BENEV
MEDICAL AFFAIRS

Letybo®: Field of Effect and Diffusion Characteristics

RELEVANT PRESCRIPTION INFORMATION LABEL INFORMATION

The information provided relates to a use for Letybo® (letibotulinumtoxinA-wlbg) that is not approved by the US Food and Drug Administration (FDA).

CLINICAL DATA

A search of the published medical literature was conducted regarding Letybo® and its field of effect and diffusion characteristics.

The relevant citations referenced in this communication are listed below. The hyperlinks to publicly available abstracts are included. Findings were limited to a randomized double-blind split face study, unpublished data on file, and a multiscale in silico model which may not be reflective of findings from controlled studies or to outcomes in a broader population, and should be considered when evaluating the data.

Some references cited in this response may discuss additional treatment areas that were not specified in this Medical Information Request.

Letybo® is an acetylcholine release inhibitor and a neuromuscular blocking agent indicated for the temporary improvement in the appearance of moderate to severe glabellar lines associated with corrugator and/or procerus muscle activity in adult patients. Hugel Inc. and BENEV Inc. do not endorse the use of Letybo® in a manner not consistent with the approved label.

Units of biological activity of Letybo® cannot be compared to nor converted into Units of any other botulinum toxin or any toxin assessed with any other specific assay method.

CITATIONS

1. Letybo® Prescribing Information, 2024.
2. Bennek M, Rudowitz D, Kerscher M. Diffusion Characteristics of LetibotulinumtoxinA, OnabotulinumtoxinA, and AbobotulinumtoxinA and its Impact on Muscle Relaxation: A Randomized Split-Face Clinical Trial. Dermatol Ther (Heidelb). Published online June 17, 2025. doi:10.1007/s13555-025-01458-3 https://pubmed.ncbi.nlm.nih.gov/40526262/

Review of Research and Clinical Practice Information

Bennek et al. 2025[2] was a randomized double-blind split-face clinical trial that consisted of 30 healthy adults that received Leti-BoNTA on one forehead side and either Ona-BoNTA or Abo-BoNTA on the other. Anhidrosis was measured via Minor's starch test over 6 months.

Leti-BoNTA had a significantly smaller maximal anhidrotic area than Ona-BoNTA (-15.1 ± 5.5 cm^2, $p < 0.001$) and Abo-BoNTA (-25.2 ± 14.5 cm^2, $p < 0.001$). An area under the curve (AUC) analysis confirmed the largest area of anhidrosis for Abo-BONTA over the 6-month period, followed by Ona-BONTA and Leti-BoNTA.

Leti-BoNTA shows limited diffusion, as assessed with the Minor's starch-iodine test. Its precise action in this trial implies to be a viable option when controlled spread is essential.

Diffusion Characteristics of LetibotulinumtoxinA, OnabotulinumtoxinA, and AbobotulinumtoxinA and its Impact on Muscle Relaxation: A Randomized Split-Face Clinical Trial[2]

To explore the clinical research and references below, please review the cited publication.

Materials and Methods: This was a double-blind, randomized, single-dose, single center study that enrolled healthy patients at the University of Hamburg. The study (EU clinical trial (CT): 2024–511047-26-01) was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice (GCP) guidelines. The research protocol was reviewed and approved by the Ethics Committee of the Medical Association of Munich (Ethikkommission bei der Medizinischen Fakultät der Ludwig- Maximilians-Universität München, Pettenkoferstraße 8, München). All participants provided written informed consent to participate in the study. Consent for publication was obtained from all participants for whom identifiable data are included. All participants provided written informed consent for publication.

Eligible study participants were healthy male and female subjects aged 18–75 with a body mass index (BMI) of 16–27 kg/m^2 and a healthy skin test area with uniform sweating activity and no areas of anhidrosis under standardized sweating conditions, assessed during the screening period (day −14 to day −1). The main inclusion and exclusion criteria are listed in Table 1.

