EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

25 July 2019
EMA/281737/2019
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Nuceiva

Common name: botulinum toxin type A

Procedure No. EMEA/H/C/004587/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

Quality Assessment

$A_{260/278}$	Absorbance at 260/278nm
A_{278}	Absorbance at 278nm
A_{420}	Absorbance at 420 nm
AA	Amino acid
AEX	Anion exchange
ANOVA	Analysis of variance
AU	Absorbance units
AUC	Analytical ultracentrifugation
BET	Bacterial endotoxin
BoNTA	Botulinum neurotoxin A
CBA	Colombia blood agar
CBPA	Cell-Based Potency Assay
CCC	Central composite circumscribed
CCI	Container closure integrity
CD	Circular dichroism
CFU	Colony-forming unit
CHMP	Committee for Medicinal Products for Human Use
CMAP	Compound muscle action potential
CPP	Critical process parameter
CQA	Critical quality attribute
CV	Column volume
Cys	Cystine
DNA	Deoxyribonucleic acid
DNAse I	Deoxyribonuclease I
DOE	Design of experiments
DP	Drug product
DS	Drug substance
EC	European Commission
ELISA	Enzyme linked immunosorbent assay
EM	Electron microscopy
EMA	European Medicines Agency

EU	Endotoxin Unit
EYA	Egg yolk agar
FDA	Food & Drug Administration
FFD	Full factorial design
FPLC	Fast Performance Liquid Chromatography
GMP	Good manufacturing practice
HA	Haemagglutinin
HAS	Human serum albumin
HCP	Host cell proteins
HDPE	High density polyethylene
HPLC	High performance liquid chromatography
HSA	Human serum albumin
ICH	International Conference on Harmonisation
IEF	Isoelectric focusing
IPC	In-process control
kDA	kilodalton
LC MS/MS	Liquid chromatography mass spectrometry
LD_{50}	Lethal dose 50%
MALDI-TOF-MS	Matrix assisted laser desorption/ionisation - time of flight - mass spectrometry
MALS	Multi angle light scattering
MCB	Master cell bank
MES	2-N-Morpholino ethanesulfonic acid
Mg	Milligram
mL	Millilitre
MO	Major objection
n/a	Not applicable
NCPP	Non-critical process parameter
NLT	Not less than
NMT	Not more than
No.	number
NOR	Normal operating range
NTNH	Non-toxic, non-haemagglutinin
OC	Other concern

OD	Optical density
OOS	Out of specification
OQ	Operational qualification
PAR	Proven acceptable range
PC	Process characterisation
PCR	Polymerase chain reaction
PDB	Protein database
Ph. Eur.	European Pharmacopoeia
pI	Isoelectric point
PMF	Plasma Master File
PO_2	Partial pressure of oxygen
PP	polypropylene
PPQ	Process performance qualification
PQ	Process qualification
PV	Process validation
QbD	Quality by design
QC	Quality control
QTPP	Quality target product profile
RA	Risk assessment
RABS	Restricted access barrier system
RNA	Ribonucleic acid
RNAse A	Ribonuclease A
RP-HPLC	Reverse phase high performance liquid chromatography
rRNA	Ribosomal RNA
RSD	Relative standard deviation
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SE	Sedimentation equilibrium
SEC-HPLC	Size exclusion chromatography high performance liquid chromatography
SV	Sedimentation velocity
TAMC	Total aerobic microbial count
TSA	Trypticase soy agar
TYMC	Total yeasts and moulds count
TYMC	Total yeast/ mold count

U	Units
USP	United States Pharmacopoeia
UV	Ultraviolet
UV/Vis	Ultraviolet/visible
WCB	Working cell bank
WC-CPP	Well controlled CPP
WFI	Water for injection

Clinical Assessment

ACh	Acetylcholine
ADA	Anti Botulinum toxin A Antibodies
ADR	Adverse Drug Reaction
CI	Confidence Interval
CMH	Cochran-Mantel-Haenszel
BLA	Biologic License Application
CDER	Center for Drug Evaluation and Research
DBPC	Double blind placebo controlled
DWP-450	Botulinum Toxin type A injection (Nuceiva)
EDC	Electronic Data Capture
EOS	End of Study
ET	Early termination of study
GAIS	Global Aesthetic Improvement Scale
GL	Glabellar lines
GLS	Glabellar Lines Scale
IA	Investigator Assessment
ITT	Intent to Treat
MAS	Modified Ashworth Score
mITT	Modified Intent to Treat
Nabs	Neutralising Antibodies
NMJ	Neuromuscular junction
PP	Per Protocol
PRO	Patient-Related Outcome
SA	Subject Assessment
SAE	Serious Adverse Event
SAS PROC	Statistical Analysis System Institute Procedure
SSS	Subject Satisfaction Scale
ULS	Upper limb spasticity

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Evolus Pharma Limited submitted on 22 June 2017 an application for marketing authorisation to the European Medicines Agency (EMA) for Nuceiva, through the centralised procedure under Article 3 (2) (a) of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 15 September 2016.

The applicant applied for the following indication:

When the severity of the following facial lines has an important psychological impact in adult patients, Nuceiva is indicated for the temporary improvement in the appearance of moderate to severe vertical lines between the eyebrows seen at maximum frown (glabellar lines).

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated that botulinum toxin type A was considered to be a new active substance.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0369/206 on the granting of a (product-specific) waiver.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

New active Substance status

The applicant requested the active substance botulinum toxin type A contained in the above medicinal product to be considered as a new active substance in comparison to botulinum toxin type A previously authorised in the European Union as the applicant claimed that botulinum toxin type A differs significantly in properties with regard to safety and/or efficacy from the already authorised active substance.

Scientific Advice

The applicant received Scientific Advice on 25 February 2016(EMEA/H/SA/3228/1/2015/III) for the development programme supporting the indication granted by the CHMP. The Scientific Advice pertained to the following quality, non-clinical, and clinical aspects:

- Suitability of the approach to demonstrate cell bank identity between Evolus and Reference Medicinal Product (RMP)- this was in relation to the initial submission strategy, as presented at the time of the Scientific advice procedure; to demonstrate equivalence of amino acid sequence; to demonstrate equivalence of higher order structure; to demonstrate equivalency in the 100 Unit vial and overall acceptability of the biosimilarity exercise and data package.
- Suitability of the approach of the stability data from the new manufacturing facility sufficient for initial MAA filing.
- Suitability of the nonclinical development to support MAA: specific head-to-head comparison studies of DWP-450 (Nuceiva) against the comparator.
- Acceptability of the clinical development program for the glabellar line and post stroke upper limb study: phase 3-comparator glabellar study in EU countries; phase 3-comparator glabellar study in South Korea; phase 3-comparator spasticity study in South Korea; two US phase II open label long term, repeat dose studies (EV-004, EV-006); two identical US phase III, placebo controlled single dose studies (EV-001, EV-002).
- Appropriateness of the dosing ranges sufficiently addressed.
- Whether it is acceptable to extrapolate the results of the Korean studies to all ethnic groups.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Greg Markey Co-Rapporteur: Peter Kiely

The application was received by the EMA on	22 June 2017
The procedure started on	13 July 2017
The Rapporteur's first Assessment Report was circulated to all CHMP members on	2 October 2017
The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on	29 September 2017
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	9 October 2017
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	9 November 2017
The applicant submitted a request for clock stop extension on	12 January 2018
The applicant submitted a 2nd request for clock stop extension on	22 March 2018
The applicant submitted the responses to the CHMP consolidated List of Questions on	17August 2018
The following GMP inspection(s) were requested by the CHMP and their outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product:	

– A GMP inspection at one manufacturing site in Republic of Korea between 29 January and 02 February 2018. The outcome of the inspection carried out was issued on	17 July 2018
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on	25 September 2018
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	4 October 2018
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	18 October 2018
The applicant submitted the responses to the CHMP List of Outstanding Issues on	27 February 2019
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	13 March 2019
The applicant submitted the responses to the CHMP List of Outstanding Issues on	21 March 2019
The outstanding issues were addressed by the applicant during an oral explanation before the CHMP during the meeting on	27 March 2019
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Nuceiva on	26 April 2019
Communication from the EC referring the CHMP opinion back to the Agency for further consideration received on	28 May 2019
Adoption of a revised opinion by CHMP recommending the granting a marketing authorisation for Nuceiva on	25 July 2019

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Glabellar lines at maximum frown are attributed to the (over-)activity of the underlying corrugator and procerus muscles. Botulinum toxin, Type A, is a neurotoxin produced by *Clostridium botulinum*, and is known to play a role in relaxing muscles by blocking the release of the neurotransmitter acetylcholine. Use of botulinum toxin in the treatment of upper facial lines that have an important psychological impact has been approved in the EU since 2002, and several botulinum toxin A products are currently approved for the following indication:

When the severity of the following facial lines has an important psychological impact in adult patients, Botulinum toxin is indicated for the temporary improvement in the appearance of moderate to severe vertical lines between the eyebrows seen at maximum frown (glabellar lines).

2.1.2. Epidemiology

Treatment with Botulinum toxin injections has become one of the most common non-surgical procedures performed. According to the American Society of Plastic Surgeons, in 2015, 6.7 million Botulinum toxin Type A procedures were performed in the United States of America (USA), making it the most common minimally invasive procedure. Risk factors include smoking, exposure to UV light, and ageing.

2.1.3. Biologic features

The glabellar complex comprises the bilateral corrugator supercilii and procerus muscles but other important factors contribute to the genesis of facial lines lipoatrophy, gravitational folds, and skin ageing with loss of elasticity.

Botulinum toxin, Type A, molecular weight 900 kDa, is a covalently bonded dimer of Neurotoxin (150 kDa) with a heavy chain, H, comprising Lectin-like, Neurospecific polysiayloganglioside binding and Translocation domains plus a Zn binding Metalloproteinase light chain; non-toxic, non-haemagglutinin, NTNH, protein; and haemagglutinin proteins (HA50, HA33, HA20 and HA17).

Botulinum toxin, Type A i.m. inhibits the release of acetylcholine, ACh, from presynaptic motor neurons at the neuromuscular junction, NMJ, to cause flaccid muscle paralysis. Botulinum H, binds selectively to receptors at the presynaptic surface of cholinergic neurons, inducing uptake into the nerve terminal by receptor mediated endocytosis. At low pH, the L chain is released into the cytosol, the inter-chain disulphide bond is cleaved, to form active metalloproteinase which cleaves synaptosomal associated protein, SNAP-25, an essential component of the protein complex promoting fusion of ACh vesicles with the presynaptic membrane. Enzymatic degradation of SNAP-25 blocks release of ACh from the nerve into the NMJ. Cleavage of 10–15% of SNAP-25 is sufficient to cause a complete blockade of neurotransmitter release and cleaved products may act as dominant negative inhibitors of the neuroexocytosis of ACh pending further degradation.

A characteristic feature of the flaccid neuroparalysis induced by botulinum toxin A is its complete reversibility. The duration of paralysis depends on dose, mode of administration, and type of nerve terminal. The duration of action of botulinum toxin type A is 3 – 4 months for skeletal muscle and about 1 year for autonomic cholinergic nerve terminals. Reversal occurs through the sprouting of new nerve terminals over weeks to several months followed by remodelling of the NMJ, resulting in gradual

recovery of muscle function. Clinical MRI studies show muscle volume loss 12 months after single and muscle volume loss with fat accumulation after multiple administrations of botulinum toxin type A.

2.1.4. Clinical presentation, diagnosis

Diagnosis is based on an assessment of the severity of the glabellar lines by the patients and the physician. In the EU diagnosis of a medical need for treatment with botulinum toxin A requires establishment of an important psychological impact associated with the glabellar lines.

2.1.5. Management

Treatment goals have evolved from muscle paralysis to modulation of muscle activity. Expert medical consensus (Global aesthetics consensus group, Sundaram 2016) regards as critical the following:

- A patient-tailored approach
- Integrated assessment of the face e.g. co-treatment of glabellar and lateral canthal lines
- Age appropriate treatment such as lower doses of toxin*
- Combination treatment with hyaluronic acid fillers (used by 21% of the expert consensus panel for glabellar lines, only 75% used botulinum toxin alone)

* For OnabotulinumtoxinA (Botox, Botox Cosmetic) doses of 2 to 4 U per injection point and 12 – 40 U in total are regarded as typical, but "doses as low as 8U may be appropriate for some patients", (Sundaram 2016).

About the product

Nuceiva is a powder for injection containing the drug substance Botulinum Toxin, Type A 100 units presented in a glass vial.

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as a powder for solution for injection containing 100 U of botulinum toxin A as active substance.

Other ingredients are human albumin and sodium chloride.

The product is available in Type I glass vials fitted with a chlorobutyl rubber stopper and aluminium seal.

Although this dossier is not considered a Quality by Design application, certain elements of an enhanced approached were applied. A design space was developed as part of control strategy development but is not being claimed.

2.2.2. Active substance

General information

Botulinum toxin, Type A is a 900 kDa, covalently bonded dimer of two complexes consisting of neurotoxin, NTNH (non-toxic, non-haemagglutinin) protein, and HA (haemagglutinin) proteins (HA50, HA33, HA20, and HA17), as illustrated in Figure 1 and Table 1.

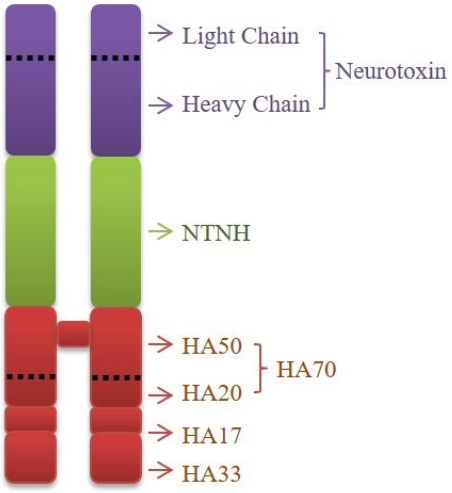


Figure 1: Theoretical Composition of Botulinum toxin, Type A (900kDa) Complex

Table 1: Composition of Botulinum toxin, Type A (900kDa) Complex

Composition of Botulinum toxin, Type A	No. of Amino Acids	Molecular Formula	Molecular Weight
Neurotoxin (Heavy chain + Light chain)	1,296	$C_{6760}H_{10447}N_{1743}O_{2010}S_{32}$	149,323
NTNH	1,193	$C_{6240}H_{9555}N_{1569}O_{1905}S_{33}$	138,093
HA70 (HA50 + HA20)	626	$C_{3200}H_{4953}N_{837}O_{999}S_{8}$	71,391
HA33	293	$C_{1515}H_{2313}N_{409}O_{468}S_{4}$	33,873
HA17	146	$C_{775}H_{1169}N_{191}O_{230}S_{6}$	17,034

Botulinum toxin, Type A, is a neurotoxin produced by *Clostridium botulinum*, and is known to play a role in relaxing muscles by blocking the release of the neurotransmitter called acetylcholine. The toxin is a two-chain polypeptide, a heavy chain joined by a bond to a light chain. The light chain is a protease enzyme that attacks fusion proteins at a neuromuscular junction, preventing the vesicles containing acetylcholine from anchoring to the pre-synaptic membrane, hence inhibiting their release. The toxin therefore interferes with nerve impulses by inhibiting the release of acetylcholine into the neuromuscular junction, causing a flaccid paralysis of muscles. The active substance has the same structural characteristics, physicochemical and biological properties as Botulinum toxin, Type A.

Manufacture, process controls and characterisation

Description of manufacturing process and process controls

The active substance is manufactured by Daewoong Pharmaceutical Co., Ltd., Republic of Korea.

The active substance manufacturing process has been adequately described. The main steps are:

- Cell Culture and Fermentation
    - Thawing of the WCB Vial
    - Seed Cultures I and II
    - Main Culture Fermentation
- Sulfuric Acid Precipitation and Harvest

- Enzyme Treatment and Toxin Extraction
- Hydrochloric Acid Precipitation and Toxin Dissolution
- Downstream and Purification Process
    - Purification Process (two steps)
    - Filling and Storage

Cells from a single thaw of an aliquot of the Working Cell Bank (WCB) are used for the seed expansion. After this cultivation, acid precipitation is used to concentrate and harvest the toxin complex from the main culture fluid. The toxin complex is then solubilized from the precipitated toxin complex in buffer and precipitated with an acid then clarified by centrifugation. After this harvest step, the toxin complex is purified and the nucleic acids are removed by chromatography.

The toxin complex is then crystallized and the neurotoxin product is separated from impurities by chromatography and subsequently is sterile filtered prior to filling and storage.

There is no pooling of sub-batches, reworking or reprocessing described in the dossier.

