for, and stockholder in, Endo Pharmaceuticals, Evolus, and Revance; a principal investigator and consultant for Allergan, Galderma, Merz, and Teoxane; a principal investigator for Croma, Cynosure, and Ulthera; a stockholder in Alpheon; and president of Innomed Tech Corp. Dr. Humphrey has been a board member of the American Society for Dermatologic Surgery; a consultant for Allergan, Galderma, L'Oréal, Merz, Revance, and Zeltiq; and on speaker bureaus for Galderma, L'Oréal, and Allergan. Dr. Biesman has been a consultant for and served on advisory boards for Allergan, Cytrellis, and Accure; been a consultant for Brera, Galderma, L'Oréal, Merz, Revance, Sienna, Suneva, and Zimmer; served on promotional speakers bureaus for Allergan, Galderma, Merz, and Revance; received grant funding from Allergan, Cutera, Cytrellis, Galderma, Jan Marini, Merz, Sienna, Suneva, and Revance; received honoraria from Allergan, Clarisonic, Dignity Health, Ferndale Pharma Group, Galderma, Intraderm, and Merz; had travel expenses paid for by Allergan, Merz, and Revance; received equipment loans from Cutera and Syneron Candela; and has an ownership interest in Cytrellis. Dr. Gallagher, Mr. Liu, and Dr. Rubio are employees of, and hold stock/stock options in, Revance.*

Inclusion and Exclusion Criteria

Adults in good general health were eligible for inclusion in the studies if, during maximum frown, they had moderate or severe glabellar lines according to both investigator and subject assessments on the validated, 4-point, Investigator Global Assessment–Frown Wrinkle Severity scale and the Patient Frown Wrinkle Severity scale, respectively (Table 1). Washout periods included 30 days for immunosuppressants and any therapy that could interfere with treatment evaluation; 3 months for retinoids in the treatment area or more than 200 U of botulinum toxin type A anywhere in the body; 6 months for facial botulinum toxin type A treatment; 9 months for forehead chemical peels; and 12 months for any procedures that may affect the glabellar region.

Treatment

Subjects were assigned randomly in a 2:1 ratio within each study center to receive a single treatment with either DAXI 40 U or placebo. Each single treatment consisted of five intramuscular 0.1-ml injections, one to each of five injection sites (two injections into each corrugator muscle and one into the procerus muscle), given by a trained physician. Both treatments were provided in sequentially numbered clinical trial kits containing masked single-use vials of DAXI or placebo that were reconstituted with sterile

unpreserved saline within 2 hours of injection by a blinded trained preparer (and provided to the investigator in a syringe for administration). All vials contained the proprietary stabilizing peptide and were identical in appearance before and after reconstitution.

Outcome Measures

Investigators and subjects were trained to rate glabellar line severity using the Investigator Global Assessment–Frown Wrinkle Severity scale and the Patient Frown Wrinkle Severity scale, respectively, both of which grade wrinkle severity as none, mild, moderate, or severe (Table 1). A photonic numeric guide was provided to each study center to help ensure consistency in ratings across investigators. The primary efficacy outcome was the percentage of subjects at week 4 achieving an improvement from baseline of at least 2 points in both Investigator Global Assessment–Frown Wrinkle Severity scale and Patient Frown Wrinkle Severity scale scores at maximum frown. This is also referred to as a 2-point composite response and is considered by the U.S. Food and Drug Administration to reflect treatment success.⁸

Other efficacy outcomes assessed at maximum frown included the proportion of subjects over time who achieved severity of none or mild on the Investigator Global Assessment–Frown Wrinkle Severity scale or the Patient Frown Wrinkle

Table 1. Efficacy and Satisfaction Rating Scales*

Score	IGA-FWS Scale	PFWS Scale	GAIS	Subject Satisfaction with Treatment†
-3	—	—	Very much worse	—
-2	—	—	Much worse	—
-1	—	—	Worse	—
0	None (no wrinkles)	None (no wrinkles)	No change	Very dissatisfied
1	Mild (very shallow wrinkles)	Mild (very shallow wrinkles)	Improved	Dissatisfied
2	Moderate (moderate wrinkles)	Moderate (moderate wrinkles)	Much improved	Somewhat dissatisfied
3	Severe (deep and furrowed wrinkles)	Severe (deep wrinkles)	Very much improved	Neither satisfied nor dissatisfied
4	—	—	—	Somewhat satisfied
5	—	—	—	Satisfied
6	—	—	—	Very satisfied

IGA-FWS, Investigator Global Assessment–Frown Wrinkle Severity; PFWS, Patient Frown Wrinkle Severity; GAIS, Global Aesthetic Improvement Scale.

*Adapted with permission from Wolters Kluwer Health, Inc.: Carruthers J, Solish N, Humphrey S, et al. Injectable daxibotulinumtoxinA for the treatment of glabellar lines: A phase 2, randomized, dose-ranging, double-blind, multicenter comparison with onabotulinumtoxinA and placebo. *Dermatol Surg*. 2017;43(11):1321–1331 (<https://journals.lww.com/dermatologicsurgery>)⁶; and Bertucci V, Humphrey S, Carruthers J, et al. Comparing injectable daxibotulinumtoxinA and onabotulinumtoxinA in moderate and severe glabellar lines: Additional analyses from a phase 2, randomized, dose-ranging, double-blind, multicenter study. *Dermatol Surg*. 2017;43(Suppl 3):S262–S273 (<https://journals.lww.com/dermatologicsurgery>).⁷ Copyright by the American Society for Dermatologic Surgery, Inc. The Creative Commons license does not apply to this content. Use of the material in any format is prohibited without written permission from the publisher, Wolters Kluwer Health, Inc. Please contact permissions@lww.com for further information.

†Subjects were asked “When you think about the results of the glabellar line treatment you received, how satisfied are you with the appearance of your frown lines?”

Severity scale, and the proportion of subjects who achieved greater than or equal to 1-point improvement on the 7-point Global Aesthetic Improvement Scale (an exploratory endpoint) (Table 1). Duration of response since treatment was evaluated as time over which glabellar line severity was maintained as none or mild on both the Investigator Global Assessment–Frown Wrinkle Severity scale and the Patient Frown Wrinkle Severity scale, and time until glabellar line severity returned to baseline levels on both the Investigator Global Assessment–Frown Wrinkle Severity scale and the Patient Frown Wrinkle Severity scale.

Subjects self-assessed their glabellar lines at maximum frown using the Patient Frown Wrinkle Severity scale daily for 2 weeks after treatment to evaluate the onset of efficacy. Onset was defined as the day at which a subject first achieved at least a 1-point improvement from baseline glabellar line severity. Subjects also reported their level of satisfaction with how the treated area of their face appeared at week 4 using a 7-point satisfaction scale (Table 1).