After receiving written informed consent, eligible patients were randomized to receive two of the three study medications on contralateral sides of the forehead. The choice of the dosage of the investigational product was based on national guidelines and the dosage recommended by the manufacturer. Each subject was randomly assigned to receive Leti-BoNTA on one side of the forehead and Ona-BoNTA or Abo-BoNTA on the other side of the forehead. An overview of the specific characteristics of each botulinum toxin formulation, including excipients and the producing cell line, is provided in Table 2.

Each of the selected products was injected on one side of the forehead with two injection points placed on each side. For each BoNTA preparation, the total injection volume of 0.2 mL was identical. In respect to the line of convergence (C-line), a template was used to ensure that the injection points were comparable (Fig. 1).

To ensure blinding, all syringes were prepared by an independent third party using identical labeling and standardized volumes, thereby masking any visual or tactile differences between products. Both the injector and participants were blinded to treatment allocation to minimize observer and subject bias.

A total dose of 4 U each was used. For Abo-BoNTA, a total of 10 U was used, on the basis of a dose conversion ratio of 1:2.5 onabotulinumtoxinA: abobotulinumtoxinA, as recommended in randomized controlled trials and in reviews, as well as the manufacturer's recommendations and the official labeling of the product. Each BoNTA formulation was reconstituted according to the package leaflet, as detailed in Table 3.

Table 1 | Eligibility Criteria for Participation in the Study

Inclusion Criteria
Fitzpatrick skin type (FST) I-VI
18-75-year-old prerequisites
Moderate-to-heavy wrinkling (in motion)
Uniform sweating activity and no areas of anhidrosis under standardized conditions
Unchanged skin care routine for at least 3 months
Healthy skin and a stable state of health

Exclusion Criteria
BMI < 16 or ≥ 27 kg/m^2
Asymmetrical forehead wrinkles
Active skin lesions/infections or irritations in the treatment area
Previous treatment (upper third of the face) with fillers of any kind within 12 months before the start of the study/permanent fillers
Pregnancy/breastfeeding
Previous treatment of the forehead within 6 months prior to the beginning of the study (upper third of the face) with botulinum toxin of any serotype prior to baseline

Exclusion Criteria (continued)
Known hypersensitivity to the investigational product or its excipient substances
Any medical condition that may pose an increased risk to the subject due to botulinum toxin, including diagnosed myasthenia gravis, Eaton–Lambert syndrome, amyotrophic lateral sclerosis, marked atrophy or weakness of the target muscles, or any other condition that may impair neuromuscular function or contraindicate botulinum toxin therapy
Inability to significantly reduce tension lines on the forehead, even if they are physically pulled apart
Planned use of a muscle relaxant within 2 weeks prior to or during the study
Pronounced asymmetry of the face or ptosis of the eyelids and of the eyebrows or current paralysis or neuromuscular connection disorders as assessed by the investigator
Use of prohibited drugs, including anticholinergics or drugs that could impair neuromuscular function, including aminoglycoside antibiotics and curare-like compounds
Use of concomitant medication: lincosamides (lincomycin, clindamycin, or pirlimycin), polymyxins (polymyxin B and E, the latter also known as colistin), spectinomycin, (aminocyclitol), and cholinesterase inhibitors rivastigmine, donepezil, galantamine, pyridostigmine, or neostigmine
Previous autoimmune disease and immunosuppressants, allergies to the botulinum toxins and its experts, previous trauma (< 6 months) in the previous 3 months, or muscular diseases diagnosed

Table 2 | Pharmaceutical Characteristics of BoNTA Formulations

BoNTA formulation	Serotype	Fermentation (strain C. *botulinum*)	MW of purified product	Excipients per 100-unit vial	Stabilization method
LetibotulinumtoxinA	Type A	CBFC26-strainA	900 kDa	HSA: 0.5 mg NaCl: 0.9 mg	Freeze-drying
Onabotulinumtoxin A	Type A	Hall (Allergan strain)	~900 kDa	HSA: 0.5 mg NaCl: 0.9 mg	Vacuum-dried
Abobotulinumtoxin A	Type A	Hall strain	300–500 kDa	HSA: 0.125 mg Lactose: 2.5 mg/500U	Freeze-drying