A batch consists of purified Botulinum Toxin, Type A from a single fermentation run and the entire content of the bioreactor is purified.

The ranges of critical process parameters and the routine in-process controls along with acceptance criteria are described for each step. The active substance manufacturing process is considered acceptable. The process has been sufficiently described and in-process controls are adequately set to control the process.

The active substance is stored in ready-to-use, sterile, 1.8 ml polypropylene (PP) tubes with high density polyethylene (HDPE) screw cap. The materials of the active substance container closure system meet the requirements of the European Pharmacopoeia (Ph. Eur.)

The active substance is transferred from the active substance manufacturing facility to the finished product manufacturing facility for processing to finished product. The shipping conditions have been validated.

Control of materials

Sufficient information on raw materials used in the active substance manufacturing process has been submitted. Compendial raw materials are tested in accordance with the corresponding monograph, while specifications (including test methods) for non-compendial raw materials are presented. No human or animal derived materials are used in the active substance manufacturing process and acceptable documents have been provided for raw materials of biological origin used in the establishment of cell substrate.

A two tiered cell banking system in accordance with the recommendations in International Conference of Harmonization (ICH) Q5D is used and sufficient information is provided regarding testing of Master Cell Bank (MCB) and WCB and release of future WCBs. Genetic stability has been demonstrated for cells at and beyond the limit of cell age. A protocol for establishment of future WCB has been provided.

The MCB was manufactured at Daewoong Pharmaceutical Co., Ltd. using the *Clostridium botulinum* bacterial strain. The MCB was tested for identity, purity, and contamination according to ICH Q5D. As *Clostridium botulinum* is an unsuitable host for infectious viral agents viral safety tests have not been performed.

Control of critical steps and intermediates

Process characterisation was carried out to identify Critical Process Parameters (CPPs) and acceptance ranges and to further develop process knowledge. Risk assessments were performed to identify process variables for evaluation and relevant response factors for given manufacturing process steps. A quality by design (QbD) concept was introduced during process development in order to define the relationship between Critical Quality Attributes (CQAs) and CPPs and to define the associated acceptance ranges for the CPPs.

The process parameters and in-process controls in place for routine control of the manufacturing process are adequately described in the dossier. The definition of the overall control strategy has been sufficiently justified and it was supported by Design of Experiments (DoE) studies. CPPs and their respective acceptance criteria have been identified and the rationale substantiating the proposed control strategy has been presented. A CPP is defined as a process parameter whose variability has an impact on a critical quality attribute and therefore should be monitored or controlled to ensure the process produces the desired quality. The assessment of risk is based on a combination of factors that include equipment design considerations, process control capability and complexity, the size and reliability of the design space, ability to detect/measure a parameter deviation, etc. Non-Critical Process Parameters (NCPPs) are defined as adjustable parameters (variable) of the process that does not have a meaningful effect on product quality or process performance.

The approach taken to define Normal Operating Ranges (NORs) is accepted as being not standard. The NORs/Proven Acceptable Ranges (PARs)/acceptance ranges were established on the basis of simulations or edge of failure experiments. The term NOR is used to define acceptable operating ranges within the defined PARs. A design space is not claimed. The term "NOR" retained in the dossier is acceptable based on the confirmation that NORs will only be varied in a univariate manner. All NORs fall within their final acceptable respective PARs. The PARs are in line with the characterised ranges established at Design of Experiments (DoE) and with additional data where necessary. The NORs will be applied in routine operation and PARs will only be used as part of a careful quality assessment in the event of an out of specification (NOR).

A comprehensive overview of critical in-process controls and critical in-process tests performed throughout the active substance manufacturing process is given. Acceptable information has been provided on the control system in place to monitor and control the active substance manufacturing process with regard to critical, as well as non-critical operational parameters and in-process tests. Actions taken if limits are exceeded are specified.

Process validation

The active substance manufacturing process has been validated adequately. Consistency in production has been shown on three full scale commercial batches. All acceptance criteria for the critical operational parameters and likewise acceptance criteria for the in-process tests are fulfilled demonstrating that the purification process consistently produces the active substance of reproducible quality that complies with the predetermined specification and in-process acceptance criteria.

Extractables/leachables for the primary packaging container have been adequately addressed.

Manufacturing process development

Minor changes were introduced during development of the active substance manufacturing process for process optimization. No major changes affecting the quality, safety and efficacy of Botulinum toxin, Type A have been implemented in the manufacturing process since it was validated. The same process was used to manufacture the batches of active substance that were used in the manufacture of finished product batches that were evaluated in the non-clinical studies and clinical studies. The comparability of the active substance has been verified and sufficiently justified.

Characterisation

The active substance was extensively characterized for structural, physicochemical, and functional comparability with the wild type toxin.

The structure and composition were determined using a number of analyses.

Amino acid composition was carried out by analysis of the N-terminal amino acid structure which was compared to the N-terminal amino acid sequence of Botulinum toxin, Type A reported in the literature and found to be identical.

Primary structure was confirmed using amino acid sequence analysis by LC-MS/MS, *i*dentification of free-thiol groups by LC-MS/MS, position and maintenance of disulfide bridges under both non-reducing and reducing conditions by SDS-PAGE analysis and LC-MS/MS.

Secondary structure prediction program was used to predict the secondary structure of 7 subunits based on known amino acid sequence of Botulinum toxin, Type A. The amino acid secondary structure of the active substance subunits was compared with reference structures and found to be consistent.

Circular dichroism (CD) spectroscopy was used to examine the folding/unfolding nature of the protein and assess secondary and tertiary structures of the protein.

Tertiary structure was confirmed by cryo-electron microscopy by capturing the microscopic image of the active substance and examining its surface structure.

The result obtained from the active substance showed tertiary structures in which 2 of the 3 arms were resolved and shown to be consistent with the published literature.

Tertiary structure was further confirmed using a light scattering machine connected to Fast Performance Liquid Chromatography (FPLC) was used to examine the molecular weight and polydispersity. Size-exclusion chromatography was conducted by enabling the fractionated sample to pass through Multi Angle Light Scattering (MALS) equipment in order to measure the absolute molecular weight. The purity of the active substance was confirmed and its sedimentation activity was associated with misfolded chains and oligomeric state in the solution.

The average molecular weight of 3 batches of the active substance was confirmed to be 901.4 kDa and the average polydispersity value of 3 batches of the active substance was confirmed. Accordingly, the measured molecular weight of 3 batches of the active substance was confirmed to be no different than the theoretical molecular weight obtained from amino acid sequence that is approximately 900 kDa.

The active substance protein in the crystal form was analyzed by X-ray diffraction.

Physiochemical properties examined were: UV Spectrum, electrophoretic pattern, molecular weight or size by mass spectrometry and patterns and purity of the active substance by size-exclusion high performance liquid chromatography.

Immunological characterization was analyzed using a western blot technique. Biological activity for the active substance was evaluated through *in vivo* activity using a mouse LD_{50} test.

Toxic Complex mass and protein aggregate levels of the active substance were analysed using an analytical ultracentrifuge technique. Based on the analytical results the molecular mass of the active substance was estimated to be 900 kDa. The results indicated that sedimentation equilibrium exists in the dimer, as illustrated in Figure 3.

Botulinum Toxin, Type A is composed of dimer structures consisting of neurotoxin, NTNH (non-toxic, non-haemagglutinin) protein covalently bonded with HA (haemagglutinin) proteins (HA50, HA33, HA20, and HA17).

The cleavage of covalent bonds in the dimer structures results in the molecular weight of the monomer structures of approximately 500 kDa consisting of neurotoxin, NTNH proteins and HA proteins (HA50, HA33, HA20, and HA17). The monomer structure of approximate 300 kDa consists of neurotoxin and NTNH proteins.

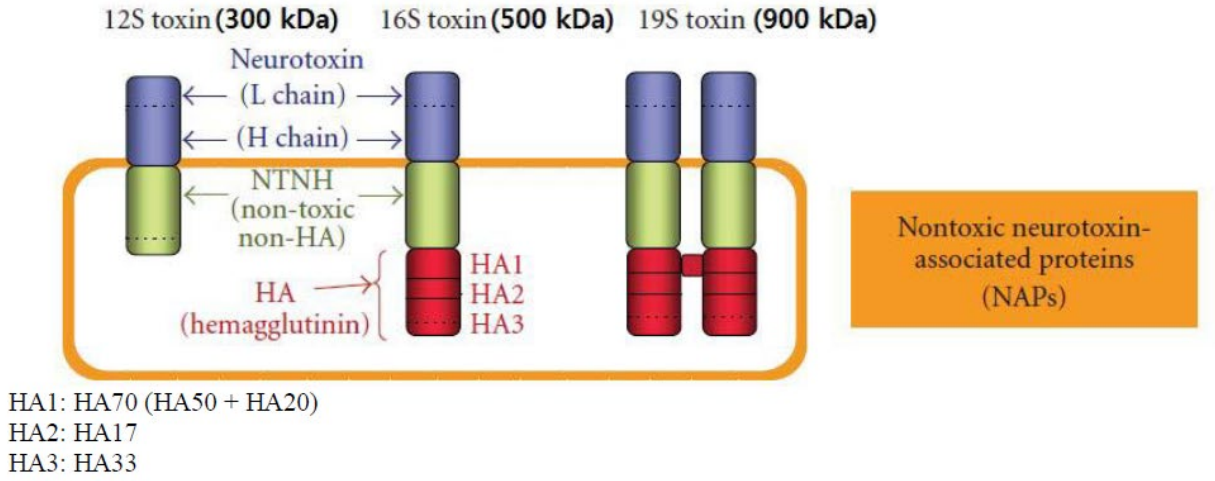


Figure 2: Structure of Botulinum Toxin Type A

The active substance has been appropriately characterised in relation to structural, physicochemical and functional comparability against theoretically expected values for a Botulinum toxin, Type A. An impurities characterisation study was also carried out to evaluate the product and process related impurities relevant for the active substance. The data presented relates to characterisation studies performed on a number of batches manufactured from the MCB between 2011 and 2014. Extended characterisation, including an evaluation of impurity profile, has been provided for WCB derived active substance in order to verify the comparability of the active substance from the MCB and the active substance derived from the WCB.

The product related impurities observed during the manufacturing stages have been identified and characterised using chromatographic and mass spectrometric tools.

The depletion of process related impurities during routine manufacture was also evaluated and a high level of clearance demonstrated. The methods used to determine clearance of process related impurities, have been confirmed as being suitable for their intended purpose. The potential for host cell proteins as a process related impurity has been discussed. SDS-PAGE analysis demonstrates that background host cell proteins (HCPs) are present in samples from the main culture, as expected, but that they are progressively removed through the process steps. While SDS-PAGE is not the most sensitive of methods for the detection of HCPs, the reference to compliance of the active substance with the Ph. Eur. monograph is noted and taking into account the toxin content per drug product vial the potential for HCPs to represent a relevant impurity is negligible.

Specification

The parameters for controlling the quality of the active substance are derived by taking into consideration the Ph. Eur. monograph for Botulinum toxin, Type A for Injection.

These tests and specifications are applied for release of each batch of active substance and also for stability testing of the active substance.

The specifications for control of the active substance are based on pharmacopoeial standards (Ph. Eur. monograph Botulinum toxin, Type A), solution properties, manufacturing process capabilities and

comparison to several reference product batches. The specifications are appropriate for control of this type of the active substance.

The neurotoxin content in terms of protein should ideally be measured by a product-specific stability indicating analytical method that reflects the current state of the art. This assay should also be included in the stability testing and in the control of the reference standards. The general protein content assay is however acceptable at this time but a recommendation is made that a suitable stability indicating analytical method that measures the Botulinum toxin type A (BoNTA) specific protein content of the active substance and finished product should be developed and implemented during the post-marketing lifecycle of the finished product.

It is also recommended to monitor active substance batches for any trend in aggregate levels. Depending on the outcome of the trend analysis in further batches the specification for aggregates could be removed or tightened, as appropriate, by way of variation.

The CHMP raised ethical concerns regarding routine control of biological activity by means of the LD50 assay. While this method is currently described in Ph. Eur., as the reference method for the potency assay of Botulinum toxin A, the monograph also explicitly recommends Applicants to develop alternative methods to be validated against this LD_{50} reference method, in accordance with the principles of Directive 2010/63/EU on the protection of animals used for scientific purposes.

As a post authorisation measure, the cell-based potency assay (CBPA) should be registered by way of variation with appropriate supportive documentation. In conclusion, in accordance with Directive 2010/63/EU on the protection of animals used for scientific purposes an appropriate *in vitro* potency assay should be developed and validated in order to reduce or replace the use of animals for the purpose of routine release of the finished product. Anticipated implementation is September 2020. This point is included as condition to the marketing authorisation of Nuceiva.

Analytical methods

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with ICH guidelines.

Batch analysis

Batch analysis data is provided for 19 commercial scale batches. The results are within the specifications and confirm consistency of the manufacturing process.

Reference materials

A two-tiered reference standard system is in use and information is provided in relation to a range of primary and working reference standards. The primary reference standard for the active substance is used to qualify subsequent primary reference standards and the active substance working reference standards which are used routinely for release of batches.

Stability

The stability results indicate that the active substance is sufficiently stable and justify the proposed shelf life in the proposed container.

Data is provided at the long-term storage condition; 36 months data is available for 3 batches of the active substance. All acceptance criteria are met.

The proposed shelf life of 36 months when stored is accepted.

The parameters tested are the same as for release.

2.2.3. Finished medicinal product

Description of the product and Pharmaceutical development

The finished product is a sterile, white to yellowish, preservative free, vacuum dried powder containing 100 units of Botulinum Toxin, Type A (active substance), 0.5 mg of human serum albumin (stabilizing agent), and 0.9 mg of sodium chloride (isotonic agent).

The powder is reconstituted with commercially available, preservative free, 0.9% sodium chloride compliant with the United States Pharmacopeia (USP) and/or European Pharmacopoeia (Ph. Eur.) to form a clear, transparent solution as per the directions for use.

The qualitative and quantitative composition of the finished product is summarized in Table 3.

The primary packaging is a type I glass vial fitted with a chlorobutyl rubber stopper and aluminium seal. The materials comply with Ph. Eur. and EC requirements. The primary glass vial container and rubber stopper are supported by extractable studies from the supplier. Container closure integrity studies and microbial challenge studies also support the use of the selected closure system. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

During the procedure CHMP raised concerns regarding the 100 U presentation, which is not considered to be in line with the maximum dose to be administered associated with this submission (20 U). The intent to extend the indications to potentially include lateral canthal lines and forehead lines utilizing a higher dose up to 65 U has been indicated and would support the use of the 100 U vial presentation in the future. The immediate concern is being addressed with the development and qualification of a more appropriate 50 U vial presentation for which a timeline for key deliverables up until its introduction has been provided. The introduction of a new vial size is expected within three months from the Commission Decision. The complexities associated with manufacturing a low dose vial due to low active substance content were presented by the applicant and acknowledged by CHMP. In order to mitigate the risk of incorrect administration the applicant has proposed warnings in the SPC section 4.3, 4.4 and 6.6. The wording proposed is considered to be acceptable to flag for healthcare professionals that each vial is to be used for a single patient only and that any unused material must be discarded. Incorrect administration due to the 100 U vial was identified as safety concern in the risk management plan (see section 2.7 of this Report). CHMP requested the applicant to submit the line extension application for the 50 U vial accompanied by appropriate supporting documentation within three months form the Commission decision. This point is further discussed later in this report.

Comparability of the clinical and commercial batches has been satisfactorily demonstrated using batch release data, comparative stability data and comparative in-process controls (IPC) data.

All excipients comply with the requirements of the Ph. Eur. Human serum albumin (HSA) is manufactured from plasma which is supported by a Plasma Master File. HSA is used as a stabiliser to reduce binding of the active substance botulinum toxin to the container surface. Confirmation that the shelf-life of albumin will be synchronised with the expiry date of the medical product in accordance with EMA/CHMP/BWP/706271/2010 is provided.

Selected quality by design (QbD) studies have been conducted by the finished product manufacturer in the current manufacturing facility at the proposed commercial batch size. Those studies ascertained the relationship between critical quality attributes (CQAs) and critical process parameters (CPPs) and define acceptable ranges for CPPs during the following key steps of the finished product manufacturing process (i.e. final bulk preparation process, filtration, immediate storage, filling processes and vacuum drying).

A Design Space has not been claimed, however design space studies have been submitted in support of the process parameter ranges for formulation and vacuum drying. The control strategy for the finished product during lifecycle has been addressed.

Normal operating ranges (NORs) and proven acceptable ranges (PARs) have been defined for the CPPs, and these ranges have generally been adequately justified from the data provided.