Subjects were evaluated for at least 24 weeks until both Investigator Global Assessment–Frown Wrinkle Severity scale and Patient Frown Wrinkle Severity scale scores had returned to baseline (to a maximum of 36 weeks). Assessments were performed at baseline and weeks 1, 2, 4, 8, 12, 16, 20, and 24 (and potentially weeks 28, 32, and 36). After completing at least 24 weeks in the study, and if Investigator Global Assessment–Frown Wrinkle Severity scale and Patient Frown Wrinkle Severity scale scores had returned to baseline, all subjects were eligible to enroll in the open-label SAKURA safety study.

Adverse events were documented, and subjects were queried in a general manner about adverse events that were potentially suggestive of the distant spread of toxin. Other safety assessments included physical examinations; clinical laboratory evaluations; electrocardiographs; and evaluations of vital signs, injection sites, cranial nerves II through VII, and facial muscle strength. Using enzyme-linked immunosorbent assay (Covance, Inc., Indianapolis, Ind.), serum from all subjects was tested at screening and weeks 2, 4, and 12 for binding antibodies to daxibotulinumtoxinA or the RTP004 excipient peptide. In those subjects positive for binding antibodies to daxibotulinumtoxinA, the mouse protection assay (Revance Therapeutics, Inc., Newark, Calif.) was performed to determine the presence of neutralizing antibodies.

Randomization and Masking

An independent statistician prepared a computer-generated randomization schedule of treatment assignments (using SAS PROC PLAN; SAS Institute, Inc., Cary, N.C.) designated by trial kit number. This was kept in a locked location, and investigators, study center staff, subjects, and the sponsor remained masked to treatment assignment throughout the study. The designated study staff member assigned subjects to treatment by selecting the next available sequentially numbered clinical trial kit.

Statistical Analyses

Estimates of efficacy from an earlier dose-ranging study⁶ showed that a sample size of 300 subjects (200 DAXI and 100 placebo) would have more than 99 percent power to detect a between-group difference in the primary efficacy outcome based on a two-sided chi-square test at an alpha level of 0.05 (assuming response rates of ≥ 50 percent versus 1 percent). A sample size of 300 also provided adequate power to detect a between-group difference in response rates for other efficacy outcomes on later visits (e.g., 90 percent power for a response rate of 8.7 percent versus 1 percent under a one-sided chi-square test at an alpha level of 0.05). A statistical analysis plan was provided before database lock, and the randomization code was unblinded and the analyses performed (using SAS version 9.4 or higher) only after all data had been included in the database and verified.

The proportions of subjects with a 2-point composite response, or glabellar line severity of none or mild on the Investigator Global Assessment–Frown Wrinkle Severity scale or the Patient Frown Wrinkle Severity scale, were compared across groups using the Cochran-Mantel-Haenszel test using a two-sided test with a type I error rate of 0.05, stratifying by study center. Differences were calculated using the Mantel-Haenszel estimate of the common risk difference, with 95 percent confidence intervals calculated using stratified Newcombe confidence limits. Duration of response data were summarized as Kaplan-Meier point estimates of median duration. A hierarchical testing procedure was used to control the overall type I error of 0.05.

Efficacy analyses were performed on the intent-to-treat population (all subjects who were randomized), according to treatment assignment. Missing Investigator Global Assessment–Frown Wrinkle Severity scale and Patient Frown Wrinkle Severity scale data were imputed at the subject

level (worst outcome for DAXI, and best outcome for placebo) up to week 24. Post hoc comparisons of baseline demographic data were performed between randomized treatment groups within each study using *t* tests for quantitative variables and chi-square or Fisher’s exact tests for categorical variables. Safety analyses were performed on all treated subjects with at least one postbaseline safety assessment, according to actual treatment received.

RESULTS

Subjects

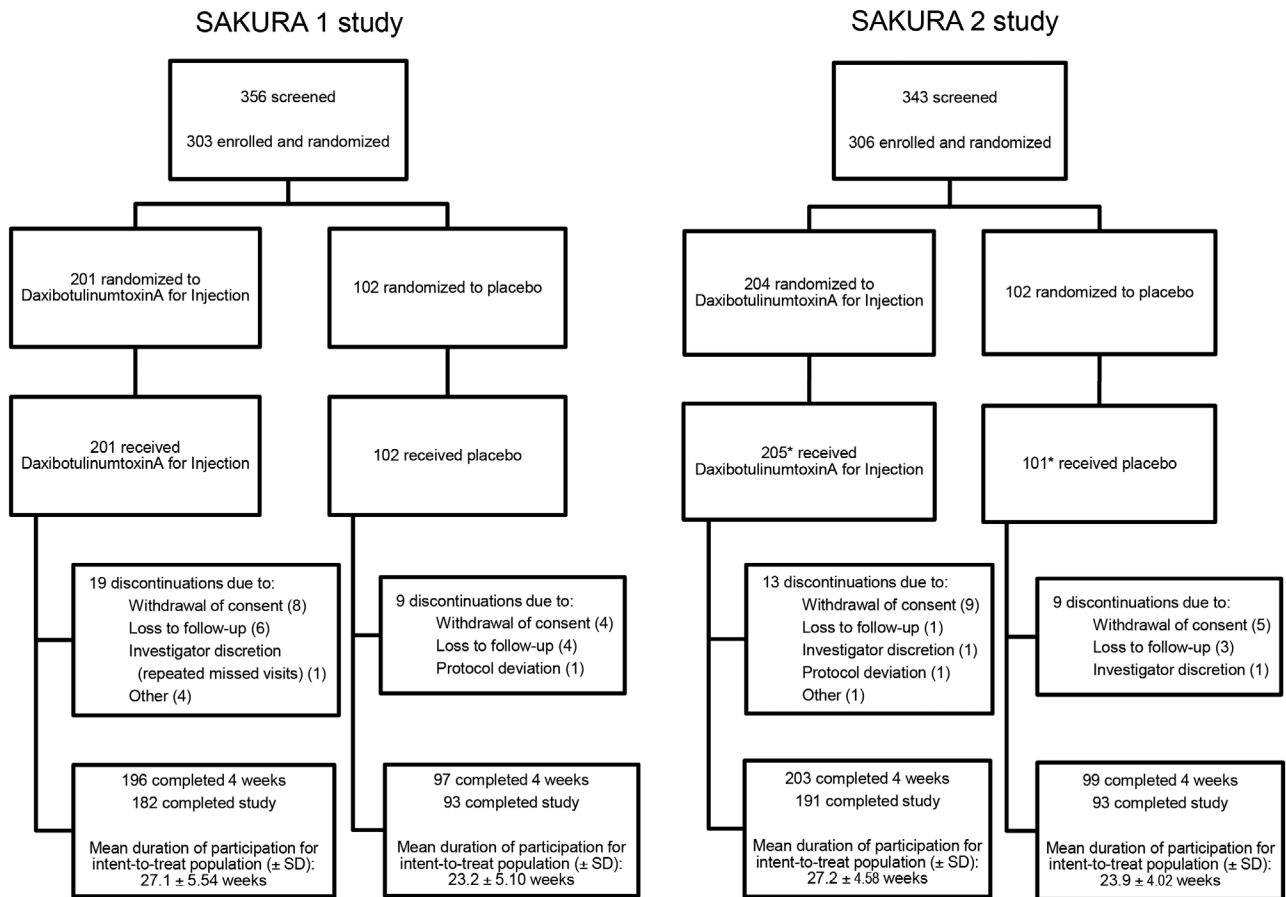
Each study was conducted at 15 experienced clinical trial centers (all in the United States for SAKURA 1, and nine in the United States and six in Canada for SAKURA 2). Investigators enrolled 303 subjects in SAKURA 1 (DAXI, *n* = 201; placebo, *n* = 102) and 306 in SAKURA 2 (DAXI, *n* = 204; placebo, *n* = 102) (Fig. 1). The first subject’s informed consent and the last subject’s last visit