HSA human serum albumin, *MW* molecular weight

Table 2 has been adapted to present only specific data relevant to this Standard Response Letter

On day 1, the intramuscular injections were applied perpendicular to the skin surface with a 30-G needle on the midpupillary line 3 cm above the orbital rim (Fig. 1). The area of anhidrosis was assessed using the starch test, according to Minor et al. Patients were instructed not to wear makeup or any dermatological products on the morning of the test. The test was conducted as follows: briefly, the skin was cleaned with 0.9% NaCl and dried thereafter. A 2% iodine solution was then applied using a swab stick and allowed to dry. Following starch application, sweating was provoked on a mechanical treadmill. Optimal development of the dark blue coloration was awaited, prior to documentation of the anhidrotic halos using standardized digital photography (VisioFace® 1000D, Courage +Khazaka electronic GmbH, Cologne, Germany). Factors such as camera angle and

parallax, lighting conditions, and the absence of calibration markers or pixel-density control were taken into account, as they may contribute to some variability in the measurements.

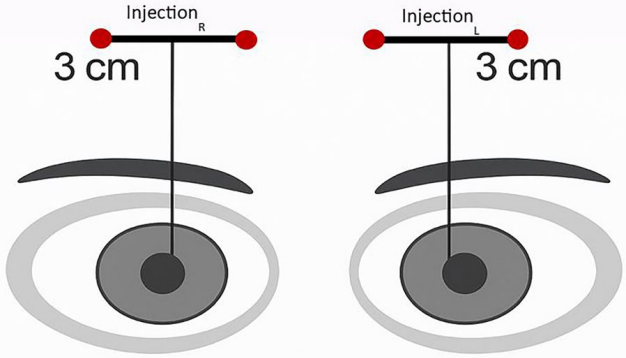

Fig. 1 Treatment injection template; adjusted from [17]

The area of anhidrosis was measured using a standardized program. The starch was removed using an alcohol swab. Spread was evaluated by analysis of the area of anhidrosis of each product. The Minor's starch test was carried out on days 4, 8, 15, 22, 29, 43, 57, 85, 113, 141, and 169.

All statistical analysis were conducted using IBM® SPSS® Version 28 (IBM Corporation, Armonk, New York, USA). The study lasted a period of 6 months (169 days). The primary outcome parameter was the maximum area of anhidrosis within 6 weeks (43 days). The secondary parameter was the area under the curve (AUC) for anhidrosis over a period of 6 months. The AUC, commonly used in clinical pharmacology to quantify overall drug exposure and compare different drugs, facilitated the comparison of the area of anhidrosis for the three BoNTA preparations over time. Safety analyses included monitoring adverse events, vital signs, physical examinations, and concomitant medications. The primary outcome parameter, the maximal area of anhidrosis within 6 weeks (43 days), was evaluated using two paired sample t-tests in the two split-face subsets. These tests compare Leti-BoNTA with Ona-BoNTA and Leti-BoNTA with Abo-BoNTA. To correct for multiple testing, $\alpha = 0.025$ was used instead of $\alpha = 0.05$. The secondary outcome, AUC for 6 months, was also assessed using two paired sample t-tests.

Results[2]:

Disposition of Subjects

Of the 35 screened subjects, 30 met the eligibility criteria on day 1 and were subsequently enrolled, as per the planned sample size of 30.

All 30 subjects were randomized and treated with study medication. As four subjects were lost to follow-up, 26 subjects belong to the safety evaluation set (SES) and the FAS. Patients in the FAS had a mean age of 35.2 years, a mean weight of 68.5 kg, and a mean BMI of 24.2 kg/m2. The subjects who completed the full 6-month study period were evenly

allocated into two groups: 13 participants received a combination of Leti-BoNTA and Ona-BoNTA (Group A), while the remaining 13 received a combination of Leti-BoNTA and Abo-BoNTA (Group B) to compare the toxin intraindividually.