In-use studies with the proposed diluent (0.9% sterile saline solution) were performed and demonstrated physico-chemical stability up to 72 hours. As the final formulation does not contain a preservative, from a microbiological perspective in-use storage should be restricted to no greater than 24 hours when stored at 2-8ºC.

Manufacture of the product and process controls

The finished product is manufactured at Daewoong Pharmaceutical Co., Ltd., Republic of Korea and released on the market at Millmount Healthcare Ltd, Ireland or Biotec Services International Ltd, United Kingdom.

The finished product manufacturing process involves preparation and release of materials, formulation, sterile filtration, aseptic filling, vacuum drying, capping (sealing), and packaging for final visual inspection and storage of the vials at refrigerated temperatures. The manufacturing process is a standard aseptic process. Final bulk can be held after formulation and prior to filtration in the agitation container. Filtered final bulk can be held after the second filtration in the intermediate storage bag. The excipients added to the active substance include human serum albumin (HSA), sodium chloride (NaCl) and water for injection (WFI).

In-process controls

The finished product is manufactured by adding the active substance to an excipient solution consisting of 0.9% sodium chloride (NaCl) and 0.5% human serum albumin (HSA) in water for injection (WFI). The finished product is manufactured under standard aseptic manufacturing procedures, and is controlled by in-process controls (IPCs). There are no intermediates in the manufacturing process.

The finished product manufacturing process has been validated using 3 consecutive commercial scale batches processed at Daewoong Pharmaceutical Co., Ltd.'s site using the same process and equipment as intended for commercial use.

The process validation batches were produced using the validated aseptic manufacturing process in full compliance with cGMP procedures.

The aseptic process used for sterilization has been validated by media fills. The process will continuously be re-validated approximately every 6 months.

The sterilization and validation information confirms the validation of the manufacturing process and qualification of equipment for manufacturing of the finished product.

The manufacturing process has been validated. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate.

Product specification

The finished product specifications include tests for: appearance pre-reconstitution and post-reconstitution (visual inspection), identity (ELISA), potency (Ph. Eur.), total protein (Ph. Eur.), neurotoxin content (ELISA), particulate contamination (Ph. Eur.), sub-visible particles (Ph. Eur.), pH

(Ph. Eur.), water determination (Ph. Eur.), solubility (in house), sterility (Ph. Eur.), bacterial endotoxins (Ph. Eur.).

Justifications for the release and end of shelf life specifications for the finished product are based on pharmacopoeial standards, solution properties, device functionality criteria, manufacturing process capabilities and comparisons to and systematic analysis of several reference product batches. Limits are set based on data collected and monitored with the generation of additional stability data and may be modified, as required.

The potential presence of elemental impurities in the finished product has been assessed on a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities. Based on the risk assessment it can be concluded that it is not necessary to include any elemental impurity controls in the finished product specification. The information on the control of elemental impurities is satisfactory.

See the discussion above in the active substance regarding the potency assay.

Analytical methods

The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with ICH guidelines.

Batch analysis

Batch analysis data on commercial scale batches of the finished product were provided. The results are within the specifications and confirm consistency of the manufacturing process.

Reference materials

See the discussion above in the active substance regarding the reference materials.

Stability of the product

Based on available stability data, the proposed shelf-life of 30 months when stored at 5°C ± 3°C as stated in the SmPC are acceptable.

Real time/real condition stability data of 3 pilot and 4 commercial scale batches of finished product for up to 36 months at the long-term storage condition (5°C ± 3°C) and for up to 6 months under accelerated conditions at 25°C ± 2°C/60% ± 5% relative humidity, according to ICH guidelines were provided. The batches of medicinal product are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing.

The parameters tested are the same as for release.

In addition, 2 batches were exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products. Based on results it was concluded that the product was stable for 150 hours under tested photostability conditions.

In-use stability of 3 batches were assessed at 5ºC ± 3ºC following reconstitution in sterile sodium chloride injection. The results indicated that there was no change in the appearance, pH and potency of the finished product over the course of the stability study. Based on these results it was concluded that the product was physically and chemically stable after reconstitution for 72 hours at 5ºC ± 3ºC.

A stress conditions study for 2 batches was conducted at 40ºC ± 2ºC/75 ± 5% RH for 14 and 28 days. The data shows that both batches had a decrease in potency but remained within specifications, consequently it was determined that there was no significant change during storage at 40 ºC ±

2ºC/75% ± 5% RH over the applied testing frequency for either batch. Continued testing of one batch is being conducted out to 24 weeks to further examine the trend in decreased potency.

Adventitious agents

Information on TSE/BSE risk has been provided in the dossier. Microbiological and fungal contamination risks have been adequately addressed. In relation to viral risk, as *Clostridium botulinum* is an unsuitable host for infectious viral agents, it is not necessary to perform viral safety tests on the MCB and WCB. The justification to not conduct studies to demonstrate the absence of phage contamination in the MCB and WCB is accepted. The applicant has provided the reports for the viral clearance studies on the active substance manufacturing process. Justification for small scale models used during viral clearance studies and how they remain representative of commercial scale processes is provided.

GMO

Not applicable.

2.2.4. Discussion on chemical, and pharmaceutical aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

The applicant has applied QbD principles in the development of the active substance and the finished product and their manufacturing process.

However, no design spaces were claimed for the manufacturing process of the active substance, nor for the finished product.

At the time of the CHMP opinion, there were a number of minor unresolved quality issues having no impact on the Benefit/Risk ratio of the product.

The CHMP raised ethical concerns regarding routine control of biological activity by means of the LD50 assay. While this method is currently described in Ph. Eur., as the reference method for the potency assay of Botulinum toxin A, the monograph also explicitly recommends Applicants to develop alternative methods to be validated against this LD50 reference method, in accordance with the principles of Directive 2010/63/EU on the protection of animals used for scientific purposes.

The CHMP raised concerns regarding the 100 U presentation, which is not considered to be in line with the maximum dose to be administered associated with this submission (20 U). The complexities associated with manufacturing a low dose vial due to low active substance content were presented by the applicant and acknowledged by the CHMP. Nonetheless, the feasibility of a more suitable 50 U formulation was agreed by CHMP and the applicant. The immediate concern on multiple use/incorrect use with the 100 U vial is therefore expected to be addressed with the development and qualification of a more appropriate 50 U vial presentation. A timeline for key deliverables up until the introduction of this new presentation has been provided. The applicant has committed to have completed the development of and submitted the 50 U vial line extension accompanied by appropriate supporting documentation within three months form the Commission decision.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

In accordance with Directive 2010/63/EU on the protection of animals used for scientific purposes an appropriate *in vitro* potency assay should be developed and validated in order to reduce or replace the use of animals for the purpose of routine release of the finished product, no later than September 2020. This point is included as condition to marketing authorisation.

In order to mitigate the risk of incorrect administration the applicant has proposed warnings in the SPC section 4.3, 4.4 and 6.6. The wording proposed is considered to be acceptable to flag for healthcare professionals that each vial is to be used for a single patient only and that any unused material must be discarded. Incorrect administration due to the 100 U vial was identified as a safety concern in the risk management plan (see section 2.7 of this Report). The applicant should submit the line extension application for the 50 U vial accompanied by appropriate supporting documentation within three months form the Commission decision.

Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends several points for investigation.

2.3. Non-clinical aspects

2.3.1. Introduction

The drug product, DWP-450, comprises of 100 U of Botulinum toxin, Type A (produced by Clostridium botulinum) complexed with 0.5 mg human serum albumin (HSA) and 0.9 mg sodium chloride. The final drug product is vacuum-dried and packaged in a single use, transparent 10 ml glass vial; each vial contains 100 Units Botulinum toxin, Type A and the concentration of the drug product is 40 U/ml or 4 U/0.1 ml after reconstitution with 2.5 ml of sterile non-preserved saline. It is intended for injection at a maximum clinical dose of 20 units (U) to be administered no more frequently than at 3 month intervals.

Initially, DWP-450 was developed by Daewoong Pharmaceuticals, South Korea as a product that was comparable to the marketed Botulinum toxin, Type A biologic product, Botox® (Onabotulinum toxin A, MAH Allergan, US). Commercially available Botulinum toxin, Type A products such as Botox® have an extensive history of clinical use. Therefore a literature review was conducted for some of the pharmacological, pharmacokinetic and toxicological aspects. In addition, pharmacology and toxicology studies were conducted with DWP-450 to support the Biologic License Application (BLA) submitted to the Korean Ministry of Food and Drug Safety. The initial BLA (100 U) was approved for the treatment of moderate to severe glabellar lines in November 2013. Consequently, additional non-clinical studies were conducted to evaluate the potential impact of these changes and to bridge the existing non-clinical toxicity and safety dataset to the final commercial product.

Hence, the non-clinical package comprises of data from the literature, and non-clinical studies conducted with DWP-450 Drug substance or various forms of DWP-450 Drug product. Scientific advice of a non-clinical nature was provided by UK in June 2014, Denmark in December 2015 and the CHMP in February 2016.

2.3.2. Pharmacology

Literature data suggest that the heavy chain of Botulinum toxin, Type A binds to the presynaptic motor neuron and aids internalisation, while the light chain cleaves synaptosomal-associated protein 25 (SNAP 25) in the cholinergic nerve terminal and prevents the anchor of vesicles to the membrane along with the release of acetylcholine into the synapse of peripheral neuromuscular junctions. However, no *in vitro* pharmacology studies were carried out by the applicant. The applicant demonstrated that the predicted amino acid sequences for the active substance within Botox® (Botulinum toxin, Type A) and DWP-450 were identical.

In vivo pharmacology studies were conducted in order to evaluate and compare the efficacy and potency of the DWP-450 DP to that of commercially available Botox®, and to evaluate potential changes in DWP-450 DP pharmacological characteristics which may have resulted from a change in manufacturing sites.

The principal characteristic of botulism in the conventional electromyograph is a diminished amplitude of the compound muscle action potential (cMAP) in response to a supramaximal stimulus to a nerve. Data in the literature suggest that latencies and conduction velocities are normal and thus not affected by Botulinum toxin, Type A. Hence, the applicant has provided the results of studies which evaluate the pharmacological effects of DWP-450.

In an initial study in the rat, the *in vivo* potency of DWP-450 was compared to that of Botox® [Study DWP450-REP-041]. A single intramuscular injection of DWP-450-GC-OL at 2, 4 and 8 U prior to sciatic nerve stimulation caused a significant reduction in cMAP amplitude (i.e. myoparalysis) which was evident from 3 days post-injection, reached its maximum at 1 week and continued to significantly reduce cMAP amplitude up until the 4-week timepoint (where the beginning of recovery was apparent). Intramuscular administration of Botox® at the same doses, caused reductions of cMAP amplitude of a similar magnitude. There were isolated incidences where the responses to lyophilised DWP-450 were of a different magnitude to Botox®; however, the observed differences were not consistent across the dose and time ranges studied.

A separate cMAP assay was conducted to bridge/compare DWP-450 DP-OV (vacuum-dried drug product manufactured in the original facility) to that of DWP-450-C (DWP-450 DP manufactured in the current facility) [Study EVL-MDD-16-001]. It was evident that a single intramuscular injection of DWP-450-OV or DWP-450-C at 1, 4 and 8 U prior to sciatic nerve stimulation caused a dose-related reduction in cMAP amplitude (i.e. myoparalysis) with a time course in line with that reported during the initial study. Although there was an instance at a single timepoint where the reduction in cMAP at 4 U, was not as pronounced for material manufactured in the new facility; overall, the reductions in cMAP amplitude observed with vacuum-dried drug product from the current facility (responsible for the production of the commercial product) was comparable to that observed with the original/old facility. No differences in latency were reported when comparing the two batches, an expected finding for the reason given above that Botulinum toxin, Type A does not affect latencies and conduction velocities. In response to questions, the applicant confirmed that animals under anaesthesia were maintained at 30°C, and not at a lower ambient temperature, sufficient to avoid concerns that lower temperatures may affect distal latency in nerve conduction studies. The applicant also addressed a concern raised to the effect that Botox® was included in the study as a reference standard but no comparison was made between the proposed formulation of Nuceiva intended to be marketed and Botox®. The applicant provided a statistical comparison: this did suggest some points of statistical difference but as the sample size was limited (n=4), the biological significance of this was discounted and the conclusion of assessment was that the statistical analysis requested supported that the pharmacological properties of DWP-450 appear consistent with those of Botox®. Actual effects on cMAP amplitude were provided on request.

No safety pharmacology studies have been conducted with DWP-450 DP; this is acceptable and in accordance with ICH S6 (R1) guideline. Effects to the core organ systems are expected to be the same as seen with Botox®. This includes a concern to the effect that some types of Botulinum Toxin Type A undergo retrograde axonal transport and may affect the Central Nervous System (CNS). Breathing difficulties and respiratory impairment, which can be life threatening, have been reported for Botulinum toxin, Type A. Risks to the respiratory and central nervous systems have been addressed by way of the SmPC.

No drug-drug interaction studies were carried out or identified in the literature for Botulinum toxin, Type A. As Botulinum toxin has anticholinergic effects, interactions may be expected with concomitant use of other anticholinergic drugs.

2.3.3. Pharmacokinetics

The applicant did not conduct any studies to demonstrate the pharmacokinetic profile of DWP-450 as it was considered that the development of reproducible, reliable, precise and accurate analytical methods was not feasible. This was due to the (i) complex nature of the product, (ii) sensitivity of the methods required to detect free toxin given the (low) predicted exposures and (iii) lack of reliable reference standards if concentrations of the free toxin were to be determined. Severe toxicity would be expected if there is systemic exposure at amounts where it can be detected. On the other hand, at tolerable doses, serum concentrations of DWP-450 (0.0002 ng/ml might be projected) would be far lower than currently available detection capabilities. It is noted that the appropriate analysis was therefore not performed for dose formulations utilised during the non-clinical studies and within the non-clinical overview (Module 2.4), the applicant suggests that the potency of the dose formulations was assessed (*in vivo* with the aid of the cMAP bioassay) instead.

In the absence of analytical methods, the conduct of absorption, distribution, metabolism, or excretion studies was not feasible. The applicant therefore relied upon data from the literature to describe the pharmacokinetic aspects for Botulinum Toxin, Type A.

The only relevant information in the public domain was a published study which described the distribution of 125I-labelled free/complexed Botulinum toxin, Type A following injection into the rat gastrocnemius muscle [Tang Liu et al, 2003]. Some spread of radioactivity to sites distal to the injected site was observed; significant amounts were recovered from thyroid, contralateral muscle, and skin and a higher rate of dispersal was observed for the free toxin. However, the radioactivity at the distal sites did not appear to represent intact neurotoxin associated with macromolecules such as proteins, DNA and RNA [as most of it could not be precipitated with trichloroacetic acid (TCA)]. Less than 1% of the injected dose of 125I-Botulinum toxin, Type A complex or free-125I-Botulinum toxin, Type A was measured at any time point in the sciatic nerve, lungs, kidneys, or brain, and most of the material could not be TCA precipitated. The data therefore demonstrate that almost no radioactivity was localized to the brain, which suggests that intramuscular injection administration of (free or) complexed Botulinum toxin, Type A should not be associated with significant effects on the central nervous system. Both 125I-labeled peptides and 125I-iodide arising from the metabolic breakdown of the radiolabelled free/complexed neurotoxin were primarily excreted in the urine (most were not TCA precipitable).

It is acknowledged that interpretation of the distribution of radioactivity is limited as radiolabelled TCA-precipitable proteins were not tested for toxin activity. However, despite these limitations, overall, the data suggest that following injection of complexed Botulinum toxin, Type A into rat gastrocnemius muscle, the majority of the neurotoxin remained at the site of intramuscular injection; this is

consistent with the high affinity binding of Botulinum toxin, Type A to specific receptors and expected minimal diffusion of toxin away from the injection site [Carli et al, 2009]. The low potential for dispersal from the injection site is an important clinical consideration and suggests a low risk for adverse events.

However, there was no mention in the Tang-Liu et al (2003) study of clinical manifestations of acute toxicity in animals. More recent publicly available preclinical literature suggests catalytically active Botulinum toxin, Type A can be actively transported by neurones away from the injection site (Antonucci et al., 2008). Moreover, *in vivo* rat and mouse studies have shown a decrease in cMAP amplitude and grip strength in the contralateral limb to the injection site.

In conclusion, despite the fact that the conduct of pharmacokinetic studies was not feasible and the information in the scientific literature was limited, the nature of the information presented was relevant to the proposed use and was considered to be adequate.