or contact occurred on December 5, 2016, and November 14, 2017, respectively, for SAKURA 1 and November 22, 2016, and November 3, 2017, for SAKURA 2.

Within each study, both groups had similar demographic characteristics at baseline, with post hoc comparisons revealing no statistically significant differences between randomized treatment groups (Table 2). The majority of subjects were female and Caucasian, and their mean age ranged from 49 to 51 years. The proportion of subjects completing the study was 90.5 percent (182 of 201) and 93.6 percent (191 of 204) in the DAXI groups, and 91 percent (93 of 102) in both placebo groups. Discontinuations were largely attributable to withdrawal of consent and loss to follow-up, and none were attributable to adverse events (Fig. 1).

Efficacy

DAXI treatment was significantly more effective than placebo in achieving the primary efficacy



* 1 patient randomized to placebo inadvertently received DaxibotulinumtoxinA for Injection

Fig. 1. Disposition of subjects in SAKURA 1 and SAKURA 2 studies.

Table 2. Baseline Demographics

Characteristic	SAKURA 1 Study			SAKURA 2 Study		
	Daxibotulinum-toxinA for Injection 40 U (%)	Placebo (%)	<i>p</i> for Difference	Daxibotulinum-toxinA for Injection 40 U (%)	Placebo (%)	<i>p</i> for Difference
No.	201	102		204	102	
Female	174 (86.6)	88 (86.3)	0.9439*	183 (89.7)	87 (85.3)	0.2588*
Mean age ± SD, yr	50.9 ± 11.22	49.0 ± 11.13	0.1602†	49.6 ± 9.84	50.5 ± 9.98	0.4167†
Racial origin/ethnicity			0.2253‡			0.9669‡
Caucasian	173 (86.1)	81 (79.4)		180 (88.2)	92 (90.2)	
Black/African American	10 (5.0)	8 (7.8)		9 (4.4)	3 (2.9)	
Asian	7 (3.5)	2 (2.0)		11 (5.4)	5 (4.9)	
Other	11 (5.5)	11 (10.8)		4 (2.0)	2 (2.0)	
Prior treatment with any BoNT	92 (45.8)	45 (44.1)	0.7846*	121 (59.3)	60 (58.8)	0.9345*
Time since last BoNT injection, mo						
Mean ± SD	32.2 (37.05)	22.6 (19.61)	0.1064†	22.7 (23.67)	23.0 (24.36)	0.9356†
Range	7–205	1–94		7–193	7–121	
IGA-FWS rating at maximum frown			0.5510*			0.6736*
Moderate	123 (61.2)	66 (64.7)		129 (63.2)	67 (65.7)	
Severe	78 (38.8)	36 (35.3)		75 (36.8)	35 (34.3)	
PFWS rating at maximum frown			0.6082*			0.5994*
Moderate	120 (59.7)	64 (62.7)		106 (52.0)	49 (48.0)	
Severe	81 (40.3)	38 (37.3)		98 (48.0)	53 (52.0)	

BoNT, botulinum toxin type A; IGA-FWS, Investigator Global Assessment–Frown Wrinkle Severity scale;

PFWS, Patient Frown Wrinkle Severity scale.

* χ^2 test.

†*t* test.

‡Fisher’s exact test.

outcome (at least a 2-point improvement in glabellar line severity according to both investigator and subject ratings at maximum frown at week 4): 73.6 percent versus 0.0 percent of subjects in SAKURA 1, and 74.0 percent versus 1.0 percent of subjects in SAKURA 2 [both $p < 0.0001$; difference, 74.2 percent (95 percent CI, 68.2 to 80.2 percent) and 72.9 percent (95 percent CI, 66.6 to 79.1 percent), respectively]. Treatment with DAXI was also significantly more effective than placebo in achieving glabellar line severity of none or mild at maximum frown according to investigator ratings ($p < 0.0001$ from weeks 2 to 24 in both studies) (Fig. 2). At week 4, the proportion of subjects achieving glabellar line severity of none or mild was 97.5 percent and 97.5 percent with DAXI in SAKURA 1 and SAKURA 2, respectively, compared with 4.9 percent and 3.9 percent with placebo ($p < 0.0001$) (Fig. 2 and Table 3). The equivalent proportions at week 24 were 35.3 percent and 29.4 percent with DAXI in SAKURA 1 and SAKURA 2, respectively, compared with 2 percent for placebo in both studies ($p < 0.0001$) (Fig. 2 and Table 3).

Subject ratings gave similar results: at week 4, the proportion of subjects reporting glabellar line severity of none or mild was 92.0 percent and 90.2 percent with DAXI in SAKURA 1 and SAKURA 2, respectively, compared with 1.0 percent and 3.9 percent with placebo (Fig. 2). The equivalent proportions at week

24 were 23.9 percent and 21.6 percent with DAXI in SAKURA 1 and SAKURA 2, respectively, compared with 1.0 percent and 2.0 percent with placebo (Fig. 2). At least a 1-point improvement in Global Aesthetic Improvement Scale score was achieved by at least 98 percent of DAXI-treated subjects in both studies at week 4, and by at least 43 percent at week 24, according to investigator ratings (Fig. 3).