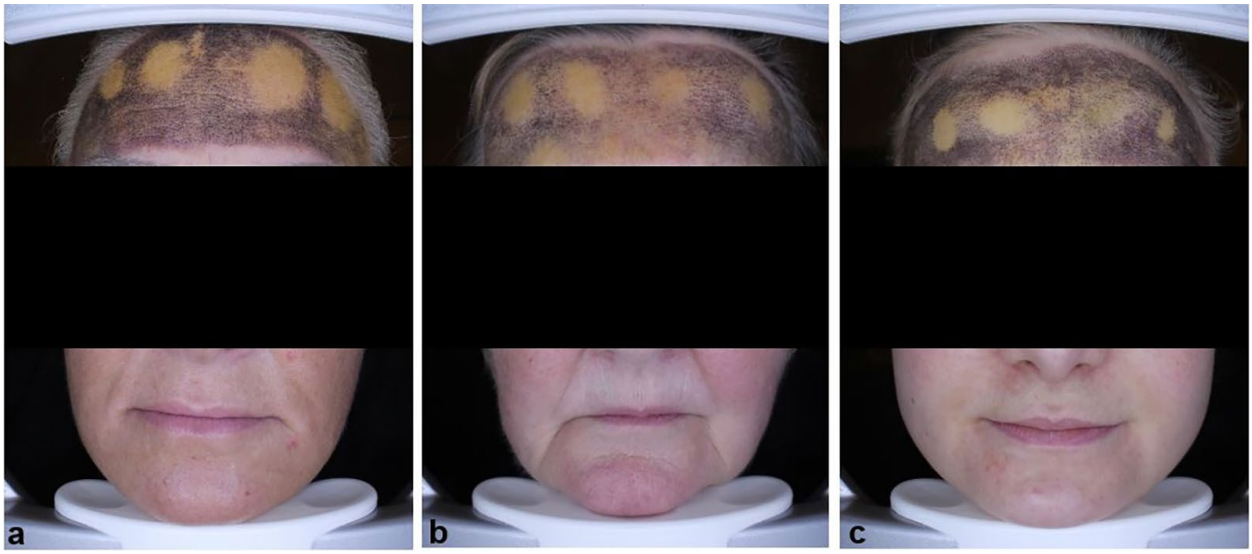

Fig. 2 Visible anhidrotic halos from three different subjects on day 43; **a** injected with letibotulinumtoxinA on the subject's right and abobotulinumtoxinA on the subject's left; **b** injected with letibotulinumtoxinA on the subject's left and onabotulinumtoxinA on the subject's right; **c** injected with letibotulinumtoxinA on the subject's left and abobotulinumtoxinA on the subject's right

Table 3 Reconstitution of Injected BoNTA Formulations

BoNTA formulation	Brand name	Total dose	Dilution
LetibotulinumtoxinA	Letybo	4 U	50 U in vial diluted in 1.25 mL of 0.9% unpreserved NaCl
OnabotulinumtoxinA	Botox	4 U	50 U in vial diluted in 1.25 mL of 0.9% unpreserved NaCl
AbobotulinumtoxinA	Dysport	10 U	500 U in vial diluted in 5.0 mL of 0.9% unpreserved NaCl

Primary Outcome Parameter Result[2]:

All patients responded to the BoNTA injection and showed a positive reaction to the Minor's test over time. (Fig. 2).

The primary outcome parameter was the maximal area of anhidrosis within 6 weeks (Fig. 3). The mean maximal areas of anhidrosis for group A were 30.7 ± 15.0 cm^2 (Leti-BoNTA) and 45.8 ± 14.7 cm^2 (Ona-BoNTA) (n = 13); for group B they were 36.2 ± 11.4 cm^2 (Leti-BoNTA) and 61.47 ± 12.9 cm^2 (Abo-BoNTA) (n = 13).