2.3.4. Toxicology

It is acknowledged that the non-clinical program consisted of studies by two separate companies and that several studies were needed to support the various manufacturing changes.

Toxicity studies were in line with ICH S6 (R1) in rats and dogs with all using intramuscular dosing, as intended in humans. Based on known biological properties of Botulinum toxin A, rats were considered to be the most relevant species for toxicological evaluation.

Single dose toxicity

Initially, a single-dose study was conducted, whereby DWP 450 drug substance (no HSA) up to 500 U/kg was injected into the rat gastrocnemius muscle. Mortality was observed at the maximum dose tested (5/5 males and 4/5 females), the most evident clinical signs were paralytic gait and abdominal distension on the injection side (which occurred within 1 day of injection) and a number of other clinical signs were observed including curling of the injected hind leg and prolapse of the penis. Microscopically, atrophy of the injected muscle fibres was observed at ≥50 U/kg and atrophy of the seminiferous tubules was noted at 200 U/kg.

Subsequently, a single dose study was conducted in the same species, to compare the effects of the drug product to that of Botox®. Interestingly, for both forms of Botulinum toxin, Type A, high rates of mortality were observed (90-100%) at ≥100 U/kg (doses that were not shown to be lethal during the first study, which was conducted with the drug substance at a different facility). At all doses, i.e. at ≥5 U/kg, impaired limb function (related to pharmacology of test article) and reduced food intake/bodyweight were observed. Other observations included dehydration at ≥50 U/kg and loss of righting reflex at ≥100 U/kg. Microscopic findings within the testes (unilateral degeneration/atrophy of the seminiferous tubules) were noted at ≥5 U/kg for both test articles i.e. at doses lower than that observed during the first study. The applicant has suggested that the no-observed-adverse-effect-level (NOAEL) for this study is 5 U/kg for males and 50 U/kg in females. The CHMP does not agree with the designation of this NOAEL, given the findings in males, where the observed tubular degeneration within the testes was marked as severe in 1/10 animals at 5 U/kg.

A comparative (single dose) study was also performed in dogs. Clinical observations were limited to intermittent vocalisation at 500 U/kg (for both lyophilised drug product, DWP-450-OL and Botox®) and histologically, minimum to mild inflammation was noted at the injection site at all doses administered (≥5 U/kg). The maximum tolerated dose (MTD) and the NOAEL were considered to be 500 U/kg and 200 U/kg, respectively.

Repeat dose toxicity

In repeat-dose general toxicity studies in rats, the first such study used weekly injections of DWP-450 DS into the left gastrocnemius muscle at 4, 12 and 32 U/kg for 4-weeks: this was associated with impaired left (injected) hind limb function (paralytic gait), curling of the left hind toes and abdominal distension (left side). Reductions in body weight were noted at ≥12 U/kg and decreased food consumption occurred at the maximum dose tested only. Reductions in the left (injected) and right (contralateral) gastrocnemius muscle weight along with bilateral muscular atrophy and/or inflammatory cell infiltration were noted at all doses; the microscopic findings were still apparent at the end of the 14-day recovery period. At the maximum dose tested, small right testis and small epididymis was noted and associated with atrophy of seminiferous tubules, inhibition of spermiation and increased cell debris in lumen of the epididymis. With the exception of the effects on the testes, all other effects related to the pharmacology of the toxin [Choi et al., 2007]. The testicular and epididymal effects are also likely linked to dysfunctional muscles as a result of the paralysis-inducing effect of Botulinum toxin, Type A. Hence, the NOAEL was considered to be 12 U/kg and 32 U/kg in males and females, respectively.

In a subsequent study, the effects of drug product were compared to that of Botox® at 30 and 60 U/kg in the rat. Following repeated weekly intramuscular administration for 5 weeks, mortality was observed (one female given Botox at 60 U/kg). For both DWP-450-OL and Botox® at ≥30 U/kg, clinical observations included paralytic gait of the left (injected) hind limb, left abdominal distention, and curling of the toes of the left hind limb and other intermittent observations of emaciation, weakening and/or irregular respiration). Decreased body weight and food intake decreased gastrocnemius weights with muscle fibre atrophy, and the testicular and epididymal findings were noted as observed during previous studies; these histological findings were still apparent after a 12-week recovery period. Bone changes included proliferation of the periosteum in the diaphysis and metaphysis of the thigh; and decreased trabecular bone and were the result of the impaired muscle function (these were partially reversible). The overall findings as observed were slightly less severe than those observed at comparable doses of Botox® on the basis of the observed mortality and effects on the testis. The NOAEL was determined to be <30 U/kg for males, and 60 U/kg for females, under the conditions of this study.

After acquisition of DWP-450, the applicant subsequently conducted a 4-week rat study to evaluate the effects of the drug product, DWP-450-OL at 4, 8 and 32 U/kg. Four repeated weekly intramuscular injections into the left biceps femoris muscle were well tolerated at 4 and 8 U/kg. However, the batch of lyophilised drug product evaluated appeared to have more profound effects than those documented for the (batches of drug substance and) batch of lyophilised product tested in the other repeat dose toxicity study. Administration of 32 U/kg was associated with premature mortality and clinical signs of toxicity including impaired limb function, hunched posture, splayed limbs swelling in the anogenital region, decreased hydration and decreased activity along with those previously described. Minimal-to-severe muscle atrophy in the left injection site and surrounding muscles and right (contralateral) biceps femoris was noted in males and females at ≥4 U/kg. In addition, there was unilateral degeneration / atrophy of seminiferous tubules in the testes, mild-to-severe unilateral oligospermia / germ cell debris in the epididymis and a mild decrease in secretory product in the prostate gland in males at 32 U/kg. Given the severity of these findings, the NOAEL was considered to be 8 U/kg.

The applicant conducted a further study, of longer duration in the same species, whereby drug product was administered via intramuscular injection to the left biceps femoris on 7 occasions, once a month for 6 consecutive months at 4, 8 and 16 U/kg. Mortality was evident in not only the 8 and 16 U/kg groups, but within the control group as well (n=1/16 males for each group) and the cause of death was unclear. The minimum dose of 4 U/kg was well tolerated. As observed during previous studies,

muscle atrophy and related changes were observed without significant effects on in-life parameters, organ or microscopic changes. At ≥8 U/kg, impaired limb function, muscle wasting, and abdominal swelling correlated with decreases in creatinine, small muscle weights and microscopic findings of myofibre atrophy of biceps femoris and skeletal muscles at the injection and contralateral sites; males were more affected than females. In addition to atrophy, adipocytes were increased in number and filled spaces formerly occupied by myofibres. Following the 2-month and 3-month recovery periods, at all dose levels, left side muscle atrophy was not reversible, while the contralateral right biceps femoris muscle atrophy was reversible at 4 U/kg, and was decreased in incidence and/or severity at ≥8 U/kg. However, the persistence of myofibre atrophy and increased adipocyte number in the injected muscle at ≥8 U/kg was considered adverse. Hence, the NOAEL was considered to be 4 U/kg.

More recently, the applicant conducted a further study to evaluate the effects of vacuum dried product containing HSA product (DWP-450 DP-C) at the current manufacturing facility. The findings were generally in line with those identified during previous studies. At 32 U/kg, no mortality was observed on this occasion (whereas animals were euthanised following administration of the same dose of lyophilised drug product); however, cross study comparisons are difficult. Repeated weekly injections of DWP-450 DP-C into the left biceps femoris muscle was associated with limb impairment and associated reductions in body weight and food consumption. At 32 U/kg, these effects were statistically significant and correlated with clinical signs of toxicity. The microscopic indicators of muscle atrophy observed in and around the injection site (injected left biceps femoris muscle) although pharmacologically mediated, was considered adverse at all dose levels. In addition, a local inflammatory response to the test article was noted in the tissues surrounding the left sciatic nerve at all doses. A NOAEL was therefore not identified during this study.

The applicant also performed repeated-dose studies in the dog. A dose-range finding study was conducted whereby DWP-450 drug substance was administered intramuscularly at 50, 100 and 200 U/kg, once weekly for 3 consecutive weeks. Muscle fibre atrophy (in the injected muscle) was noted as the only treatment-related finding and the maximum dose recommended for the subsequent pivotal study was said to be 200 U/kg. Interestingly, the maximum dose evaluated during the subsequent pivotal 4-week study was only 32 U/kg and the applicant failed to provide adequate justification for the dose selection; the histological findings (minimal to mild muscle fibre atrophy) were reproduced and shown to be reversible after 2 -week recovery period. Although the dose evaluated is not considered to be sufficiently high, it is noted that this is the less sensitive species evaluated and thus, no further data are requested.

Head-to head comparison single dose toxicity

Given the slight differences as observed during the repeated-dose studies observed with different batches at different test facilities, the applicant performed a head-to head comparison of single doses of DWP 450 that have been evaluated previously, along with Botox®. Following single intramuscular injection, there were no apparent toxicologically significant differences between the test articles; this could indicate that the manufacturing process change had no effect on the biological activity and toxicological profile of the toxin, but it is possible that the study is not capable of detecting such differences. In addition, neither of the DWP-450 test articles showed differences in toxicological profile to that of Botox®. The NOAEL was determined to be 8 U/kg for DWP-450 in female SD rats.

The applicant also performed a separate head to head comparison study, to compare the effects of a single dose of drug product from the original facility to product from the current facility. Intramuscular injection of both test articles into the left biceps femoris produced clinical signs that were pharmacologically mediated and similar for the groups at 4 and 8 U/kg. At the maximum dose tested, the test articles were not well tolerated and treatment was associated with adverse clinical

observations, effects on body weight and food consumption and anatomical pathology changes as described previously. At termination on Day 15 (i.e. 14 days post-dose), the applicant noted a slightly higher incidence of mild atrophy of the injection site muscle in animals dosed with DWP-450 from the current facility when compared to those dosed with DWP-450 from the original facility but came to the conclusion that the observed differences were not toxicologically significant. However, it is noted that the differences observed between the DWP 450 from the current facility were consistently different with respect to significantly lower body weights, the macroscopic observation of smaller contralateral muscle, the trend towards a lower absolute and relative muscle weight and the microscopic findings indicative of atrophy in both the injected and the contralateral muscle. The applicant addressed a question on this point and it is possible that the differences seen are not indicative of a true difference in profile of the product, but rather, the lack of effect in some animals may be due to administration errors.

Some of the later studies were conducted only in female rats: as toxicity had been seen in male rats at lower doses in earlier studies, the applicant was required to explain the choice of use of female rats only in later studies. The applicant was also asked to consider whether the toxicity seen in males could reflect systemic anticholinergic toxicity, possibly indicating biological consequences of the drug after it had distributed away from its local site of intramuscular injection. On this latter point the applicant responded to the effect that its product was not different in this respect from the profile seen with other botulinum toxin preparations and clinical evidence of concern is lacking. Thus it was concluded noting that for the indication sought and the location of the injection for glabellar lines in the face, the dose and the distance between the injection site and the groin area is large, there is no significant concern regarding diffusion for testicular effects. On the latter of use only of females, the applicant explained that the choice to use females only reflected a desire to minimise the number of animals used and the information that might have been lost by so doing was considered minimal. It might have been more relevant to consider use of males only, but the point was considered resolved as additional toxicity studies are not needed.

Finally, the applicant was asked to justify a lack of immunogenicity evaluations. Its response related that this was studied clinically and the applicant considered this to be sufficient. Animal immunogenicity is accepted as not predictive of such effects in humans but the lack of evaluation of immunogenicity in animals might have undermined the evaluation of toxicity in animals.

Genotoxicity and carcinogenicity

No data from genotoxicity or carcinogenicity studies were provided. It is acceptable given the nature of the product and is in keeping with the recommendations of the ICH S6 (R1) guideline.

Reproduction Toxicity

Data from the literature suggest that in rats, Botulinum toxin Type A (Botox) causes reduced fertility in males at 8 and 16 U/kg and in females at 16 U/kg. No effects were reported at 4 U/kg and 8 U/kg in males and females, respectively. However the potential impact of Nuceiva on fertility has not been investigated. The effect on fertility and the data are correctly reflected in the SmPC.

An embryofetal development study was conducted in which pregnant rats were given DWP-450 DS drug substance (no HSA) via intramuscular injection into the left gastrocnemius muscle at 0, 0.5, 1 and 4 U/kg, once daily from Days 6 to 15 of gestation. While some of the typical clinical signs were apparent, there were no effects on food consumption or body weight and no effects on embryofetal development were reported at 4 U/kg. Peri and post-natal studies were not conducted, which is acceptable.

2.3.5. Ecotoxicity/environmental risk assessment

In accordance with the CHMP guideline for Environmental risk assessment of medicinal products for human use [EMEA/CHMP/SWP/4447/00 corr 2], as the proposed product falls within the classification of a products containing vitamins, electrolytes, amino acids, peptides, proteins, carbohydrates and lipids as active pharmaceutical ingredient(s), an environmental risk assessment (ERA) is not required.

2.3.6. Discussion on non-clinical aspects

Overall, the approach for the pharmacology aspects was deemed acceptable. However, it is noted that *in vivo* testing was the only means by which the potency of the proposed product could be demonstrated. Development of an *in vitro* assay may have reduced the number of animals used but the use of *in vivo* methods can be accepted. The applicant provided reassurance that the assay is sufficiently robust, noting low maintenance temperature used (22 or 23 ± 3°C); in fact, the description did not make clear that animals were maintained at 30°C, and at this temperature, an effect on neuromuscular junction function was not expected to be obscured. The applicant also clarified that although effects on latency were observed sporadically, this parameter is not sensitive to effect of Botulinum Toxin Type A and was not used to provide evidence comparing Nuceiva with Botox. The applicant also provided control data on cMAP and latency generated with Botox.

No safety pharmacology studies have been conducted with DWP-450 DP; this is acceptable and in accordance with ICH S6 (R1) guideline. Clinical data suggest potential risks to the cardiovascular, respiratory and central nervous systems. This is appropriately reflected in the Product Information and, from the non-clinical perspective, the issue has been considered satisfactory.

The applicant did not conduct any studies to demonstrate the pharmacokinetic profile of DWP-450 as it was considered that the development of reproducible, reliable, precise and accurate analytical methods was not feasible. It is noted that the appropriate analysis was therefore not performed for dose formulations utilised during the non-clinical studies.

In order to bridge the bulk of the toxicology studies that were performed with drug product produced at older facilities, the applicant performed a separate head-to-head comparison study, to compare the effects of a single dose of vacuum dried drug product from the original facility (DWP-450 DP-OV) to vacuum dried product from the current facility (DWP-450 DP-C). The applicant noted a slightly higher incidence of mild atrophy of the injection site muscle in animals dosed with DWP-450 DP-C when compared to those dosed with DWP-450 DP-OV but came to the conclusion that the observed differences were not toxicologically significant. However, it is noted that the differences observed between the vacuum-dried product from the current facility were consistently different with respect to significantly lower body weights, the macroscopic observation of smaller contralateral muscle, the trend towards a lower absolute and relative muscle weight and the microscopic findings indicative of atrophy in both the injected and the contralateral muscle. It is acknowledged that the differences are small. However, comparison of a separate sensitive endpoint may have clarified whether the observed difference is likely to occur via chance. Comparison of the effect on the testes would have been a useful tool to determine whether the vacuum-dried drug product is associated with a slightly different toxicity profile and a higher propensity for adverse events in the clinic but the applicant has conducted the comparative studies in females only.

The applicant did not supply data from studies into immunogenicity, genotoxicity, carcinogenicity or reproductive toxicity. This is acceptable, the product is considered to pose no risk to the environment.

2.3.7. Conclusion on the non-clinical aspects

Overall, the application is approvable from a non-clinical point of view.

2.4. Clinical aspects

2.4.1. Introduction

Glabellar lines at maximum frown are attributed to the (over-) activity of the underlying corrugator and procerus muscles.

The applicant conducted a pivotal, European and Canadian efficacy and safety study EVB003, designed as an active treatment (Botox) and placebo controlled randomised trial of non-inferiority of a single treatment with DWP-450 for the temporary improvement in moderate to severe glabellar lines, where the adult subjects confirm their glabellar lines have an important psychological impact (on mood, anxiety and/or depressive symptoms).

There are two supporting US safety and efficacy studies, EV001, EV002, designed as placebo superiority trials of single treatment with DWP-450 for the temporary improvement in moderate to severe glabellar lines.

Two additional USA-performed safety studies, EV004, EV006, are also presented as open-label, repeat treatment studies of DWP-450 for the temporary improvement in moderate to severe glabellar lines.