Duration of Response

The median time since DAXI treatment over which glabellar line severity of none or mild at maximum frown was maintained according to composite investigator and subject ratings was 24.0 weeks (SAKURA 1) and 23.9 weeks (SAKURA 2) (Fig. 4). Similarly, among the subgroup who achieved a 2-point composite response at week 4 (SAKURA 1, $n = 145$ subjects; SAKURA 2, $n = 150$ subjects), the median duration for maintaining glabellar line severity of none or mild was 24.0 weeks in both studies (95 percent CI, 23.4 to 24.1 weeks and 23.7 to 25.0 weeks, respectively). The median time for return to baseline glabellar line severity at maximum frown was 27.7 weeks in SAKURA 1 and 26.0 weeks in SAKURA 2 (Fig. 5). Photographic documentation of a long-term maintenance of response to DAXI (where a 1-point improvement in Investigator Global Assessment–Frown Wrinkle

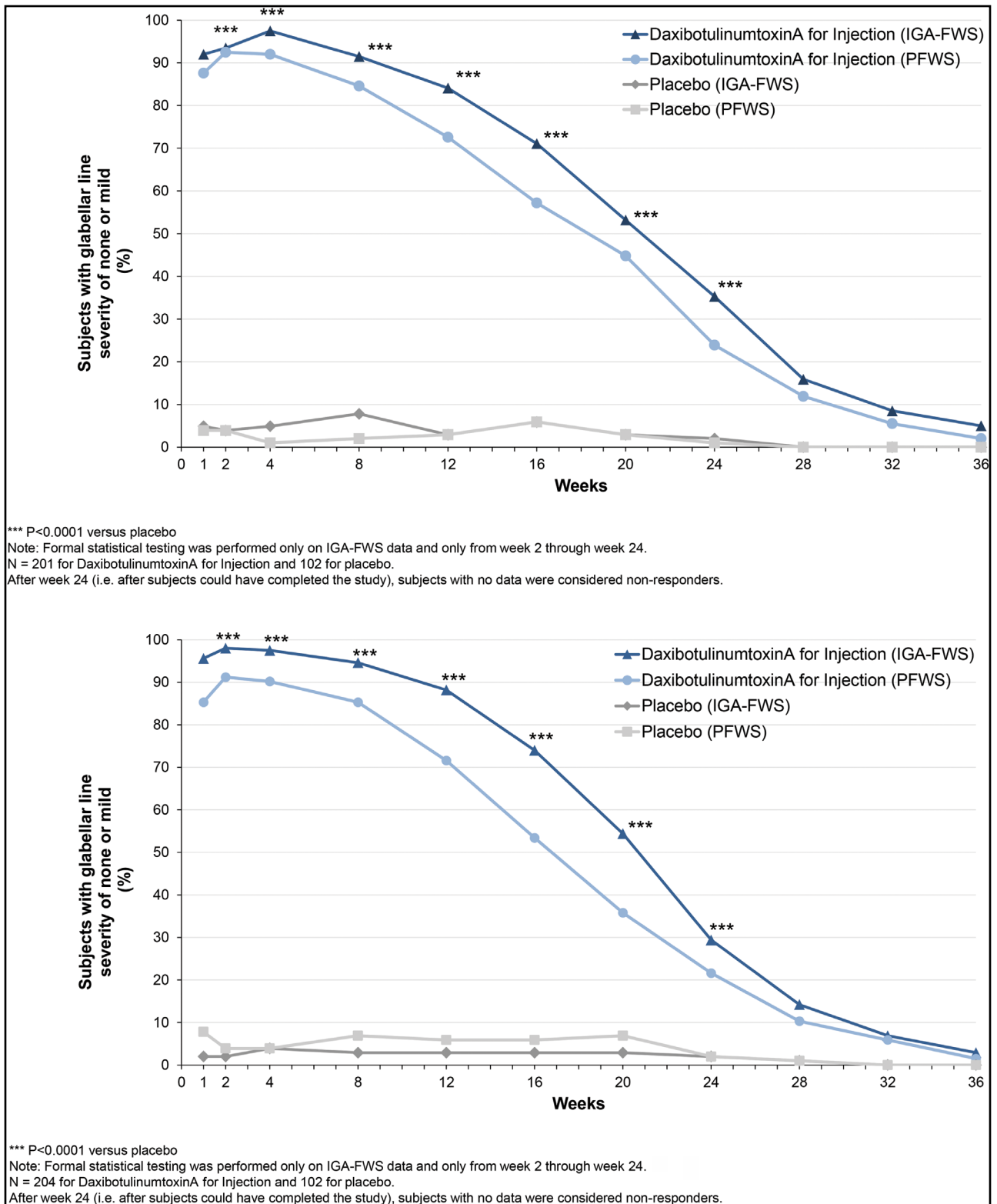


Fig. 2. Proportion of subjects with glabellar line severity of none or mild at maximum frown assessed by investigators using the Investigator Global Assessment–Frown Wrinkle Severity (IGA-FWS) scale or by subjects using the Patient Frown Wrinkle Severity (PFWS) scale. (Above) SAKURA 1 study; (below) SAKURA 2 study.

Table 3. Proportion of Subjects with Glabellar Line Severity of None or Mild at Maximum Frown According to Investigator Ratings after Treatment with DaxibotulinumtoxinA for Injection or Placebo

Time Point	SAKURA 1 Study				SAKURA 2 Study			
	No. of Subjects (%)		Difference (95% CI), <i>p</i>	Placebo	No. of Subjects (%)		Difference (95% CI), <i>p</i>	Placebo
	DaxibotulinumtoxinA for Injection 40 U	Placebo			DaxibotulinumtoxinA for Injection 40 U	Placebo		
Baseline	0/201 (0)	0/102 (0)	—	0/204 (0)	0/102 (0)	—	—	
Wk 1	185/201 (92.0)	5/102 (4.9)	—	195/204 (95.6)	2/102 (2.0)	—	—	
Wk 2	188/201 (93.5)	4/102 (3.9)	89.9% (85.0–94.8%), <0.0001	200/204 (98.0)	2/102 (2.0)	96.1% (92.7–99.4%), <0.0001	—	
Wk 4	196/201 (97.5)	5/102 (4.9)	92.7% (88.1–97.3%), <0.0001	199/204 (97.5)	4/102 (3.9)	93.6% (89.3–98.0%), <0.0001	—	
Wk 8	184/201 (91.5)	8/102 (7.8)	83.8% (77.5–90.1%), <0.0001	193/204 (94.6)	3/102 (2.9)	91.6% (87.0–96.3%), <0.0001	—	
Wk 12	169/201 (84.1)	3/102 (2.9)	81.3% (75.5–87.1%), <0.0001	180/204 (88.2)	3/102 (2.9)	85.3% (79.7–90.9%), <0.0001	—	
Wk 16	143/201 (71.1)	6/102 (5.9)	65.5% (57.8–73.2%), <0.0001	151/204 (74.0)	3/102 (2.9)	70.9% (64.0–77.9%), <0.0001	—	
Wk 20	107/201 (53.2)	3/102 (2.9)	50.6% (43.0–58.3%), <0.0001	111/204 (54.4)	3/102 (2.9)	51.5% (44.0–59.0%), <0.0001	—	
Wk 24	71/201 (35.3)	2/102 (2.0)	33.6% (26.4–40.7%), <0.0001	60/204 (29.4)	2/102 (2.0)	27.4% (20.6–34.3%), <0.0001	—	
Wk 28	32/93 (34.4)	0/7 (0)	—	29/89 (32.6)	1/4 (25.0)	—	—	
Wk 32	17/50 (34.0)	0/2 (0)	—	14/47 (29.8)	0/1 (0)	—	—	
Wk 36	10/28 (35.7)	0/0 (0)	—	6/26 (23.1)	0/0 (0)	—	—	

Severity scale score was maintained until at least week 36) is shown in [Figure 6](#).

