Phase I/III Clinical Trial to Evaluate the Safety and Efficacy of a New Botulinum Toxin (HU-014) Versus OnabotulinumtoxinA in Subjects With Moderate-to-Severe Crow's Feet Lines

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BACKGROUND HU-014, a newly introduced botulinum toxin type A, has not been investigated for its efficacy and safety in crow's feet line (CFL) treatment.

OBJECTIVE Here, we compared the efficacy and safety of HU-014 and onabotulinumtoxinA in CFL treatment.

METHODS This was a randomized, double-blind, active drug–controlled, multicenter, 16-week, Phase I/III study designed to determine the noninferiority of HU-014 compared with onabotulinumtoxinA in moderate-to-severe CFL treatment. In the Phase III study, 290 subjects were randomized at a 1:1 ratio to receive a single treatment of HU-014 or onabotulinumtoxinA. The primary endpoint was the proportion of subjects achieving Grade 0 or 1 in the facial wrinkle scale on maximum smile at Week 4.

RESULTS The primary endpoint was achieved by 72% of the subjects with HU-014 and onabotulinumtoxinA treatments, supporting the noninferiority of HU-014 compared with onabotulinumtoxinA. All secondary efficacy outcomes were achieved by the subjects. The 2 groups showed no significant differences in the safety analysis.

CONCLUSION HU-014 has noninferior efficacy and safety compared with onabotulinumtoxinA in the treatment of CFL.

Botulinum toxin type A (BoNT-A) is extensively used in nonsurgical esthetic procedures. OnabotulinumtoxinA (Botox, Allergan Inc., Irvine, CA) is the first processed BoNT-A product approved by the FDA for upper face rejuvenation (glabella frown lines and crow's feet lines [CFLs]) and is considered as the market leader in the aesthetic field. Recently, numerous BoNT-A products have been developed by several pharmaceutical companies to cater medical needs. However, not all these agents have proven efficacy and safety, and their imprudent use could cause adverse effects. Because BoNT-A drugs are unique and not interchangeable,[1] clinical trials are required to demonstrate the efficacy and safety of a new BoNT-A.

Crow's feet lines are formed by repeated contractions of the orbicularis oculi muscle. Moderate-to-severe CFLs can be observed even if there are no facial expressions (i.e., at rest), causing individuals to appear older.[2] BoNT-A injection limits the activity of lateral orbicularis oculi, leading to an effective and safe CFL treatment.[3] A randomized, placebo-controlled trial of onabotulinumtoxinA demonstrated its efficacy in improving moderate-to-severe CFLs.[4,5] In most cases, BoNT-A treatment of CFLs shows improvements within 4 weeks and shows superior efficacy over alternatives, such as facial lifting, laser dermabrasion, and chemical peeling.

HU-014 (Hutox, Huons Global Co., Ltd., Seongnam, Korea), a newly introduced BoNT-A, has shown its efficacy and safety for moderate-to-severe glabellar frown line treatment in previous Phase I-III clinical studies (*ClinicalTrials.gov identifier: NCT02961673, NCT03440671*). However, its efficacy and safety in CFL treatment have not been investigated. Therefore, we aimed to investigate the efficacy and safety of HU-014 for CFL treatment by comparing it with onabotulinumtoxinA.

Materials and Methods

Study Design

This was a Phase I/III study designed to evaluate the noninferiority of HU-014 compared with onabotulinumtoxinA. The Phase I study was performed in a small number of subjects with a shorter follow-up time to evaluate the safety of HU-014. This was followed by a multicenter, randomized, double-blinded, active-controlled Phase III study conducted in 2 medical centers (Konkuk University Hospital and Chung-Ang University Hospital) between November 2018 and August 2019.

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Ethics Approval

The institutional review board at Chung-Ang University Hospital and Konkuk University Hospital approved the study protocols, informed consent forms, and relevant supporting data. The study complied with the guidelines of the Korea Good Clinical Practice, International Council for Harmonization-Good Clinical Practice, and the Declaration of Helsinki. Written informed consent was obtained from all subjects.