GCP

The clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies

	EV-001	EV-002	EVB-003	EV-004	EV-006
Phase	US pivotal Phase III safety and efficacy	US pivotal Phase III safety and efficacy	EU pivotal Phase III safety and efficacy	US Phase II long-term safety	US Phase II long-term safety
CSR Location	• Section 5.3.5.1	• Section 5.3.5.1	• Section 5.3.5.1	• Section 5.3.5.2	• Section 5.3.5.2
Population	• healthy adults (≥18 years) who had moderate to severe glabellar lines (GLS score ≥2) at maximum frown, as independently agreed by both IA and SA	• healthy adults (≥18 years) who had moderate to severe glabellar lines (GLS score ≥2) at maximum frown, as independently agreed by both IA and SA	• healthy adults (≥18 years) who had moderate to severe glabellar lines (GLS score ≥2) at maximum frown by IA only **AND** who felt that their glabellar lines had an important psychological impact	• healthy adults (≥18 years) who had moderate to severe glabellar lines (GLS score ≥2) at maximum frown by IA only	• healthy adults (≥18 years) who had moderate to severe glabellar lines (GLS score ≥2) at maximum frown, as independently agreed by both IA and SA
Design, including duration	• multicenter • randomized (3:1) • double blind • placebo controlled • single dose • 150 days duration	• multicenter • randomized (3:1) • double blind • placebo controlled • single dose • 150 days duration	• multicenter • randomized (5:5:1) • double blind • placebo and active controlled • single dose • 150 days duration	• multicenter • non-randomized • open label • repeat dose (the initial treatment plus up to 3 repeat treatments) [a] • 365 days duration	• multicenter • non-randomized • open label • repeat dose (the initial treatment plus up to 3 repeat treatments) [a] • 365 days duration
Treatments	Single treatment of: • 20 U DWP-450 or • 0.5mL saline (Placebo)	Single treatment of: • 20 U DWP-450 or • 0.5mL saline (Placebo)	Single treatment of: • 20 U DWP-450 or • 20 U BOTOX or • 0.5mL saline (Placebo)	• 20 U DWP-450/treatment, up to a maximum of 4 treatments – i.e., 80 U total (IT, RT1, RT2 and RT3)	• 20 U DWP-450/treatment, up to a maximum of 4 treatments – i.e., 80 U total (IT, RT1, RT2 and RT3)
Number of Subjects	330 randomized (3:1): • 246 DWP-450 • 84 Placebo	324 randomized (3:1): • 246 DWP-450 • 78 Placebo	540 randomized (5:5:1): • 245 DWP-450 • 246 BOTOX • 49 Placebo	352 treated with DWP-450 • 33 received 1 treatment • 57 received 2 treatments • 108 received 3 treatments • 154 received 4 treatments	570 treated with DWP-450 • 46 received 1 treatment • 93 received 2 treatments • 217 received 3 treatments • 214 received 4 treatments
Number of Sites	• 10 US sites	• 10 US sites	• 19 sites total: 4 Canada; 5 France; 7 Germany; 2 Sweden; 1 UK	• 11 US sites	• 18 US sites
Visits	• Screening/D0 • D2, D7, D14, D30, D90, D120 and D150/ET	• Screening/D0 • D2, D7, D14, D30, D90, D120 and D150/ET	• Screening/D0 • D2, D14, D30, D90, D120 and D150/ET (no D7)	• Screening/IT D0 • IT D3, IT D7, IT D14, IT D30, IT D90 and MFVs • RT1-3 D0, D3 [b], D7, D14 [b], D30, D90 and MFVs	• Screening/IT D0 • IT D2, IT D7, IT D14, IT D30, IT D90 and MFVs • RT1-3 D0, D2 [b], D7, D14 [b], D30, D90 and MFVs
Efficacy Assessments	• GLS, by IA and SA • GAIS, by IA and SA • SSS by SA	• GLS, by IA and SA • GAIS, by IA and SA • SSS by SA	• GLS, by IA and SA • HADS-A and HADS-D by SA • GAIS, by IA and SA • SSS by SA; 7 day diary by SA	• GLS by IA • GAIS by SA • SSS by SA	• GLS, by IA and SA • GAIS, by IA and SA • SSS by SA
1° Endpoint	• yes	• yes	• yes	• no, all efficacy endpoints were exploratory	• no, all efficacy endpoints were exploratory

2.4.2. Pharmacokinetics

The lack of conventional PK studies was accepted by the CHMP during scientific advice, based on the evidence available. The justification for not carrying out clinical PK studies is based on the limitations of the available at the time assays for theoretical concentrations of 0.2 picograms/mL (200 femtograms/ml) and the absence of evidence that efficacy correlates with blood levels. It has since come to light that there are Botulinum toxin A assays in the femtogram range, including the BoNT ALISSA (Bagramyan et al., 2008; Bagramayan et al., 2013). However this assay remains in the research phase and the actual accuracy is unclear.

2.4.3. Pharmacodynamics

Similarly, no pharmacodynamics studies were conducted *in vitro* or clinically such as receptor binding or attempts to correlate pharmacodynamic effects to dose or plasma concentrations, based on the understanding that there are no sensitive analytical methods to support this.

2.4.4. Discussion on clinical pharmacology

No pharmacokinetics and pharmacodynamics studies were conducted. This was considered acceptable by the CHMP.

2.4.5. Conclusions on clinical pharmacology

Following CHMP's scientific advice in Feb 2016, EMA/CHMP/SAWP/130926/2016, accepting the absence of conventional PK studies, it has come to light that there are immunoassays available for botulinum toxin type A with a detection margin of >100 times the estimated blood concentrations. It is agreed that an appropriate assay for botulinum toxin blood levels would have helped to support the investigations of DWP-450 efficacy over safety, including the impact of Anti-Botulinum toxin A Antibodies (ADA). However the lack of pharmacokinetics and pharmacodynamics studies is acceptable.

2.5. Clinical efficacy

Clinical efficacy data are provided on >2100 healthy subjects treated for Glabellar Lines (GLs) within the Evolus programme including 540 (DWP-450 = 245) subjects recruited to the pivotal single administration active comparator and placebo controlled European study; 654 (DWP-450 = 492) subjects across the single administration placebo-controlled US studies; and 912 (DWP-450 = 912) subjects in the open-label repeat administration US studies with exploratory efficacy endpoints.

2.5.1. Dose response studies

No clinical dose response studies were performed. The same Unit dose as Botox was selected with the justification of pharmacologic and non-clinical comparability, together with clinical comparability in the Korean clinical registration studies DWP-450 for the treatment of GL with Botox as an active comparator. The 3 controlled, single dose EV-001, EV-002 and EVB-003 studies used a single treatment of DWP-450 as 5x 4U (0.1ml) i.m. doses.

A comparison of effective doses for different botulinum toxin A preparations based on experimental models is problematic and does not accommodate potential differences in drug diffusion or spreading from the injection site.

2.5.2. Main studies

Pivotal Study EVB003

A phase III, multi-centre, randomised double blind, active and placebo control, single dose trial to demonstrate the efficacy and safety of DWP-450 in adult subjects for the treatment of moderate-to-severe glabellar lines.

Methods

Subjects were screened and assessed then block randomized in a 1:5:5 ratio to receive Placebo, BOTOX® or DWP-450 in a single treatment, with follow-up on days 2, 7, 14, 30 and then every 30 days to day 150.

Study Participants

Participants were selected from healthy adults age ≥18 years

Entry criteria were moderate (GLS=2) to severe (GLS=3) glabellar lines at maximum frown as assessed by the investigator using the 4-point photonumeric GLS.

Subjects verbally confirmed that their glabellar lines had an important psychological impact (on mood, anxiety and/or depressive symptoms).

Exclusion criteria included previous treatment with any botulinum toxin in the last 6 months; planned treatment with any botulinum toxin in any other body region during the study; participation in any other interventional clinical study within 30 days; other previous permanent or planned facia aesthetic procedures; and local or systemic conditions likely to increase risks of the procedure.

Treatments

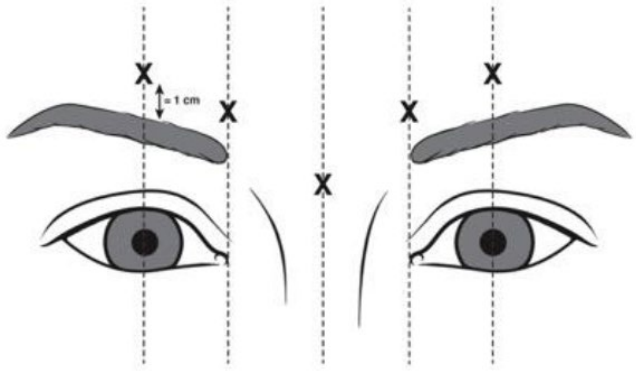
A single treatment (0.1 mL im into each of 5 sites) of DWP-450 (20 U in total, administered as 4 U/0.1 mL) or BOTOX (20 U in total, administered as 4 U/0.1 mL) or Placebo (0.9% saline) on Day 0.

Study EVB003: Investigational product

Product	Formulation
DWP-450	100U vacuum dried botulinum toxin type A, 0.5mg HAS stabiliser, 0.9mg NaCl isotonic agent
Botox (Allergan Inc)	100U botulinum toxin type A with 0.5mg HAS stabiliser, 0.9mg NaCl isotonic agent
Placebo	Empty colourless transparent vial

2.5ml 0.9% preservative free saline was added to the vial to reconstitute without shaking to a final dilution of 4U to 0.1ml. Each of 5 sites was injected with 0.1ml im using a 30G needle and a 1ml syringe with topical anaesthesia used if required. Target sites for injection were:

- Superior middle aspect of each corrugator muscle, ≥1cm above the bony orbital rim
- Inferomedial aspect of each corrugator muscle
- Midline of the procerus



Objectives

Primary objective is to show the safety and efficacy of DWP-450 in the treatment of moderate to severe glabellar lines associated with corrugator and/or procerus muscle activity in adult subjects at maximum frown.

Outcomes/endpoints

Efficacy

Primary endpoints

- The proportion of responders at day 30 by Investigator Assessment (IA), where glabellar lines at maximum frown score 0 or 1 on the GLS

Secondary endpoints

- Proportion of subjects with a GLS score of 0 or 1 on day 30 at maximum frown by Subject Assessment (SA)
- Proportion of subjects with ≥1 point in Subject Satisfaction at day 30
- Change from baseline to day 90 in HADS Anxiety score
- Change from baseline to day 90 in HADS Depression score
- Proportion of subjects with ≥1point improvement in GLS from day 0 to day 2 at maximum frown by Investigator Assessment
- Proportion of subjects with ≥1point improvement in GLS from day 0 to day 150 at maximum frown by Investigator Assessment

Exploratory Efficacy Endpoints

- Global aesthetic improvement scale (GAIS) scores by Investigator and Subject Assessment on Days 2, 14, 30, 90, 120 and 150
- Subject Satisfaction Score (SSS), Day 2, 14, 30, 90, 120 and 150
- ≥1point improvement in GLS score at maximum frown by IA on days 2, 14, 30, 90, 120, 150
- ≥2point improvement in GLS score at maximum frown by IA on days 2, 14, 30, 90, 120, 150
- 3 point improvement in GLS score at maximum frown by IA on days 2, 14, 30, 90, 120, 150
- 1, 2, 3 point improvement in GLS score at maximum frown by SA on days 2,14,30, 90, 120, 150 by IA, and likewise by SA
- Change from baseline to day 90, baseline to day 30, in HADS-A, HADS-D scores for subjects with abnormal baseline score
- Change from baseline to day 30 in HADS-A, HADS-D scores for all subjects
- Subject 7 day daily diary for onset assessment

Safety Endpoints (summarised by treatment), including:

- Exposure to treatment
- AEs including drug relates AES, serious AEs, AEs of special interest, AEs resulting in discontinuation, and AEs with an incidence of ≥5%, ≥10%
- Vital signs and laboratory tests

Assessments Methods

- Glabellar Line Scale (GLS) score at maximum frown and at rest by IA and SA.

GLS comprises 2 photonumeric scales for assessing GLs at maximum frown and at rest over a 4-point scale of 0=none, 1=mild, 2=moderate, 3=severe. The GLS was validated using 4 physicians assessing photographs of subjects at rest (33) and at maximum frown (38).

Investigators were trained and certified when achieving ≥80% in a grading test. All subjects received onsite training and a training manual for reference at each visit.

- Global Aesthetic Improvement Scale (GAIS) scores by IA and SA.

Score	Grade	Description
2	Much Improved	Marked improvement in appearance
1	Improved	Improved in appearance, but would like more
0	No Change	The appearance is essentially the same as the original condition
-1	Worse	The appearance is worse than the original condition
-2	Much Worse	The appearance is much worse that the original condition

- Subject Satisfaction Scale (SSS) by SA.

Score	Grade	Description
2	Very Satisfied	I am very satisfied with the treatment
1	Satisfied	I am satisfied with the treatment
0	Indifferent	I am indifferent with the treatment
-1	Unsatisfied	I am unsatisfied with the treatment
-2	Very Unsatisfied	I am very unsatisfied with the treatment

- Hospital Activity and Depression Scale (HADS) Score

The HADS has 14 questions each on a 0-3 scale, with 7 questions assessing each of anxiety and depression to generate HADS-Anxiety, HADS-Depression subscales where 0–7 = normal, 8–10 = mild, 11–14 = moderate, 15–21 = severe (Zigmond and Snaith 1983).

Sample size

The sample size of 540 subjects was designed for a power of 80% with a one-sided type 1 error of 2.5% to demonstrate a non-inferiority margin of 0.10 for DWP-450 versus Botox in the proportion of responders at day 30, assuming a response rate of 0.85 for DWP-450, 0.85 Botox, O.15 Placebo, AND superiority of DWP-450 versus Placebo, Botox versus Placebo with a two-sided type I error rate of 2.5% for each of the superiority tests. The initial total sample size of 497 subjects including 45 placebo subjects was increased to 540 subjects to allow for 10% missing data at day 30 - day 30 assessments missed or assessed outside the ±3day permitted window.

Randomisation

Subjects were randomised 5:5:1 ratio to receive DWP-450, BOTOX® or placebo using a blinded randomisation scheme where each subject is assigned a unique subject study number using an Interactive Voice Response System (IVRS) to provide the study site with a randomisation number. A block randomisation scheme was prepared by the CRO. Random numbers were generated in SAS using PROC PLAN. Randomisation was implemented through an Interactive Voice Response System (IVRS). No stratification was applied in the block randomisation.

Blinding (masking)

Allocation is randomised and administration of the DWP-450, active comparator and placebo will be double-blinded.

Statistical methods

Efficacy Analysis Sets

Study populations were defined as:

ITT – all subjects randomised to treatment.

PPP – all randomised subjects who received the protocol required single treatment of 5 injections of the correct drug by randomisation and had the primary outcome measure assessed at both baseline Day 0 and Day 30 $\pm$3 days.

Safety Population

- All subjects who were randomised and received treatment.

Statistical tests

Efficacy data were summarised by: number and proportion of subjects in each group with exact 95% confidence intervals (CIs); and, the difference in the proportions between groups with the 2-sided 95% level CI. Efficacy analyses were performed using the PP and ITT Populations.

Using the Per Protocol population, the tests of superiority for DWP-450 versus placebo and for BOTOX® versus placebo will be performed using unconditional exact test by inversion of two one-sided tests using standardised statistics.

Analysis of the primary efficacy endpoint

The proportion of subjects with a GLS score of 0 or 1 at maximum frown on Day 30 by IA will be tested against the primary non-inferiority hypothesis that the proportion of responders in the DWP-450 PP group is no more than 0.10 lower than that in the BOTOX® group. A two-sided 95% Wald asymptotic confidence interval for risk difference between the proportion of responders in each group (DWP-450 and Botox) will be provided . Non-inferiority of DWP-450 compared to BOTOX® was concluded if the lower bound of the 95% confidence interval of the difference in proportions is greater than -0.10. The ITT population will be used to confirm the stability of the conclusion, and missing Day 30 data imputed using a tipping point analysis. No adjustments for covariates were factored in any of the analyses. There was no stratification for study centre, prior exposure to botulinum toxin, nor ADA status.

Tests of superiority for DWP-450 versus Placebo and for BOTOX versus Placebo for the primary endpoint were performed using the unconditional exact test, by inversion of two one-sided tests using standardized statistics. A p-value <0.025 was required for each test to conclude that DWP-450 and BOTOX were each superior to Placebo.

Superiority of DWP-450 over BOTOX was to be tested last using the same 95% two-sided CI (lower bound of 95% two-sided CI was equal to the lower bound of 97.5% one-sided CI) employed for testing non-inferiority. If the lower bound was above zero, DWP-450 was demonstrated as superior to BOTOX.