Onset of Response

The median time to onset of a clinically meaningful response was 3 days in each study.

Subject Satisfaction

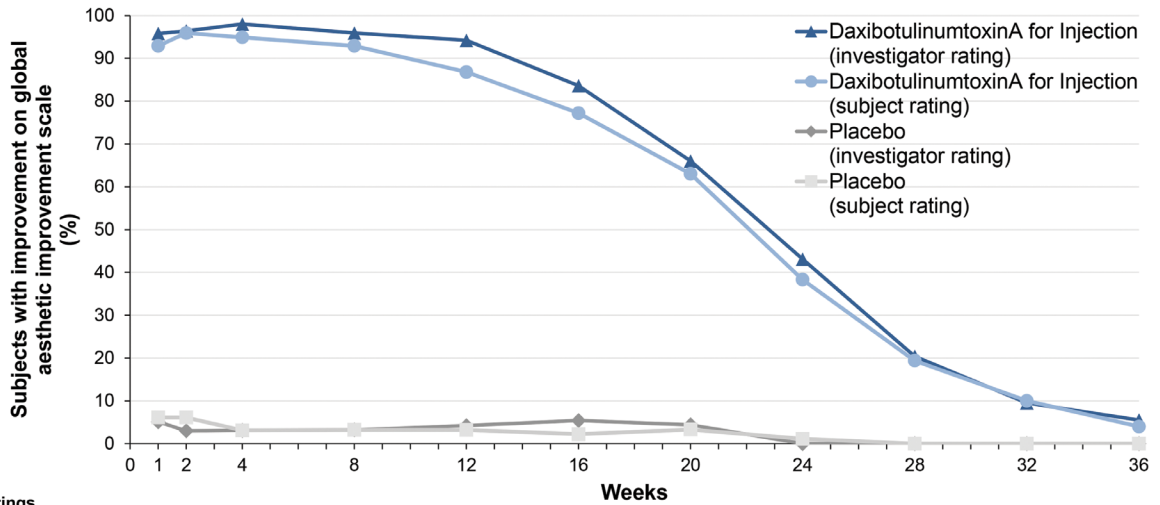
The proportion of subjects at week 4 who rated their global satisfaction with treatment as very satisfied, satisfied, or somewhat satisfied totaled 95 percent and 96 percent with DAXI in SAKURA 1 and SAKURA 2, respectively, compared with 4 percent and 6 percent with placebo ([Fig. 7](#)).

Safety and Tolerability

There were no discontinuations caused by adverse events, no deaths, and no serious adverse events that were considered related to treatment. The most common treatment-related events in the DAXI and placebo groups were headache and injection-site pain ([Table 4](#)). The incidence of eyelid ptosis in DAXI-treated subjects was 2.5 percent and 2.0 percent in SAKURA 1 and SAKURA 2, respectively. All but three subjects who experienced eyelid ptosis were treated with apraclonidine or related agents, and all cases resolved without sequelae. Other safety evaluations (i.e., clinical laboratory investigations; physical examinations; and evaluation of vital signs, electrocardiographs, cranial nerves, and facial muscle strength) were largely normal. No subject developed neutralizing antibodies to DAXI or any appreciable increase in antibody titers to RTP004.

DISCUSSION

The results from both of these studies show that DAXI was significantly more effective than placebo in achieving glabellar line severity of none or mild ($p < 0.0001$ at every time point for which statistical analyses were performed; i.e., weeks 2 to 24). Although statistical testing was not performed at week 1, results were also similar at this time point. According to composite data from investigators and subjects, glabellar line severity of none or mild was sustained for a median duration of 24.0 and 23.9 weeks (SAKURA 1 and SAKURA 2, respectively), and the median time to return to baseline levels was 27.7 and 26.0 weeks. Furthermore, at week 24, investigators considered that at least 43 percent of subjects had an improvement in their Global Aesthetic Improvement Scale



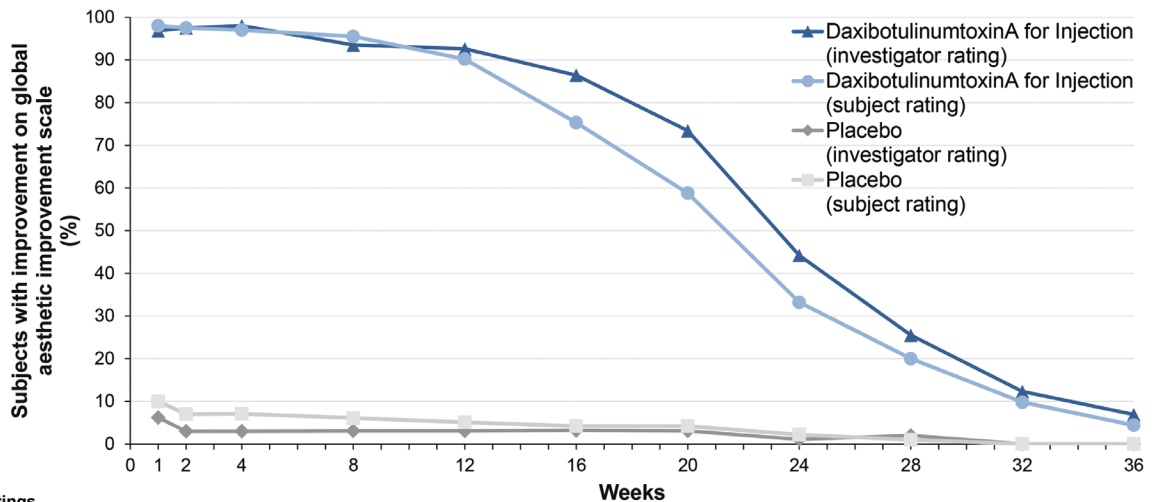
Investigator ratings

n for DaxibotulinumtoxinA for Injection =	191	195	196	196	191	189	188	188	201	201	201
n for placebo =	98	99	97	94	95	93	91	91	102	102	102

Subject ratings

n for DaxibotulinumtoxinA for Injection =	196	195	196	196	190	189	189	188	201	201	201
n for placebo =	99	99	97	95	95	93	91	91	102	102	102

Note: For weeks 1–24, percentages were calculated based on the number of subjects with data at each timepoint. For weeks 28–36, percentages were calculated based on the number of subjects at baseline and subjects with no data were considered non-responders.