The mean difference in maximal area of anhidrosis from Leti-BoNTA to Ona-BoNTA was −15.1 ± 5.5 cm^2 (95% confidence intervals [CI]: −18.5, −11.8) and was significantly smaller ($p < 0.001$) than that of Ona-BoNTA. The mean difference in maximal area of anhidrosis from Leti-BoNTA to Abo-BoNTA was −25.2 ± 14.5 cm^2 (95% CI: −34.0, −16.5) and was significantly smaller ($p < 0.001$) than that of Abo-BoNTA.

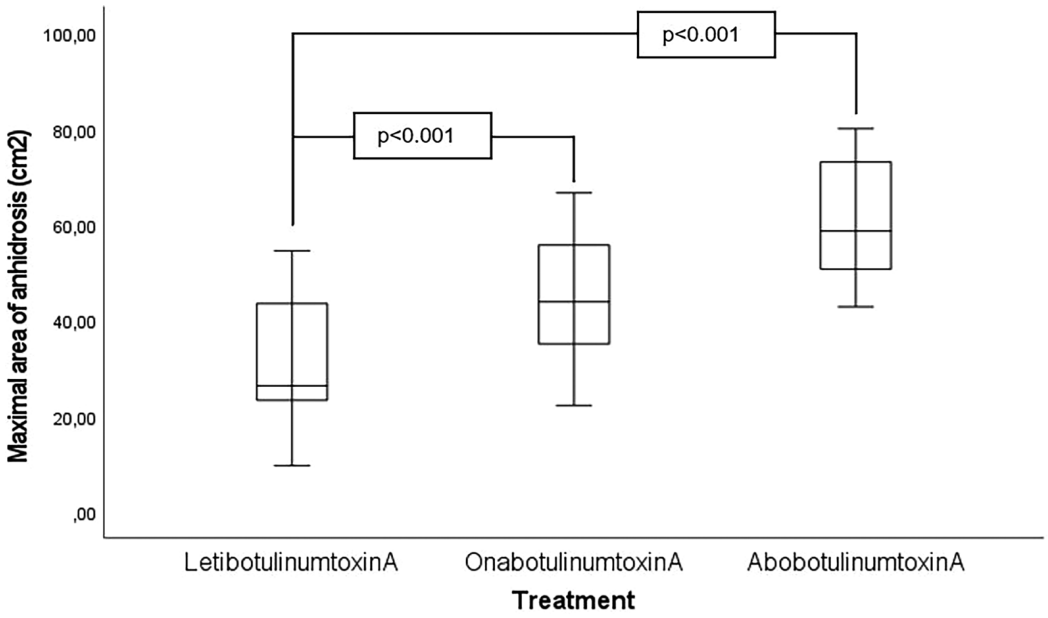

Fig. 3 The maximal area of anhidrosis of letibotulinumtoxinA, onabotulinumtoxinA, and abobotulinumtoxinA within 6 weeks

Secondary Outcome Parameter Result[2]:

A secondary outcome parameter was the AUC of anhidrosis for Leti-BoNTA, Ona-BoNTA, and Abo-BoNTA over 6 months. The difference between the mean AUC of Leti-BoNTA and that of Ona-BoNTA and Abo-BoNTA was calculated. Over 6 months, the mean difference in AUC from Leti-BoNTA was −2056.7 d cm^2 (95% CI: −2531.9, −1581.5) for Ona-BoNTA and −3172.6 d cm^2 (95% CI: −4082.0, −2263.2) for Abo-BoNTA.

The area of anhidrosis for Leti-BoNTA was significantly smaller than that of Ona-BoNTA ($p < 0.001$) and also smaller than that of Abo-BoNTA ($p < 0.001$) over 6 months. A linear mixed model was used to examine the effects of treatment, time, and their interaction on diffusion characteristics. Both treatment and time had statistically significant main effects ($p < 0.001$), indicating a meaningful relationship between these variables. In addition, a significant linear decline was observed across the overall sample (regression coefficient [RC]: −2.715; $p < 0.001$), suggesting that diffusion characteristics decreased over time.