Subjects

We enrolled subjects aged 19 to 65 years with moderate-to-severe bilaterally symmetrical CFLs at maximum smile, as assessed by the investigators. The following were excluded: (1) subjects with a history of botulinum toxin hypersensitivity, facial nerve palsy or ptosis, and neuromuscular disorders (myasthenia gravis, Lambert–Eaton myasthenic syndrome, amyotrophic lateral sclerosis, or motor neuropathy); (2) subjects who used medications that could interfere with the neuromuscular function, such as aminoglycoside antibiotics and curare-like agents, within 4 weeks before screening; (3) subjects injected with BoNT-A in the past 6 months; and (4) subjects who underwent cosmetic procedures associated with CFLs (any surgery in the periocular area, laser rejuvenation, or augmentation) within 12 months before screening.

Materials and Intervention

HU-014 is an investigational drug product that has not been approved for CFL treatment. Each vial of HU-014 and onabotulinumtoxinA was composed of 100-U *Clostridium* BoNT-A, stored at 4°C and reconstituted with 2.5 mL of preservative-free, sterile 0.9% sodium chloride immediately before injection.

Using a double-blind design, the subjects were randomly assigned codes at a 1:1 ratio to determine who would receive HU-014 or onabotulinumtoxinA injection. An independent provider reconstituted the medications and prepared 2 identical syringes that were labeled with the assigned codes. Using a 30 to 33-gauge needle, 0.1 mL of 4-U BoNT-A per site was injected at 3 sites on each side of the lateral orbicularis oculi muscle (a total dose of 24-U). If the CFL was primarily below the lateral canthus, the injector had the option to inject below the lateral canthus (See **Supplemental Digital Content 1**, Figure, http://links.lww.com/DSS/A556, showing the injection pattern and allowed modification for lateral canthal line treatment).

Efficacy Assessment

The severity of CFL was evaluated in all subjects at baseline and at 4, 8, 12, and 16 weeks postinjection. All subjects were assessed twice at each time point: one face-to-face assessment by the investigating clinician and one standardized photograph assessment by an independent clinician blinded to the treatment groups and study time point. Photographs of CFLs were captured under standard conditions (identical camera equipment, positioning, lighting, and settings) at each visit. CFL severity was assessed using a facial wrinkle scale (FWS) with grades of 0 (none), 1 (mild), 2 (moderate), and 3 (severe) at maximum smile and rest.

An improvement in CFL and subjects' satisfaction with CFL treatment were also assessed. Subjective global assessment was used to determine the improvement in CFL severity, with 9 grades (+4, 100% improvement; +3, 75% improvement; +2, 50% improvement; +1, 25% improvement; 0, no change; −1, 25% worsening; −2, 50% worsening; −3, 75% worsening; and −4, 100% worsening). Subjects' satisfaction with the treatment was evaluated using 7 grades (from Grade 1 [very dissatisfied] to Grade 7 [very satisfied]).

For assessments at maximum smile, treatment response was defined as an improvement in CFL severity to none or mild (Grade 0 or 1) on both sides. For assessments at rest, the treatment response was defined as an improvement in CFL severity by at least 1 grade from baseline on both sides.

The primary efficacy outcome was the proportion of treatment responders with maximum smile at Week 4, based on the face-to-face assessment. The secondary efficacy outcomes were the (1) proportion of responders at maximum smile at Weeks 8, 12, and 16 based on the face-to-face assessment; (2) proportion of responders at maximum smile at Weeks 4, 8, 12, and 16 based on blinded photographic assessment; (3) proportion of responders at rest at Weeks 4, 8, 12, and 16 based on the face-to-face assessment; (4) proportion of responders at rest at Weeks 4, 8, 12, and 16 based on blinded photographic assessment; (5) proportion of subjects with an improvement of Grade +2 or more at maximum smile in the subjective global assessment at Weeks 4, 8, 12, and 16; and (6) proportion of subjects with an improvement of Grade +2 or more at rest in the subjective global assessment at Weeks 4, 8, 12, and 16; and (7) satisfaction of subjects.