Investigator ratings

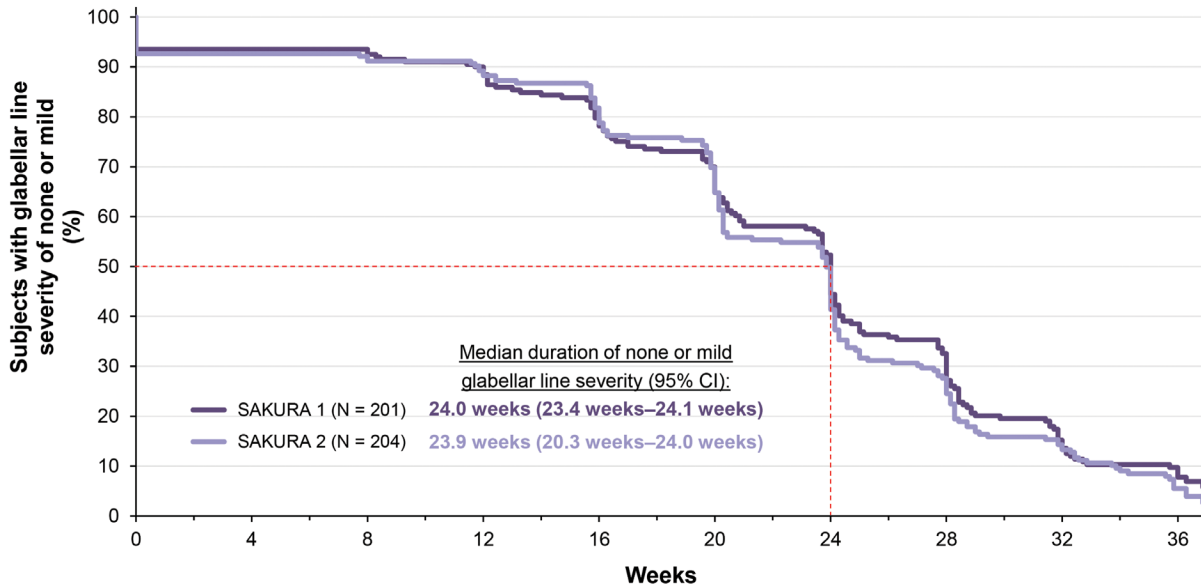
n for DaxibotulinumtoxinA for Injection =	190	201	203	201	204	198	199	199	204	204	204
n for placebo =	97	100	99	98	97	95	97	93	102	102	102

Subject ratings

n for DaxibotulinumtoxinA for Injection =	198	201	203	201	204	198	199	199	204	204	204
n for placebo =	100	100	99	98	98	95	96	93	102	102	102

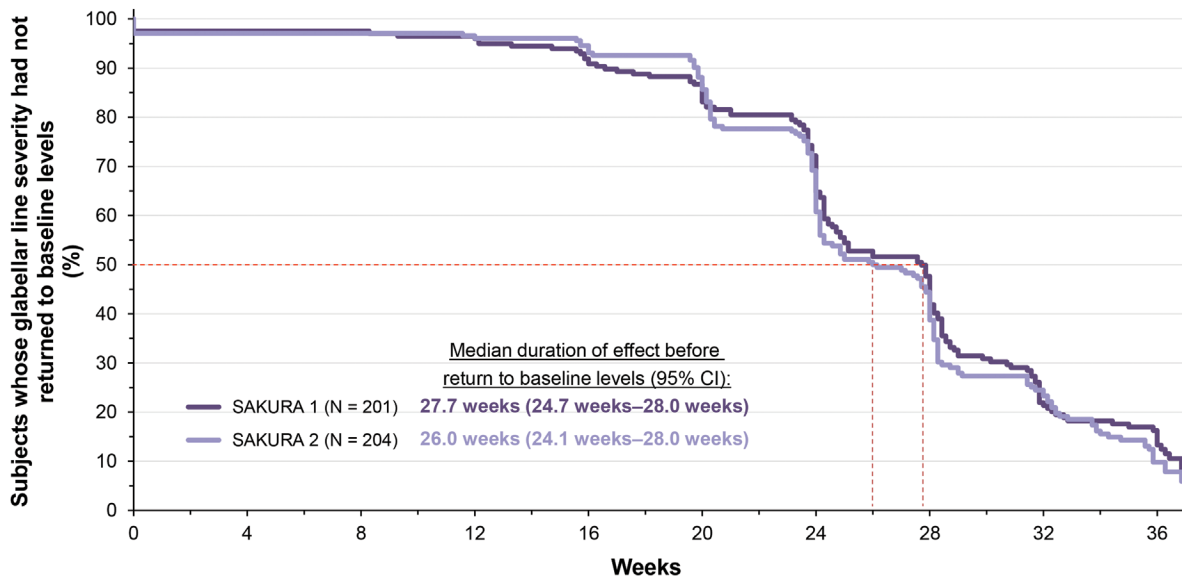
Note: For weeks 1–24, percentages were calculated based on the number of subjects with data at each timepoint. For weeks 28–36, percentages were calculated based on the number of subjects at baseline and subjects with no data were considered non-responders.

Fig. 3. Proportion of subjects with an improvement in Global Aesthetic Improvement Scale score at maximum frown. In the two studies, investigator ratings showed that the proportion of subjects who were improved, much improved, or very much improved in the DaxibotulinumtoxinA for Injection group was 98.0 percent and 98.0 percent at week 4, and 43.1 percent and 44.2 percent at week 24, compared with placebo group values of 3.1 percent and 3.0 percent at week 4, and 0 percent and 1.1 percent at week 24. (Above) SAKURA 1 study; (below) SAKURA 2 study.



Time to return to moderate or severe glabellar line severity was the number of days since treatment until the first visit at which IGA-FWS and PFWS scores had both returned to moderate or severe levels after an improvement from baseline to none or mild levels had been observed at week 1, 2, or 4. If no such return to moderate or severe level occurred, censoring occurred at the latest visit for which both IGA-FWS and PFWS data were available. In the absence of an improvement in both IGA-FWS and PFWS scores at weeks 1, 2 or 4, the time to return to baseline was 0 days.

Fig. 4. Duration for which glabellar line severity of none or mild at maximum frown was maintained after DaxibotulinumtoxinA for Injection treatment in SAKURA 1 and SAKURA 2 studies according to wrinkle severity assessments from both investigators and subjects [i.e., on both the Investigator Global Assessment–Frown Wrinkle Severity (IGA-FWS) scale and the Patient Frown Wrinkle Severity (PFWS) scale].



Time to return to baseline level was the number of days since treatment until the first visit at which IGA-FWS and PFWS scores had both returned to baseline levels after an improvement from baseline had been observed at week 1, 2, or 4. If no such return to baseline level occurred, censoring occurred at the latest visit for which both IGA-FWS and PFWS data were available. In the absence of an improvement in both IGA-FWS and PFWS scores at weeks 1, 2 or 4, the time to return to baseline was 0 days.

Fig. 5. Time for glabellar line severity at maximum frown to return to baseline levels after DaxibotulinumtoxinA for Injection treatment in SAKURA 1 and SAKURA 2 studies according to wrinkle severity assessments from both investigators and subjects [i.e., on both the Investigator Global Assessment–Frown Wrinkle Severity (IGA-FWS) scale and the Patient Frown Wrinkle Severity (PFWS) scale].

score. Subject ratings of global satisfaction with treatment at week 4 were overwhelmingly positive (i.e., satisfied) for DAXI and negative (i.e., dissatisfied) for placebo.