The primary efficacy endpoint will also be analysed by age group ($\geq$65 vs. <65).

Analysis of Secondary Efficacy Endpoints

Secondary efficacy endpoints were tested, for all subjects in the ITT Population with non-missing assessments at the respective visits, in a closed sequential process using gatekeeping methods to

maintain the overall study Type 1 error rate of 0.05 – i.e., each endpoint was only tested if the p-value for the previous test was <0.05:

• In the case of secondary endpoints based on the GLS and SSS, the differences between groups in the proportions of responders were tested using the same exact test as the primary analysis; sequential testing was limited to testing the superiority of DWP-450 versus Placebo.

• In the case of secondary endpoints based on the HADS, the Day 90 HADS scores were compared to baseline scores within each group using the paired t-test; sequential testing was limited to testing the improvement of HADS scores from baseline to Day 90 for the DWP-450 group. Sequential testing of secondary efficacy endpoints for HADS scores was based on the mean changes in HADS-A and HADS-D scores from baseline to Day 90 for all DWP-450 subjects, not on comparisons with Placebo. P-Value was based on paired t-test.

For the comparison of DWP-450 versus Placebo, the exact confidence interval and associated test were based unconditional exact test by inversion of two-one sided tests. For DWP-450 vs Placebo and BOTOX vs Placebo, the 95% confidence interval for the mean was calculated based on two-independent t-test.

Sensitivity analyses

Missing Day 30 primary efficacy outcome data were imputed using a tipping point. This analysis consisted of determining if, for subjects with a missing Day 30 primary outcome, there was a combination of assigning a failure to those in the DWP-450 group and a success to those in the Placebo group, which resulted in a non-statistically significant test of the null hypothesis. Using the general concepts outlined by Yan et al. (2009), the additional number of responders required in the Placebo group compared to the DWP-450 group among subjects with missing data was determined to achieve a p-value of ≥0.025.

Subgroup analyses

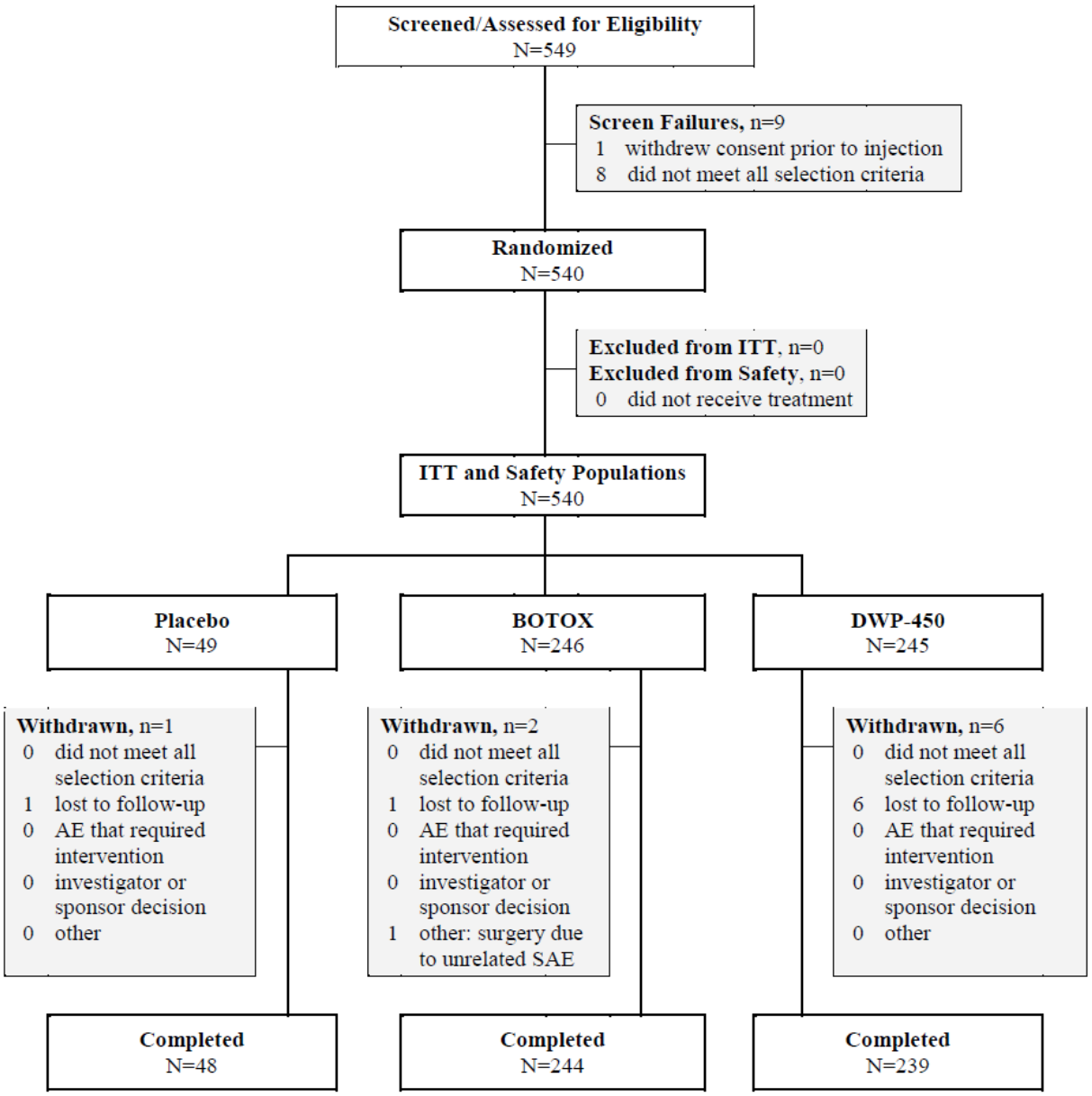
No test for homogeneity of treatment effects across study sites was otherwise performed, and no analyses were performed that controlled for differences by study site.

The primary efficacy endpoint was analysed by age groups of <65 years and ≥65 years. PP population was used.

Results

Participant flow

Study EVB-003: Summary of Participant Flow and Primary Populations for Analysis



Study EVB-003: Disposition of Subjects

Site	Treatment	All (N=540)	Completed (N=531)	Discontinued/Withdrawn (N=9)	Subject	A/S/R[a]	Day	Primary Reason for Withdrawal
ALL	Placebo	49	48	1	---	---	---	1 Lost to follow-up prior to Day 30 visit
	BOTOX	246	244	2	---	---	---	1 Lost to follow-up after Day 30 visit
								1 Other: surgery due to an unrelated SAE
	DWP-450	245	239	6	---	---	---	6 Lost to follow-up: 3 prior to the Day 30 visit; 3 after the Day 30 visit

Recruitment

Subjects were recruited from 19 centres in Canada, France, Germany, Sweden and the UK.

	Placebo n=49	**BOTOX** n=246	**DWP-450** n=245
Randomised	49 (100%)	246 (100%)	245 (100%)
ITT	49 (100%)	246 (100%)	245 (100%)
Per-protocol	48 (97.9%)	244 (99.2%)	235 (95.9%)
Safety			
Completed Day 150	48 (98.0%)	244 (99.2%)	239 (97.6%)

Conduct of the study

Protocol amendments

The original Clinical Study Protocol dated September 10, 2014 was revised once with advice from the Clinical Trials Facilitation Group (Protocol Amendment 1, January 29, 2015). All subjects were enrolled under the revised protocol.

Changes in the planned analyses

The study commenced on 11 June 2015 and completed on 27 April 2016. The Statistical Analysis Plan was drafted to address all changes incorporated in the amended protocol. The Statistical Analysis Plan, Final Version 3.0, is dated June 15, 2016.

Study EVB-003: Summary of Protocol Deviations

Site	Treatment	All (N=540)	Significant Protocol Deviation (N=80)	Subject	Description of Deviation
ALL	Placebo	49	11	---	2 Missing endpoint assessments (MEA)
					2 Study procedures/assessments (SP/A)
					8 Visit scheduling (VS), Visit 5 outside ±3 day window
	BOTOX	246	29	---	2 Exclusion criteria (EC)
					7 Missing endpoint assessment (MEA)
					8 Study procedures/assessments (SP/A)
					15 Visit scheduling (VS), Visit 5 outside ±3 day window
	DWP-450	245	40	---	2 Exclusion criteria (EC)
					1 Safety Reporting
					10 Missing endpoint assessment (MEA)
					15 Study procedure/assessment (SP/A)
					16 Visit scheduling (VS), Visit 5 outside ±3 day window

Baseline data

Study EVB-003: Demographic characteristics

Characteristic	Placebo (N=49)	BOTOX (N=246)	DWP-450 (N=245)
Age (years)			
mean ± SD	48.4 ± 10.84	49.7 ± 10.41	48.8 ± 10.73
[min, max]	[26, 71]	[24, 75]	[22, 79]
< 65, n (%)	45 (91.8)	227 (92.3)	228 (93.1)
≥ 65, n (%)	4 (8.2)	19 (7.7)	17 (6.9)
Sex, n (%)			
Male	8 (16.3)	31 (12.6)	25 (10.2)
Female	41 (83.7)	215 (87.4)	220 (89.8)
Fertility Status of Women, n/N (%)			
Post-menopausal	17/41 (41.5)	85/215(39.5)	91/220(41.4)
Potentially able to bear children	24/41 (58.5)	113/215(52.6)	110/220(50.0)
Sterile	0/41 (0.0)	17/215 (7.9)	19/220 (8.6)
Race, n (%)			
White	36 (73.5)	183 (74.4)	165 (67.3)
Black or African American	1 (2.0)	1 (0.4)	3 (1.2)
Asian	1 (2.0)	5 (2.0)	6 (2.4)
Multiple	0 (0.0)	1 (0.4)	1 (0.4)
Other	1 (2.0)	2 (0.8)	8 (3.3)
Missing [a]	10 (20.4)	54 (22.0)	62 (25.3)

a All 126 subjects for which race was "missing" were from Sites 305-309 – all were located in France; Per national laws, France does not permit listing the race of subjects participating in clinical trials.

Study EVB-003: Skin Type, IA and SA of GL at Maximum Frown and at Rest for ITT/Safety Population

Characteristic	Placebo (N=49)	BOTOX (N=246)	DWP-450 (N=245)
Fitzpatrick Type,[b] n (%)			
I	2 (4.1)	4 (1.6)	9 (3.7)
II	16 (32.7)	84 (34.1)	84 (34.3)
III	24 (49.0)	118 (48.0)	116 (47.3)
IV	5 (10.2)	36 (14.6)	31 (12.7)
V	2 (4.1)	2 (0.8)	4 (1.6)
VI	0 (0.0)	2 (0.8)	1 (0.4)
Investigator Assessment of Glabellar Lines			
GLS Score at Maximum Frown, n (%)			
None	0 (0.0)	0 (0.0)	0 (0.0)
Mild	0 (0.0)	0 (0.0)	0 (0.0)
Moderate	13 (26.5)	70 (28.5)	62 (25.3)
Severe	36 (73.5)	176 (71.5)	183 (74.7)
GLS Score at Rest, n (%)			
None	4 (8.2)	15 (6.1)	10 (4.1)
Mild	17 (34.7)	80 (32.5)	94 (38.4)
Moderate	15 (30.6)	105 (42.7)	97 (39.6)
Severe	13 (26.5)	46 (18.7)	44 (18.0)

Subject Assessment of Glabellar Lines			
GLS Score at Maximum Frown, n (%)			
None	0 (0.0)	0 (0.0)	0 (0.0)
Mild	0 (0.0)	2 (0.8)	0 (0.0)
Moderate	9 (18.4)	44 (17.9)	44 (18.0)
Severe	40 (81.6)	200 (81.3)	201 (82.0)
GLS Score at Rest, n (%)			
None	3 (6.1)	13 (5.3)	9 (3.7)
Mild	8 (16.3)	50 (20.3)	56 (22.9)
Moderate	26 (53.1)	105 (42.7)	114 (46.5)
Severe	12 (24.5)	78 (31.7)	66 (26.9)

Study EVB-003: Summary of HADS at Baseline

Characteristic	Placebo (N=49)	BOTOX (N=246)	DWP-450 (N=245)
HADS-A			
Score, mean ± SD	5.2 ± 2.74	5.6 ± 3.53	5.3 ± 3.20
Score, [min, max]	[0, 10]	[0, 17]	[0, 15]
Abnormal (score >7), n (%)	14 (28.6)	64 (26.0)	59 (24.1)
HADS-D			
Score, mean ± SD	2.7 ± 2.43	3.1 ± 2.93	2.4 ± 2.45
Score, [min, max]	[0, 10]	[0, 14]	[0, 15]
Abnormal (score >7), n (%)	3 (6.1)	21 (8.5)	12 (4.9)

Study EVB-003: Medical History

Body System/Question	Placebo (N=49) n	(%)	BOTOX (N=246) n	(%)	DWP-450 (N=245) n	(%)
Medical History						
Any Body System	36	(73.5)	165	(67.1)	169	(69.0)
Body Systems of Particular Interest[a]						
Cardiovascular	6	(12.2)	31	(12.6)	29	(11.8)
Dermatological	10	(20.4)	42	(17.1)	38	(15.5)
Head and Neck	13	(26.5)	62	(25.2)	59	(24.1)
Musculoskeletal	7	(14.3)	30	(12.2)	22	(9.0)
Neurological	3	(6.1)	24	(9.8)	22	(9.0)
Respiratory	3	(6.1)	20	(8.1)	24	(9.8)
Other Body Systems[b]	23	(46.9)	104	(42.3)	103	(42.0)
Directed Questions						
Eyebrow Drooping	0	(0.0)	0	(0.0)	1	(0.4)
Eyelid Drooping	0	(0.0)	2	(0.8)	0	(0.0)
Headaches	2	(4.1)	8	(3.3)	8	(3.3)
Problems with Breathing	1	(2.0)	0	(0.0)	2	(0.8)
Problems with Speaking	0	(0.0)	0	(0.0)	0	(0.0)
Problems with Swallowing	0	(0.0)	0	(0.0)	0	(0.0)
Directed Review,[c] abnormal						
Body Systems of Particular Interest[a]						
Cardiovascular	0	(0.0)	5	(2.0)	3	(1.2)
Dermatological	1	(2.0)	8	(3.3)	9	(3.7)
Head and Neck	0	(0.0)	0	(0.0)	1	(0.4)
Musculoskeletal	0	(0.0)	3	(1.2)	1	(0.4)
Neurological	0	(0.0)	2	(0.8)	1	(0.4)
Respiratory	0	(0.0)	2	(0.8)	2	(0.8)
Other Body Systems[b]	0	(0.0)	2	(0.8)	3	(1.2)
Physical Examination,[c] abnormal						
Body Systems of Particular Interest[a]						
Cardiovascular	0	(0.0)	2	(0.8)	0	(0.0)
Dermatological	1	(2.0)	9	(3.7)	7	(2.9)
Head and Neck	0	(0.0)	0	(0.0)	0	(0.0)
Musculoskeletal	0	(0.0)	3	(1.2)	1	(0.4)
Neurological	0	(0.0)	3	(1.2)	0	(0.0)
Respiratory	0	(0.0)	0	(0.0)	0	(0.0)
Other Body Systems[b]	0	(0.0)	0	(0.0)	0	(0.0)

Note: At each level of summarization, a subject is counted once if the subject reported one or more findings.

a Body systems of particular interest were those associated with FDA warnings regarding the distant spread of botulinum toxin effects

Numbers analysed

- 540 were randomised as ITT and safety populations, as 9 of 549 failed screening
- 527 were PP population, with 13 excluded due to missed assessments or assessments outside the ±5 day window, 48 placebo 48, 244 Botox, 235 DWP-450.