Safety Assessments

All adverse events (AEs) were monitored and divided according to the severity and relationship with the study intervention. All subjects were observed for 30 minutes post-treatment to confirm acute AEs. Vital signs were measured at each visit, and laboratory tests and physical examinations were performed at baseline and at the end of the study. The AEs were classified according to their severity using a 5-point grading scale (mild, moderate, severe, life-threatening consequences, and death).

Statistical Analysis

The efficacy assessment was performed on the full analysis set (FAS) and per-protocol sets (PPS), whereas the safety assessment was performed on the safety analysis set (SAS). Statistical analyses were performed using SAS Version 9.4 (SAS Institute, Cary, NC). To evaluate the primary endpoint, the lower limit of the 97.5% 1-sided confidence interval (CI) for the difference in the proportion of responders between the HU-014 and onabotulinumtoxinA groups was calculated. If the lower limit of the estimated CI

surpassed the limit of −18%, HU-014 could be considered noninferior to onabotulinumtoxinA. For the secondary end points, 2-sample *t*-test, Pearson chi-squared test, or Fischer exact test was used. Safety analysis was performed using the paired *t*-test or Wilcoxon signed-rank test for continuous variables and the Pearson chi-squared or Fisher exact test for categorical variables. All *p*-value were considered significant at <.05.

Results

Subjects

In the Phase I study, 12 subjects were screened; all of them received HU-014 treatment. In the Phase III study, 290 subjects were screened and randomized. The safety set comprised 290 subjects. However, 12 subjects were not included in the efficacy outcome analysis (FAS = 278 subjects). Within the FAS, 18 subjects were excluded (PPS = 272 subjects) (See **Supplemental Digital Content 2**, Figure, http://links.lww.com/DSS/A557, showing the disposition of the subjects [Phase III]). No differences were found in demographic information and baseline characteristics, including CFL severity at baseline between the 2 groups in the Phase III study (See **Supplemental Digital Content 3**, Table, http://links.lww.com/DSS/A558, showing demographic and baseline characteristics).

Efficacy Outcome Assessments

For the Phase I primary efficacy endpoint at Week 4, the proportion of responders from the FAS was 83.33% (10/12). For the Phase III primary efficacy endpoint at Week 4, the proportion of responders from the FAS was 72.14% for the HU-014 group and 72.46% for the onabotulinumtoxinA group. The difference between the 2 groups was −0.32% (95% CI, −10.84% to 10.20%), and the lower limit of 97.5% CI was higher than the noninferior limit of −18%, supporting the noninferiority of HU-014 compared with onabotulinumtoxinA (Figure 1A). The results of the PPS population were similar.

In all secondary efficacy outcome assessments of the FAS and PPS, HU-014 treatment showed results comparable with those of onabotulinumtoxinA treatment. The proportion of responders at maximum smile at Weeks 8, 12, and 16 based on the face-to-face assessment was 61%, 34%, and 19% for the HU-014 group and 57%, 35%, and 17% for the onabotulinumtoxinA group, respectively. There was no significant difference ($p > .05$) between the 2 groups at each evaluation point (Figure 1A).

The proportion of responders at maximum smile at Weeks 4, 8, 12, and 16 based on the blinded photographic assessment was 81%, 66%, 51%, and 41% for the HU-014 group and 78%, 64%, 54%, and 29% for the onabotulinumtoxinA group, respectively. There was no significant difference ($p > .05$) between the 2 groups at Weeks 4, 8, and 12, but there was a significant difference ($p = .0402$) between the 2 groups at Week 16 (Figure 2A).

The proportion of responders at rest based on the face-to-face assessment and the blinded photographic assessment were similar for both the HU-014 group and the onabotulinumtoxinA group. There was no significant difference ($p > .05$) between the 2 groups at each evaluation points (Figures 1B,2B).

The proportion of subjects with an improvement of Grade +2 at maximum smile assessed by the subjective global assessment at Weeks 4, 8, 12, and 16 was 98%, 96%, 87%, and 51% for the HU-014 group and 99%, 97%, 88%, and 53% for the onabotulinumtoxinA group, respectively. Similar results were found for the results at rest in both groups. There was no significant difference ($p > .05$) between the 2 groups at each evaluation point (See **Supplemental Digital Content 4**, Figure, http://links.lww.com/DSS/A559, illustrating the proportion of subjects [%] with an improvement of Grade +2 [50% improvement] or more in subjective global assessment from baseline at [A] maximum smile and [B] rest).