Perhaps of greatest clinical importance is the fact that DAXI treatment achieved a more prolonged clinical benefit than would be anticipated from initial treatment with any of the currently



Fig. 6. Subject treated with 40 U of DaxibotulinumtoxinA for Injection showing a 2-point reduction in glabellar line severity at maximum frown at week 4 that was sustained through week 24 according to both investigator and subject ratings. A 1-point reduction in glabellar line severity was sustained until at least week 36 according to investigator ratings and until at least week 32 according to subject ratings. Investigators used the Investigator Global Assessment–Frown Wrinkle Severity (*IGA-FWS*) scale and subjects used the Patient Frown Wrinkle Severity (*PFWS*) scale.

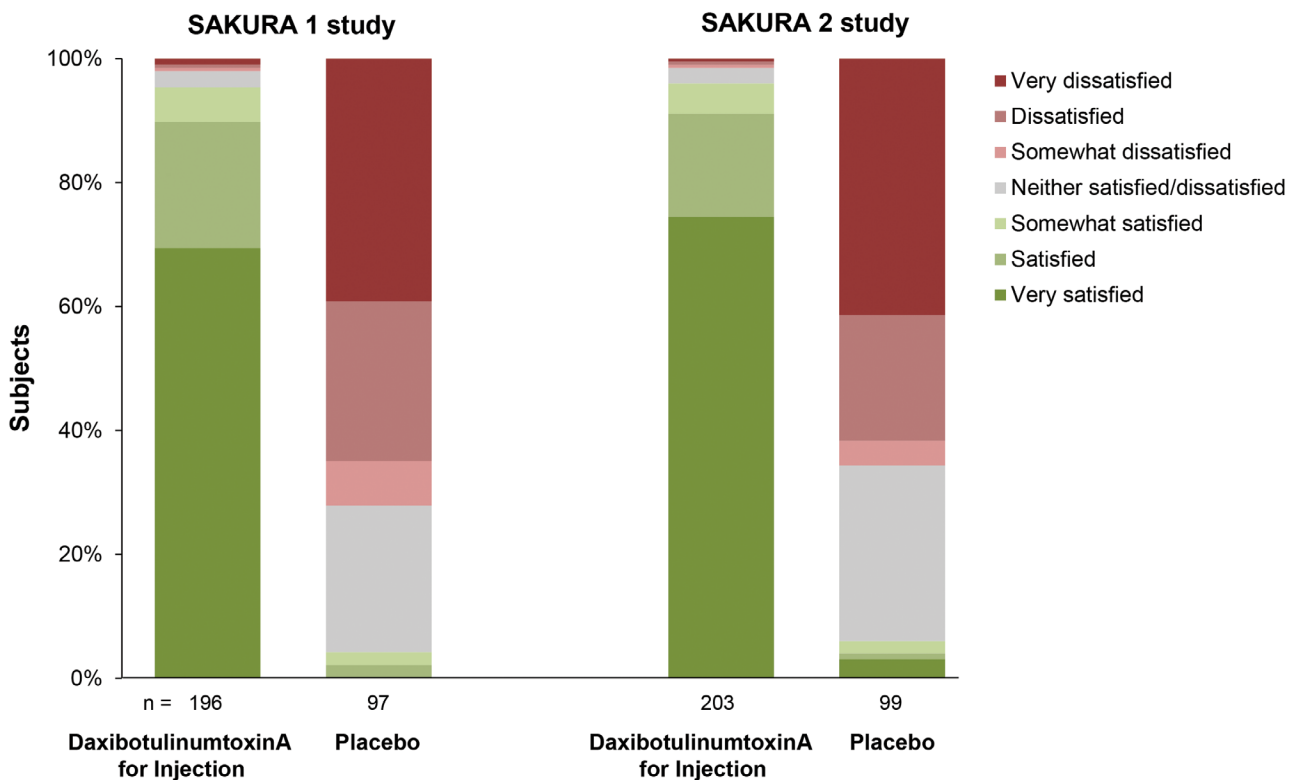


Fig. 7. Subject global satisfaction with treatment at week 4. The proportion of subjects who were satisfied or very satisfied was 89.8 percent and 91.1 percent in the DaxibotulinumtoxinA for Injection groups (in the SAKURA 1 and SAKURA 2 studies, respectively) compared with 2.1 percent and 4.0 percent in the placebo groups.

available botulinum toxin type As. Although the efficacy of different treatments can only be properly compared in direct comparative studies, when such data are lacking, we can attempt to evaluate our findings in the context of the existing literature. Previous registration studies have reported that glabellar line severity of none or mild is sustained for a median of 12.1 (85 days)^{9,10} to 16.7 weeks (117 days) with 50 U of abobotulinumtoxinA¹⁰ and 17.1 weeks (120 days) with 20 U of

onabotulinumtoxinA.¹¹ Glabellar line severity of none or mild was selected as a key measure of efficacy in our trial because of its clinical relevance in everyday practice—it reflects the true clinical goal of treatment, and a relapse from none or mild lines to moderate or severe lines will be perceived by the patient and trigger a desire for retreatment.

The validity of our results is strong, as data from both studies are not only remarkably consistent with each other but are also highly consistent

Table 4. Adverse Events*

	SAKURA 1 Study		SAKURA 2 Study	
	DaxibotulinumtoxinA for Injection 40 U (%)	Placebo (%)	DaxibotulinumtoxinA for Injection 40 U (%)	Placebo (%)
No. of subjects	201	102	205	101
All adverse events				
Any	72 (35.8)	25 (24.5)	94 (45.9)	24 (23.8)
Mild	51	20	73	16
Moderate	17	4	19	7
Severe	4	1	2	1
Leading to discontinuation	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Serious†	2 (1.0)	1 (1.0)	2 (1.0)	1 (1.0)
Deaths	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Treatment-related‡ adverse events				
Any	35 (17.4)	8 (7.8)	43 (21.0)	10 (9.9)
Mild	25	6	37	8
Moderate	10	2	6	2
Severe	0	0	0	0
Serious	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Important treatment-related‡ adverse events (incidence of ≥2% in any group)				
Headache	14 (7.0)§	3 (2.9)	12 (5.9)§	1 (1.0)
Mild	12	2	10	0
Moderate	2	1	2	1
Severe	0	0	0	0
Injection-site pain	10 (5.0)	4 (3.9)	5 (2.4)	4 (4.0)
Mild	5	2	5	3
Moderate	5	2	0	1
Severe	0	0	0	0
Injection-site erythema	0 (0.0)	0 (0.0)	5 (2.4)	4 (4.0)
Mild	0	0	5	4
Moderate	0	0	0	0
Severe	0	0	0	0
Injection-site edema	1 (0.5)	0 (0.0)	5 (2.4)	3 (3.0)
Mild	1	0	5	3
Moderate	0	0	0	0
Severe	0	0	0	0
Eyelid ptosis	5 (2.5)¶	0 (0.0)	4 (2.0)¶	0 (0.0)
Mild	3	0	3	0
Moderate	2	0	1	0
Severe	0	0	0	0

*Worst severity reported for each subject.