Study EVB-003: Summary of Subjects Excluded from PP Analysis

Site	Treatment	ITT (N=540)	Excluded from PP Population (N=13)	Subject	A/S/R[a]	Description of Deviation[b]
ALL	Placebo	49	1	---	---	1 Missing baseline/Day 30 GLS measure
	BOTOX	246	2	---	---	2 MPD VS, Visit 5 outside ± 7 day window
	DWP-450	245	10	---	---	3 Missing baseline/Day 30 GLS measure
						1 MPD VS, missing baseline/Day 30 GLS measure as subject did not attend Visit 5/Day 30 visit
						5 MPD VS, Visit 5 outside of ± 7 day window
						1 MPD, Exclusion criteria (EC)

Outcomes and estimation

Primary endpoint

Study EVB003: Summary of Primary Endpoints: GLS score of 0 or 1 at Maximum Frown by IA, Day 30

Responders for the Primary Efficacy Endpoint[a]	Placebo	BOTOX	DWP-450	Absolute Difference: BOTOX Vs. Placebo	Absolute Difference: DWP-450 Vs. Placebo	Absolute Difference: DWP-450 Vs. BOTOX
PP Population (Primary)						
Number[b]	2/48	202/244	205/235			
Percentage, %	4.2	82.8	87.2	78.6	83.1	4.4
(% CI)[c,d]	(0.0, 9.8)[c]	(78.1, 87.5)[c]	(83.0, 91.5)[c]	(66.5, 85.5)[d]	(70.3, 89.4)[d]	(-1.9, 10.8)[c]
P-Value				<0.001	<0.001	
Subjects <65 Years						
Number[b]	2/44	190/226	192/219			
Percentage, %	4.5	84.1	87.7	79.5	83.1	3.6
(% CI)[c,d]	(0.0, 10.7)[c]	(79.3, 88.8)[c]	(83.3, 92.0)[c]	(66.5, 86.7)[d]	(70.3, 89.8)[d]	(-2.9, 10.1)[c]
P-Value				<0.001	<0.001	
Subjects ≥65 Years						
Number[b]	0/4	12/18	13/16			
Percentage, %	0.0	66.7	81.3	66.7	81.3	14.6
(% CI)[c,d]	(0.0, 0.0)[c]	(44.9, 88.4)[c]	(62.1, 100.0)[c]	(-6.7, 90.0)[d]	(14.8, 97.3)[d]	(-14.4, 43.6)[c]
P-Value				0.023	0.009	
ITT Population (Sensitivity)						
Number[b]	2/48	204/246	209/241			
Percentage, %	4.2	82.9	86.7	78.8	82.6	3.8
(% CI)[c,d]	(0.0, 9.8)[c]	(78.2, 87.6)[c]	(82.4, 91.0)[c]	(66.5, 85.7)[d]	(70.2, 89.1)[d]	(-2.6, 10.2)[c]
P-Value				<0.001	<0.001	

c Within each of the Placebo, BOTOX and DWP-450 treatment groups, and for the comparison of DWP-450 versus BOTOX, the confidence interval was a 2-sided 95% asymptotic CI.

To conclude non-inferiority of DWP-450 versus BOTOX, the lower bound of the 95% CI for the difference between groups in the proportion of primary efficacy endpoint responders was required to be greater than -0.10 (i.e., > -10.0%).

d For each of the comparisons of BOTOX versus Placebo, and DWP-450 versus Placebo, the confidence interval was a 97.5% Exact CI, for which the exact CI and the associated unconditional exact test were based on the inversion of 2 one-sided tests.

To conclude superiority of BOTOX to Placebo, and of DWP-450 to Placebo, a p-value <0.025 was required for each test.

The absolute differences between BOTOX and Placebo, DWP-450 and Placebo, were 78.6% and 83.1%, respectively (both p<0.001). The absolute difference between DWP-450 and BOTOX in percentages of responders was 4.4% (95% CI -1.9, 10.8), consistent with non-inferiority given that the lower bound of the 95% CI interval was greater than -10.0%, and similarly for ITT. The impact of missing primary endpoint data was minimal.

Less than 10% were ≥65 years of age; % responders for the primary efficacy endpoint after Placebo, BOTOX and DWP-450 were greater in the 489 PP subjects <65 years vs 38 PP subjects ≥65 years old at 4.5%, 84.1%, 87.7% versus 0.0%, 66.7% and 81.3%, respectively.

The proportion of responders to Botox, DWP-450 varied between sites from 7/13 (54%) to 11/11 (100%), 12/16 (75%) to 20/20 (100%) with a relative risk differences from -11.9 (95% -36.4, 13.0) to 20.0 (-4.8, 44.8).

Sensitivity analysis of the primary efficacy endpoint

Responders Based on the GLS at Maximum Frown by Visit	Placebo (N=48)		BOTOX (N=244)		DWP-450 (N=235)	
	n/N	(%)	n/N	(%)	n/N	(%)
≥1 Point Improvement by IA						
Day 30	3/48	(6.3)	229/244	(93.9)	224/235	(95.3)
≥2 Point Improvement by IA						
Day 30	0/48	(0.0)	168/244	(68.9)	181/235	(77.0)
3 Point Improvement by IA						
Day 30	0/35	(0.0)	39/175	(22.3)	41/175	(23.4)
≥1 Point Improvement by SA						
Day 30	7/48	(14.6)	224/244	(91.8)	215/235	(91.5)
≥2 Point Improvement by SA						
Day 30	2/48	(4.2)	164/242	(67.8)	166/235	(70.6)
3 Point Improvement by SA						
Day 30	1/39	(2.6)	43/199	(21.6)	54/191	(28.3)

Note: Baseline was defined as the last non-missing measurement prior to randomization. Denominators are the number of subjects with a non-missing assessment at the corresponding visit. In addition, denominators for a 3 point improvement are limited to subjects with a baseline GLS score of 3 at maximum frown. IA=Investigator assessment;

Secondary Endpoints

Study EVB003: Summary of Secondary Endpoints Based on GLS at Maximum Frown – ITT

Responders Based on the GLS at Maximum Frown	Placebo (N=49)	BOTOX (N=246)	DWP-450 (N=245)	Absolute Difference DWP-450 Vs. Placebo	Absolute Difference DWP-450 Vs. BOTOX
GLS Score of 0 or 1 on Day 30 by SA					
Number [a]	3/48	187/246	190/241		
Percentage, %	6.3	76.0	78.8	72.6	2.8
(95% CI) [c,d]	(0, 13.1) [c]	(70.7, 81.4) [c]	(73.7, 84.0) [c]	(60.8, 79.9) [d]	(-4.6, 10.2) [c]
P-Value				<0.001	
≥1 Point Improvement on the GLS From Day 0 to Day 2 by IA					
Number [b]	6/49	139/244	130/240		
Percentage, %	12.2	57.0	54.2	41.9	-2.8
(95% CI) [c,d]	(3.1, 21.4) [c]	(50.8, 63.2) [c]	(47.9, 60.5) [c]	(28.7, 51.8) [d]	(-11.7, 6.1) [c]
P-Value				<0.001	
≥1 Point Improvement on the GLS From Day 0 to EOS (Day 150)/ET by IA					
Number [b]	4/48	84/244	90/239		
Percentage, %	8.3	34.4	37.7	29.3	3.2
(95% CI) [c,d]	(0.5, 16.2) [c]	(28.5, 40.4) [c]	(31.5, 43.8) [c]	(17.0, 38.2) [d]	(-5.3, 11.8) [c]
P-Value				<0.001	

The absolute difference in responders judged by a GLS Score of 0 or 1 on Day 30 by SA on ITT is similar to IA on PP for DWP-450 vs Placebo at 72.6 (95% CI 60.8, 79.9), 83.1 (70.3, 89.4) & DWP-450 vs Botox at 2.8 (-4.6, 10.2), 4.4 (-1.9, 10.8) respectively.

Study EVB003: Summary of the Subject Satisfaction Scale, SSS, Response by ITT

Responders Based on the SSS	Placebo (N=49)	BOTOX (N=246)	DWP-450 (N=245)	Absolute Difference DWP-450 Vs. Placebo	Absolute Difference DWP-450 Vs. BOTOX
≥1 Point Improvement in Subject Satisfaction from Day 0 to Day 30 [a]					
Number [b]	3/48	213/246	219/240		
Percentage, %	6.3	86.6	91.3	85.0	4.7
(95% CI) [c,d]	(0, 13.1) [c]	(82.3, 90.8) [c]	(87.7, 94.8) [c]	(74.2, 91.1) [d]	(-0.9, 10.2) [c]
P-Value				<0.001	

The improvement in SSS was 80% - 85% higher for Botox and DWP-450 respectively than Placebo.

Study EVB003: Summary of the Change in HADS-A and HADS-D Scores by ITT

Change from Baseline to Day 90 in HADS Scores	Placebo (N=49)	BOTOX (N=246)	DWP-450 (N=245)	Absolute Difference: DWP-450 Vs. Placebo	Absolute Difference: DWP-450 Vs. BOTOX
HADS-A Score, All Subjects					
N [a]	47	239	231		
Mean Change ± SD	-0.9 ± 2.50	-0.9 ± 2.72	-1.1 ± 2.40	-0.2	-0.2
95% CI [b]	(-1.7, -0.2)	(-1.3, -0.6)	(-1.4, -0.8)	(-0.9, 0.6)	(-0.6, 0.3)
P-Value [c]	0.013	<0.001	<0.001		
HADS-A Score, Subjects with Abnormal Scores at Baseline					
N [a]	14	63	55		
Mean Change ± SD	-2.2 ± 2.33	-2.8 ± 3.03	-2.3 ± 2.86	-0.1	0.5
95% CI [b]	(-3.6, -0.9)	(-3.6, -2.0)	(-3.1, -1.5)	(-1.7, 1.6)	(-0.6, 1.6)
P-Value [c]	0.003	<0.001	<0.001		
HADS-D Score, All Subjects					
N [a]	47	239	231		
Mean Change ± SD	-0.5 ± 1.98	-0.6 ± 2.15	-0.6 ± 2.19	-0.1	0.0
95% CI [b]	(-1.1, 0.0)	(-0.9, -0.3)	(-0.9, -0.3)	(-0.7, 0.6)	(-0.4, 0.4)
P-Value [c]	0.071	<0.001	<0.001		
HADS-D Score, Subjects with Abnormal Scores at Baseline					
N [a]	3	21	12		
Mean Change ± SD	-2.3 ± 1.53	-3.6 ± 2.60	-4.3 ± 3.84	-1.9	-0.7
95% CI [b]	(-6.1, 1.5)	(-4.8, -2.4)	(-6.7, -1.8)	(-6.9, 3.1)	(-3.0, 1.6)
P-Value [c]	0.118	<0.001	0.003		

Exploratory Efficacy Endpoints (GAIS and SSS)

Study EVB003: Global Aesthetic Improvement Scale (GAIS) Scores by IA ,SA at Day 30, Day 90 by PP

		Investigator Assessment			Subject Assessment		
		Placebo (N=48)	BOTOX (N=244)	DWP-450 (N=235)	Placebo (N=48)	BOTOX (N=244)	DWP-450 (N=235)
Visit	GAIS Score	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Day 30	N	48	244	235	48	244	235
	Much Improved	0 (0.0)	154 (63.1)	156 (66.4)	0 (0.0)	132 (54.1)	156 (66.4)
	Improved	2 (4.2)	75 (30.7)	70 (29.8)	4 (8.3)	96 (39.3)	66 (28.1)
	No Change	46 (95.8)	15 (6.1)	9 (3.8)	44 (91.7)	16 (6.6)	13 (5.5)
	Worse	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Much Worse	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Day 90	N	48	241	232	48	241	232
	Much Improved	0 (0.0)	57 (23.7)	56 (24.1)	0 (0.0)	67 (27.8)	69 (29.7)
	Improved	3 (6.3)	129 (53.5)	134 (57.8)	2 (4.2)	107 (44.4)	111 (47.8)
	No Change	45 (93.8)	54 (22.4)	42 (18.1)	45 (93.8)	63 (26.1)	49 (21.1)
	Worse	0 (0.0)	1 (0.4)	0 (0.0)	1 (2.1)	4 (1.7)	3 (1.3)
	Much Worse	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Study EVB003: Summary of Subject Satisfaction Scores on Day 30, Day 90 by PP

Visit	SSS Score	Placebo (N=48) n	(%)	BOTOX (N=244) n	(%)	DWP-450 (N=235) n	(%)
Day 30	N	48		244		234	
	Very Satisfied	1	(2.1)	133	(54.5)	145	(62.0)
	Satisfied	2	(4.2)	78	(32.0)	69	(29.5)
	Indifferent	24	(50.0)	26	(10.7)	15	(6.4)
	Unsatisfied	12	(25.0)	7	(2.9)	5	(2.1)
	Very Unsatisfied	9	(18.8)	0	(0.0)	0	(0.0)
Day 90	N	48		241		232	
	Very Satisfied	2	(4.2)	88	(36.5)	79	(34.1)
	Satisfied	1	(2.1)	86	(35.7)	108	(46.6)
	Indifferent	25	(52.1)	39	(16.2)	29	(12.5)
	Unsatisfied	10	(20.8)	23	(9.5)	14	(6.0)
	Very Unsatisfied	10	(20.8)	5	(2.1)	2	(0.9)

Ancillary Analyses

Study EVB003: Response as >/=2point Improvement in GLS at Maximum Frown, Day 0 to Day 30, PP

Responders at Day 30 [a]	Placebo (N=48)	BOTOX (N=244)	DWP-450 (N=235)	Absolute Difference DWP-450 Vs. Placebo	Absolute Difference DWP-450 Vs. BOTOX
By Both IA and SA					
Number [b]	0/48	135/244	145/235		
Percentage, %	0.0	55.3	61.7	61.7	6.4
(95% CI) [c,d]	(0.0, 0.0)	(49.1, 61.6)	(55.5, 67.9)	(54.2, 68.0)	(-2.4, 15.2)
P-Value				<0.001	0.157
By IA Only					
Number [b]	0/48	168/244	181/235		
Percentage, %	0.0	68.9	77.0	77.0	8.2
(95% CI) [c,d]	(0.0, 0.0)	(63.0, 74.7)	(71.6, 82.4)	(69.8, 82.3)	(0.3, 16.1)
P-Value				<0.001	0.044
By SA Only					
Number [b]	2/48	164/244	166/235		
Percentage, %	4.2	67.2	70.6	66.5	3.4
(95% CI) [c,d]	(0.0, 9.8)	(61.3, 73.1)	(64.8, 76.5)	(55.9, 73.6)	(-4.9, 11.7)
P-Value				<0.001	0.418

Study EVB003: Kaplan-Meier Analysis of >/=1 Point Improvement in GLS at Maximum Frown for ITT

	Placebo (N=49)	BOTOX (N=246)	DWP-450 (N=245)
By Investigator Assessment			
Responders, n (%) [a]	14/49 (28.6)	236/246 (95.9)	235/245 (96.7)
Did Not Stop Responding, n/N (%)	3/14 (21.4)	78/236 (33.1)	81/235 (34.5)
Stopped Responding, n/N (%)	11/14 (78.6)	158/236 (66.9)	154/235 (65.5)
Duration of Response, days [b,c]			
25% stop responding	29	99	106
[95% CI]	[13, 52]	[91, 107]	[91, 113]
50% stop responding	52	132	139
[95% CI]	[13, 85]	[121, 144]	[131, 143]
75% stop responding	85	154	155
[95% CI]	[30, 96]	[151, NE]	[153, NE]
By Subject Assessment			
Responders, n (%) [a]	14/49 (28.6)	237/246 (96.3)	230/245 (94.7)
Did Not Stop Responding, n/N (%)	4/14 (28.6)	74/237 (31.2)	89/230 (38.7)
Stopped Responding, n/N (%)	10/14 (71.4)	163/237 (68.8)	141/230 (61.3)
Duration of Response, days [b,c]			
25% stop responding	32	89	86
[95% CI]	[13, 65]	[79, 93]	[81, 93]
50% stop responding	65	124	136
[95% CI]	[13, 85]	[116, 136]	[121, 145]
75% stop responding	85	155	156
[95% CI]	[65, NE]	[149, NE]	[153, 191]

Study EV001

A phase III, multi-centre, randomised, double-blind placebo controlled, single dose trial to demonstrate the safety and efficacy of DWP-450 in adults for the treatment of moderate-to-severe glabellar lines (GL).

Methods

- **Study design**

Subjects were screened and assessed then randomised 3:1 to undergo a single treatment with DWP-450 or placebo, with follow-up on days 2, 7, 14, 30 and then every 30 days to day 150.

- **Study participants**

Participants were selected from healthy adults age ≥18 years

Entry criteria were moderate (GLS=2) to severe (GLS=3) glabellar lines at maximum frown as assessed by the investigator using the 4-point photonumeric GLS.

Subjects verbally confirmed that their glabellar lines had an important psychological impact (on mood, anxiety and/or depressive symptoms).

Exclusion criteria included previous treatment with any botulinum toxin in the last 6 months; planned treatment with any botulinum toxin in any other body region during the study; participation in any other interventional clinical study within 30 days; other previous permanent or planned facia aesthetic procedures; and local or systemic conditions likely to increase risks of the procedure.