Discussion[2]:

The study aimed to compare the efficacy and diffusion characteristics of Leti-BoNTA, Ona-BoNTA, and Abo-BoNTA in the treatment of forehead lines and wrinkle severity. The maximal area of anhidrosis was significantly larger for Ona-BoNTA and Abo-BoNTA compared with Leti-BoNTA. Specifically, the mean difference in maximal anhidrotic area from Leti-BoNTA to Ona-BoNTA was −15.1 ± 5.5 cm^2 and to Abo-BoNTA was −25.2 ± 14.5 cm^2, both reaching statistical significance ($p < 0.001$). This suggests a lower diffusion capacity under standardized conditions, which may translate into a more confined therapeutic effect. Such a profile could be particularly relevant in anatomical regions where precise localization is desired to reduce the risk of diffusion-related adverse effects. At the same time, it is important to note that more limited diffusion may also affect the extent of muscle coverage, particularly in broader treatment areas.

The AUC analysis over 6 months revealed significant differences in the accumulated anhidrotic effects. The mean AUC differences from Leti-BoNTA were −2056.7 $d \cdot cm^2$ for Ona-BoNTA and −3172.6 $d \cdot cm^2$ for Abo-BoNTA ($p < 0.001$). This indicates that Abo-BoNTA results in the largest overall anhidrotic effect, followed by Ona-BoNTA, whereas Leti-BoNTA demonstrates the least overall anhidrotic effect. These results align with previous studies indicating that Abo-BoNTA has a higher diffusion potential compared with Ona-BoNTA. As Leti-BoNTA has been reported to be noninferior to Ona-BoNTA, these findings further support the hypothesis that Leti-BoNTA follows a similar trend.

This study shows that Leti-BoNTA is a precise and targeted toxin with predictable diffusion properties, making it a viable option for applications where controlled diffusion is essential—without being limited solely to wrinkle reduction.

Conclusions[2]:

Overall, the findings demonstrate that all three botulinum toxin formulations effectively induce anhidrosis. These results suggest that the choice of formulation should be tailored to the desired clinical outcome—particularly in applications where predictable diffusion with effective wrinkle reduction are essential. Conversely, this also implies that injection points should be selected according to individual diffusion profiles to achieve optimal clinical outcomes and high responder rates.

Approved Indication for Letybo®

Letybo® (letibotulinumtoxinA-wlbg) is an acetylcholine release inhibitor and a neuromuscular blocking agent indicated for the temporary improvement in the appearance of moderate to severe glabellar lines associated with corrugator and/or procerus muscle activity in adult patient[1].

Important Safety Information:

WARNING: DISTANT SPREAD OF TOXIN EFFECT

Postmarketing safety data from other approved botulinum toxins suggest that botulinum toxin effects may be observed beyond the site of local injection. The symptoms are consistent with the mechanism of action of botulinum toxin and may include asthenia, generalized muscle weakness, diplopia, ptosis, dysphagia, dysphonia, dysarthria, urinary incontinence, blurred vision and breathing difficulties. These symptoms have been reported hours to weeks after injection. Swallowing and breathing difficulties can be life threatening and there have been reports of death related to spread of toxin effects. In unapproved uses and approved indications, symptoms consistent with spread of toxin effects have been reported at doses comparable to or lower than the maximum recommended total dose. LETYBO is not approved for any conditions other than glabellar lines. Patients or caregivers should be advised to seek immediate medical care if swallowing, speech or respiratory difficulties occur.

Letybo is contraindicated in individuals with known hypersensitivity to any botulinum toxin preparation or to any of the components in the LETYBO formulation and/or have an infection at the injection site.

The potency Units of Letybo® are specific to the preparation and assay method utilized. Letybo® is not equivalent to other preparations of botulinum toxin products, and therefore, Units of biological activity of Letybo® cannot be compared to nor converted into Units of any other botulinum toxin products assessed with any other specific assay method.

This response contains information that is not included in the approved Product Information label. Hugel Inc. and BENEV Inc. do not endorse the use of its products in a manner not consistent with the approved label. For approved products, please refer to the full Prescribing Information for additional information. The Prescribing Information label for Letybo® is available at: bit.ly/3UbRZtP