In the FAS, the difference in all primary and secondary efficacy outcomes between the study and control groups was not significant ($p > .05$) except for the proportion of responders at maximum smile at Week 16 based on the blinded photographic assessment.

Patient Satisfaction

Participants who rated themselves 6 (satisfied) and 7 (totally satisfied) in the subject satisfaction assessment were classified as "satisfied." The proportion of "satisfied" subjects with Grade 6 or 7 at Weeks 4, 8, 12, and 16 was 95%, 89%, 68%, and 41% in the HU-014 group and 96%, 86%, 62%, and 41% in the onabotulinumtoxinA group, respectively. There was no significant difference between the 2 groups at each visit (See **Supplemental Digital Content 5**, Figure, http://links.lww.com/DSS/A560, illustrating the

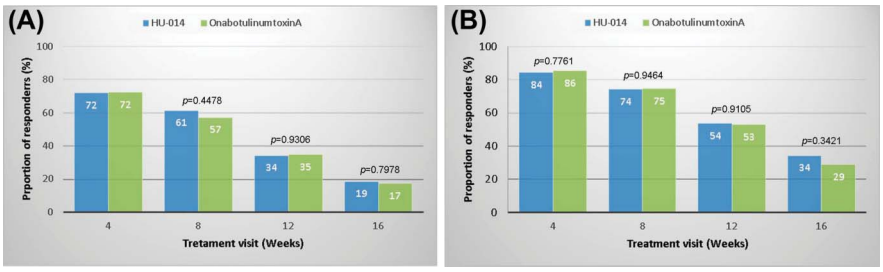

Figure 1. Proportion of responders based on investigator's face-to-face assessment at (A) maximum smile and (B) rest.

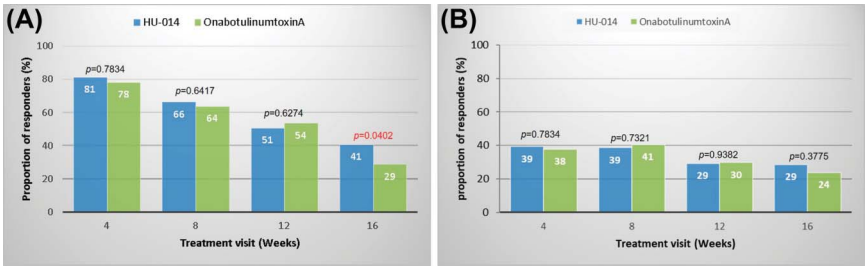

Figure 2. Proportion of responders based on blinded photographic assessment at (A) maximum smile and (B) rest.

proportion of subjects [%] rating themselves as "satisfied" or "very satisfied" on the satisfaction scale).

Safety Assessments

In the Phase I cohort, the incidence rate of AEs was 16.67%, and the severity of all reported AEs was Grade 2, "moderate." No adverse drug reaction (ADR), serious AE (SAE), or serious ADR was observed in the patients (Table 1). In the Phase III study, 23 subjects (15.86%) from the HU-014 group and 20 (13.79%) from the onabotulinumtoxinA group reported 30 and 28 AEs, respectively. The incidence rate of ADRs was 2.07% (4 cases) for HU-014% and 2.76% (4 cases) for onabotulinumtoxinA. There were 3 cases of injection site bruising and 1 case of injection site swelling in the HU-014 group, and 3 cases of injection site bruising and 1 case of injection site pruritus in the onabotulinumtoxinA group. The difference in the incidence rate of AEs and ADRs was not significant between the groups. There were no reports of SAEs in the onabotulinumtoxinA group, but there was 1 case of SAE in the HU-014 group, but it was determined to be unrelated to the investigational product. Furthermore, there were no significant findings with respect to vital signs, laboratory tests, and physical examinations (Table 1).

Discussion

The noninferiority of HU-014 compared with onabotulinumtoxinA was determined during the Phase III study, which was conducted after the successful assessment of safety of HU-014 for CFL treatment in the Phase I study. In the Phase III study, the primary and secondary efficacy endpoints were satisfied in both groups, confirming the clinical equipotency of both products when used at a 1:1 dose ratio.