†SAKURA 1: anxiety (placebo group), sepsis (DaxibotulinumtoxinA for Injection [DAXI] group), bone marrow failure (DAXI group). SAKURA 2: recurrent leiomyosarcoma (placebo group), uterine perforation (DAXI group), uterine leiomyoma (DAXI group).

‡Possibly, probably, or definitely related.

§Median duration of headache: SAKURA 1, 2 days; SAKURA 2, 1.5 days. Durations are calculated by excluding start day and including end day.

¶Median duration of eyelid ptosis: SAKURA 1, 77 days; SAKURA 2, 34 days. Durations are calculated by excluding start day and including end day.

with those from the earlier phase 2 dose-ranging study.⁶ For example, investigator ratings at week 4 showed the proportion of subjects with glabellar line severity of none or mild to be 97.5 percent in both SAKURA 1 and SAKURA 2, and 97.4 percent in the dose-ranging study with the same 40-U dose of DAXI.⁶ In addition, at week 24, the proportion of subjects with glabellar line severity of none or mild was 35.3 percent in SAKURA 1, 29.4 percent in SAKURA 2, and 30.8 percent in the dose-ranging study.⁶ In contrast, the proportion of such responders in the 20-U onabotulinumtoxinA arm of the phase 2 dose-ranging study at week 24 was 11.9 percent (the proportion having declined below 30 percent after the week-16 visit).⁶ It is noteworthy that the 40-U dose of DAXI contains

0.18 ng of core 150-kDa neurotoxin (data on file; Revance Therapeutics, Inc.), an amount identical to that in a 20-U dose of onabotulinumtoxinA¹² (which contains 0.18 ng of core neurotoxin within 1 ng of neurotoxin complex). Nevertheless, even though both these doses contain the same amount of active neurotoxin, the products have shown significant differences in efficacy and duration of effect.⁶ Furthermore, a 20-U dose of DAXI contains only 0.09 ng of core neurotoxin, yet treatment with 20 U of DAXI has significantly exceeded the response seen with 20 U of onabotulinumtoxinA in terms of the proportion of subjects showing glabellar line severity of none or mild on the Investigator Global Assessment–Frown Wrinkle Severity scale at week 16.⁶ It has also resulted

in a comparable proportion of subjects with a 1- or 2-point improvement in Investigator Global Assessment–Frown Wrinkle Severity scale score and a comparable duration of response.⁶

That the 0.18-ng dose of DAXI (40 U) produced a significantly greater response and duration than the similar amount of onabotulinumtoxinA suggests that formulation differences between the products, including the presence of the proprietary stabilizing excipient peptide in DAXI, contribute to the observed differences in clinical performance. This underscores the established fact that potency units of botulinum toxin type As are not interchangeable and units of DAXI should not be compared to those of other products. Relative potencies cannot be established, and dosing should be based on the available clinical data for individual products.

Although it has been suggested with other botulinum toxin type A products that increasing dose may lead to increased duration, there are few dose-ranging studies published in this indication. A small study with onabotulinumtoxinA failed to find increased efficacy or duration at doses above 20 U in women.¹³ The mean onabotulinumtoxinA dose used for glabellar lines in women in the United States is 17 U.¹⁴ This suggests that in current clinical practice dose is not being increased to attempt to drive increased duration, perhaps because of the risk of increasing adverse events.

In our study, the inclusion of subjects with a history of botulinum toxin type A treatment was novel and the washout period for facial botulinum toxin type A treatment was set at 6 months. As it is possible that a subclinical chemodeneration may persist following the restoration of muscle function, this may be considered to be a confounding factor. Approximately 50 percent of subjects had a history of botulinum toxin type A treatment (roughly comparable between the DAXI and placebo groups) and the mean time since last botulinum toxin type A treatment (for any indication) was 32 and 23 months in SAKURA 1 and SAKURA 2, respectively, suggesting that there was little effect of carryover from prior treatments. Additional limitations of the SAKURA studies include a preponderance of women and Caucasians, and it would be interesting to extend this research into other patient populations to ensure widespread applicability. It could also be useful to evaluate satisfaction beyond week 4, as satisfaction could increase further once patients have begun to appreciate the long duration of clinical benefit.

CONCLUSIONS

These studies with DAXI (SAKURA 1 and SAKURA 2) are the only large well-controlled botulinum toxin type A studies in subjects with moderate or severe glabellar lines that demonstrate a clinically meaningful benefit with a median duration of at least 24 weeks. This prolonged duration of action may help sustain efficacy between treatments and lessen the frequency of retreatment. No new safety signals were detected and no immunogenicity to daxibotulinumtoxinA or the stabilizing peptide was observed.

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The SAKURA 1 and SAKURA 2 Investigator Group includes the following: **AboutSkin Dermatology and Dersurgery, PC:** Joel L. Cohen, M.D. (principal investigator); Stephen Ho, M.D. (subinvestigator). **Aesthetic Solutions, PA:** Sue Ellen Cox, M.D. (principal investigator); John Soderberg, M.D. (subinvestigator). **Arthur Swift Research, Inc.:** Arthur Swift, M.D. (principal investigator); Daniel Borsuk, M.D. (subinvestigator); Vasilios Papanastasiou, M.D. (subinvestigator). **ATS Clinical Research:** Ava Shamban, M.D. (principal investigator); Soheil Simzar, M.D. (subinvestigator). **Bertucci MedSpa, Inc.:** Vince Bertucci, M.D. (principal investigator); Brittany Waller, M.D. (subinvestigator). **BOYD:** Charles Boyd, M.D. (principal investigator). **Brian S. Biesman, M.D.:** Brian S. Biesman, M.D. (principal investigator); Molly Katz, R.N. (subinvestigator); Lauren Churchill, B.S.N. (subinvestigator). **California Dermatology & Clinical Research Institute:** Stacy Smith, M.D. (principal investigator). **Center for Dermatology and Dermatologic Surgery:** Cheryl Burgess, M.D. (principal investigator). **Clinical Testing of Beverly Hills:** John Joseph, M.D. (principal investigator); Karan Dhir, M.D. (subinvestigator). **Dermatology Consulting Services, PLLC:** Zoe Draelos, M.D. (principal investigator); Michael Draelos, M.D. (subinvestigator). **Dermetics:** Nathan Rosen, M.D. (principal investigator); Channy Muhn, M.D. (subinvestigator). **Dr. Jean Carruthers Cosmetic Surgery, Inc.:** Jean Carruthers, M.D. (principal investigator); Shannon Humphrey, M.D. (subinvestigator). **Dr. Shannon Humphrey, Inc.:**

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