The response rate for primary efficacy endpoints at Week 4 in both HU-014 and onabotulinumtoxinA groups was 72%, supporting the noninferiority of HU-014 compared with onabotulinumtoxinA. This finding is consistent with that of 2 randomized, double-blind, placebo-controlled studies that used the same dose of onabotulinumtoxinA and 4-grade FWS (68.3% in a Japanese study and 66.7% in a Caucasian study).[4,6]

Our results suggest that the injection of HU-014 or onabotulinumtoxinA into CRLs improves both dynamic and static wrinkles. The proportion of responders assessed using the face-to-face assessment at Week 4 was 84% in the HU-014 group and 86% in the onabotulinumtoxinA group at rest, in comparison with 72% in both HU-014 and onabotulinumtoxinA groups at maximum smile. However, the proportion of responders assessed using the blinded photographic assessment at rest was significantly lower than that assessed using the face-to-face assessment. This is because the FWS mean score determined using the baseline blinded photographic assessment was significantly lower than that determined using the face-to-face assessment. These results are also consistent with those of previous studies, indicating that photographic assessments tend to be skewed downward.[7,8] These results suggest that 2-dimensional photographic analysis minimizes the 3-dimensional (depth) wrinkle structure determined by visual assessment, yielding artificially low scores.

Of note, the proportion of responders in the HU-014 group was significantly higher than that of the onabotulinumtoxinA group at maximum smile at Week 16 based on the blinded photographic assessment ($p = .0402$). In

TABLE 1. Incidence of Adverse Drug Reactions (Phase III)

	Phase I	Phase III		
Adverse Drug Reaction Term	**HU-014 ($n = 12$), N (%)**	**HU-014 ($n = 145$), N (%)**	**OnabotulinumtoxinA ($n = 145$), N (%)**	**p**
Treatment emergent adverse event	2 (16.67)	23 (15.86)	20 (13.79)	.7414
Adverse drug reaction	0	3 (2.07)	4 (2.76)	1.0000
Serious adverse event	0	1 (0.69)	0	1.0000
Serious adverse drug reaction	0	0	0	NA

general, similar trends were noted in other secondary outcomes as well; the proportion of responders was higher in the HU-014 group than that of the onabotulinumtoxinA group at maximum smile at Week 16 based on face-to-face assessment and at rest at Week 16. Thus, it may be possible that HU-014 has longer efficacy than onabotulinumtoxinA. However, because the differences were not statistically significant at other occasions, no conclusion can be drawn and further evaluation of HU-014 is needed.

Patient satisfaction is one of the most important factors to evaluate the efficacy of a cosmetic treatment. In this study, high patient satisfaction rates were reported which is also consistent with the results of a previous study, which reported that approximately 50% subject satisfaction was observed 12 weeks post-treatment.[9] These results suggest that both HU-014 and onabotulinumtoxinA can be effective treatments for moderate-to-severe CFLs, providing satisfactory results for patients.

The safety analysis results showed no significant differences in the incidence of AEs and ADRs between the HU-014 and onabotulinumtoxinA groups. There were 3 cases of injection site bruising and 1 case of injection site swelling in the HU-014 group, and 3 cases of injection site bruising and 1 case of injection site pruritus in the onabotulinumtoxinA group. However, the severity of all ADRs was Grade 1, and all reported ADRs were predictable adverse reactions that commonly occur with BoNT-A injection.

This study also has several limitations. All of the subjects were Koreans, and a majority of enrolled patients were women. Furthermore, the total duration of the study was 16 weeks. Therefore, further studies with larger patient population of various ethnic groups with longer follow-up periods are warranted in the future.

Conclusions

The differences in the efficacy of HU-014 and onabotulinumtoxinA, as assessed using the investigator-evaluation and subject-evaluation tools, were statistically insignificant, supporting the noninferiority of HU-014 compared with onabotulinumtoxinA in treating CFL. The 2 groups also showed no significant difference in the safety analysis. Thus, HU-014 can be a new alternative BoNT-A currently on the market for the treatment of CFL.

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