- **Treatments**

Study EV001: Investigational product

Product	Formulation
DWP-450	100U vacuum dried botulinum toxin type A, 0.5mg HAS stabiliser, 0.9mg NaCl isotonic agent
Placebo	Empty colourless transparent vial

2.5ml 0.9% preservative free saline was added to the vial to reconstitute without shaking to a final dilution of 4U to 0.1ml. Each of 5 sites was injected with 0.1ml im using a 30G needle and a 1ml syringe with topical anaesthesia used if required. Target sites for injection were:

- Superior middle aspect of each corrugator muscle, ≥1cm above the bony orbital rim
- Inferomedial aspect of each corrugator muscle
- Midline of the procerus

- **Objectives**

Primary objective

Efficacy of DWP-450 versus placebo in the treatment of moderate to severe glabellar lines associated with corrugator and/or procerus muscle activity in adults

Secondary objective

Safety and immunogenicity of DWP-450 versus placebo in the treatment of moderate to severe glabellar lines associated with corrugator and/or procerus muscle activity in adults

- **Outcomes/endpoints**

Schedule of Assessments

Study Phase	Screening	Treatment	Monitoring Safety and Efficacy							
Visit Number	1 [a]	2 [a]	3	4	5	6	7	8	9 [k]	Early Termin-ation [k]
Scheduled Assessment Day	-14	0	2 (+/-1)	7 (+/-3)	14 (+/-3)	30 (+/-3)	90 (+/-7)	120 (+/-7)	150 (+/-7)	
Informed Consent	X									
Inclusion / Exclusion Criteria	X									
Demographics	X									
Safety Evaluations										
Medical History	X									
Directed Questionnaire	X		X	X	X	X	X	X	X	X
Directed Review of Systems	X		X	X	X	X	X	X	X	X
Physical Examination [b]	X		X	X	X	X	X	X	X	X
Vital Signs [c]	X		X	X	X	X	X	X	X	X
Urine Pregnancy Test (as applicable)	X									
Toxin Antibody Test	X					X	X		X	X
Laboratory Tests [d]	X								X	X
ECG	X					X			X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X
Adverse Events	X	X	X	X	X	X	X	X	X	X
Injection (DWP-450 or Placebo) [e]		X								
Efficacy Evaluations										
Investigator Assessments										
GLS [f] at Maximum Frown	X	X	X	X	X	X	X	X	X	X
GLS [g] at Rest	X	X	X	X	X	X	X	X	X	X
Global Aesthetic Improvement			X	X	X	X	X	X	X	X
Subject Assessments										
GLS [f] at Maximum Frown	X	X	X	X	X	X	X	X	X	X
GLS [g] at Rest	X	X	X	X	X	X	X	X	X	X
Global Aesthetic Improvement [h]			X	X	X	X	X	X	X	X
Subject Satisfaction Scale [i]			X	X	X	X	X	X	X	X
Photography of Brow Area [j]	X		X	X	X	X	X	X	X	X

Key a. Screening and treatment visits could take place the same day. e. Randomisation occurred prior to injection.

Efficacy

Primary endpoints

Primary endpoint = a composite endpoint of the proportion of responders at day 30, by both Investigator Assessment and Subject Assessment, where glabellar lines at maximum frown showed a ≥2point improvement in GLS,

- By ITT and age <65 years versus ≥65 years
- Sensitivity analysis of PP, mITT, varying approaches to missing data on day 30

Secondary endpoints by Investigator Assessment & Subject Assessment

- ≥2point improvement of GLS at maximum frown on day 90 vs day 0 by ITT (post-hoc analysis)
- ≥2point improvement of GLS at maximum frown on day 120 vs day 0 by ITT
- ≥2point improvement of GLS at maximum frown on day 150 vs day 0 by ITT

- ≥2point improvement of GLS at maximum frown on day 30 vs day 0 by modified ITT

Exploratory Efficacy Endpoints

- Global aesthetic improvement scale (GAIS) scores (-2 to +2) by Investigator and Subject assessment
- Subject Satisfaction Scale (-2 to +2)
- ≥1point improvement in the GLS score at maximum frown and at rest by IA & SA
- Individual GLS scores by Investigator, Subject assessment at each visit by ITT

Safety Endpoints - including

- Exposure to treatment
- AEs including drug related AES, serious AEs, AEs of special interest, AEs resulting in discontinuation, and AEs with an incidence of ≥5%, ≥10%
- Vital signs and laboratory tests
- Anti-botulinum toxin Abs

- **Assessment Methods**

- Glabellar Line Scale (GLS) score

- Global Aesthetic Improvement Scale (GAIS)

- Subject Satisfaction Scale (SSS)

- Anti-Botulinum toxin A Antibodies (ADA)

ADA was determined using a 2-tiered approach of screening then confirmation using a bridging ELISA. Botulinum toxin A immobilised on the assay plate is linked by ADA or positive control Ab to biotinylated botulinum toxin A, with the immune complex detected by the action on substrate of Streptavidin conjugated horseradish peroxidase. Confirmation of ADA was dependent on neutralisation of the signal by pre-incubation of botulinum toxin A with test serum or positive control.

Confirmed positive ADA were tested for neutralisation of toxicity in a mouse LD50 toxicity assay using botulinum toxin A.

- **Sample size**

Sample size was determined based on safety considerations. A sample size of 324 subjects randomised 3:1 to DWP-450:Placebo with 10% dropout rate would yield 219:73 subjects. If no adverse events were observed after a single treatment with DWP-450, upper bound of 95% CI =0.016, the likely true incidence is <1.6%.

- **Randomisation**

Subjects were block randomised without stratification in fours, 3:1 to DWP-450:placebo

- **Blinding (masking)**

The study was double-blinded including provision of the loaded syringe to the investigator

- **Statistical methods**

Study populations were defined as:

ITT – all subjects randomised to treatment.

mITT – all randomised subjects who had a moderate or severe GLS score at rest on Day O by IA & SA.
Safety population – all randomised subjects who received at least one injection of DWP-450 or placebo.
PPP – all randomised subjects who received the protocol required single treatment of 5 injections of the correct drug by randomisation and had the primary outcome measure assessed at both baseline Day 0 and Day 30 $\pm$7 days.

The primary endpoint was assessed in a superiority design with alpha 0.05 using the stratified CMH test, stratified by investigator site, testing the primary null hypothesis that there was no difference in rates of responders between DWP-450 and placebo. The hypothesis was tested using the exact unconditional test, with its exact confidence interval (CI) calculated by inversion of two one-sided intervals. The overall study wide Type 1 error rate (alpha) was 0.05.

Sensitivity analyses of the primary efficacy endpoint were performed to assess the impact of missing data. These included 3 scenarios: a worst case scenario in which missing data in the DWP-450 arm were treated as "non responders" and missing data in the Placebo arm were treated as "responders"; a best case scenario in which missing data in the DWP-450 arm were treated as "responders" and missing data in the Placebo arm were treated as "non responders"; and, using Days 14 and 90 data. The principal sensitivity analysis for the primary outcome was a tipping point analysis.

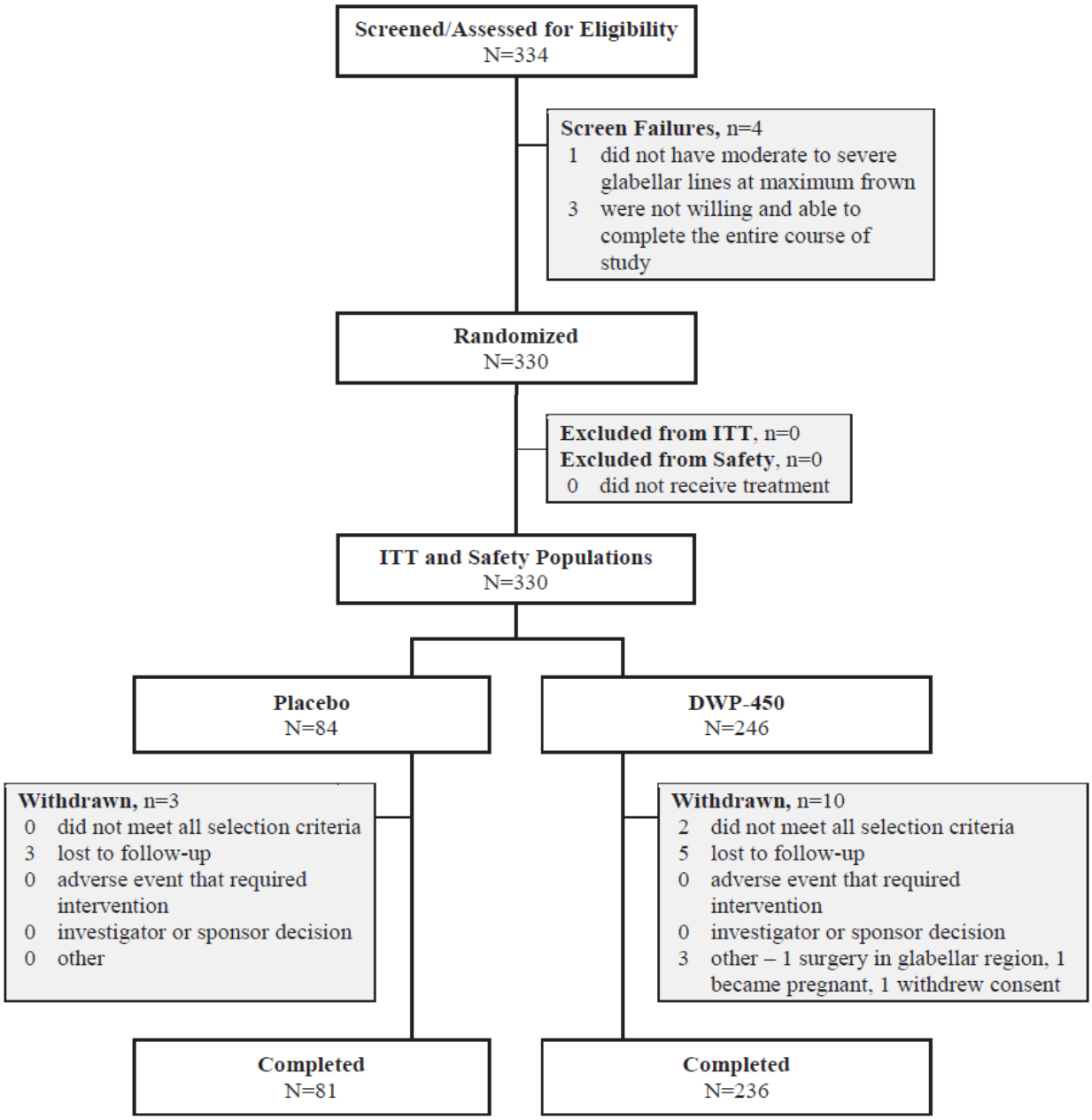
Additional sensitivity analyses were performed using each of the per protocol (PP) and the modified ITT (mITT) Populations. The mITT population was defined as all randomised subjects who had moderate or severe GLS score at rest on Day 0, as assessed by both the investigator and subject.

The secondary endpoints were tested sequentially using gatekeeper methods to maintain the overall study Type 1 error rate of 0.05 – i.e., each endpoint was only tested if the p-value for the previous exact test was <0.05.

Results:

Study EV001

- **Participant flow**



Study EV001: Disposition of Subjects

Site	Treatment	All (N=330)	Completed (N=317)	Discontinued/Withdrawn (N=13)	Subject	A/S/R[a]	Day	Primary Reason for Withdrawal
ALL	Placebo	84	81	3	---	---	---	3 Lost to follow-up
	DWP-450	246	236	10	---	---	---	1 Became pregnant
								1 Withdrew consent
								1 Had surgery in glabellar region
								2 Did not meet all selection criteria
								5 Lost to follow-up

- **Recruitment**

Subjects were recruited from 10 sites in the US

- **Conduct of the study**

The study was conducted according to the ethical principles established in the Declaration of Helsinki and ICH GCP. All subjects were recruited under the revised protocol incorporating changes recommended by the FDA, EV-001 protocol amendment 1, Oct 27th 2014. The revised statistical analysis plan 3.3 23 Feb 2016, prior to breaking the blinding, allowed assessment of the primary endpoint without the restriction of ±3 days for the day 30 timepoint. The CMH test was rendered inappropriate with the small number of placebo subjects noting a ≥2point improvement, prompting the post hoc change to analysis by exact unconditional test with exact confidence intervals by inversion of two one sided intervals.

A study vial was selected out of sequential order at 4 sites affecting 18 subjects, 6 Placebo & 12 DWP-450; the influence of this error was assessed in the sensitivity analysis.

Study EV001: Summary of Protocol Deviations

Site	Treatment	All (N=330)	Significant Protocol Deviation (N=52)	Subject	Description of Deviation
ALL	Placebo	84	16	---	1 Study procedure/assessment (SP) not performed
					1 Visit scheduling (VS) – missed visit
					14 VS – Visit 6 outside ±3 day window
	DWP-450	246	36	---	2 Exclusion criteria (EC)
					4 VS – missed visit
					30 VS – Visit 6 outside ±3 day window

- **Baseline data**

Study EV001: Demographic characteristics

Characteristic	Placebo (N=84)	DWP-450 (N=246)
Age (years)		
mean ± SD [min, max]	50.4 ± 11.95 [23, 74]	50.2 ± 11.76 [22, 81]
< 65, n (%)	75 (89.3)	220 (89.4)
≥ 65, n (%)	9 (10.7)	26 (10.6)
Sex, n (%)		
Male	5 (6.0)	19 (7.7)
Female	79 (94.0)	227 (92.3)
Fertility Status of Women, n/N (%)		
Post-menopausal	33/79(41.8)	114/227(50.2)
Potentially able to bear children	37/79(46.8)	92/227(40.5)
Sterile	9/79(11.4)	21/227 (9.3)
Race, n (%)		
White	63 (75.0)	205 (83.3)
Black or African American	7 (8.3)	18 (7.3)
Asian	4 (4.8)	2 (0.8)
Multiple	2 (2.4)	7 (2.8)
Other	8 (9.5)	14 (5.7)

Study EV001: Skin Type, Previous Treatment, IA/SA of GL at Maximum Frown and at Rest by ITT

Characteristic	Placebo (N=84)	DWP-450 (N=246)
Fitzpatrick Type,[a] n (%)		
I	6 (7.1)	9 (3.7)
II	29 (34.5)	81 (32.9)
III	31 (36.9)	98 (39.8)
IV	12 (14.3)	41 (16.7)
V	6 (7.1)	13 (5.3)
VI	0 (0.0)	4 (1.6)
Prior History of Botulinum Toxin Treatment, n (%)	31 (36.9)	103 (41.9)
Investigator Assessment of Glabellar Lines on the GLS,[b] n (%)		
At Maximum Frown		
Moderate	28 (33.3)	78 (31.7)
Severe	56 (66.7)	168 (68.3)
At Rest		
None	13 (15.5)	37 (15.0)
Mild	30 (35.7)	68 (27.6)
Moderate	25 (29.8)	91 (37.0)
Severe	16 (19.0)	50 (20.3)
Subject Assessment of Glabellar Lines on the GLS,[b] n (%)		
At Maximum Frown		
Moderate	18 (21.4)	56 (22.8)
Severe	66 (78.6)	190 (77.2)
At Rest		
None	8 (9.5)	20 (8.1)
Mild	13 (15.5)	48 (19.5)
Moderate	36 (42.9)	91 (37.0)
Severe	27 (32.1)	87 (35.4)

a Type I=always burns, never tans (pale white skin); Type II=usually burns, tans minimally (white skin); Type III=sometimes burns, tans uniformly (cream/light brown skin); Type IV=rarely burns, always tans well (moderate brown skin); Type V=very rarely burns, tans very easily (dark brown skin); Type VI=never burns, deeply pigmented (dark brown to black skin). b baseline assessments of glabellar lines were performed prior to randomization.

Source: End-of-Text Tables 14.1.1.4 and 14.1.1.6; Appendix Listings 16.2.4.1 and 16.2.4.3

Treatment groups were well matched for age (mean age 50 years, ~11% ≥65 years, sex (7% male), race (81% White) and Fitzpatrick skin type (72% type I or II), whilst 40.6% were previous recipients of botulinum toxin treatment. Some 42% of women were potentially able to bear children.

Investigator assessment noted 32% had moderate GLs at maximum frown, whilst 45% had mild or no GLs at rest. Subject assessment noted 22% had moderate GLS at maximum frown whilst 27% had no or mild GLS at rest.

Medical History

On direct questioning, headaches were reported in 11 (13%) and 25 (10%) of subjects in the placebo and DWP-450 treatment groups respectively. No subjects reported problems with eyebrow/eyelid drooping or speaking or swallowing at baseline.

Study EV001: Summary of Medical History

	Placebo (N=84)	DWP-450 (N=246)
Medical History, n (%)		
Any Body System	73 (86.9)	210 (85.4)
Body Systems of Particular Interest[a]		
Cardiovascular	18 (21.4)	48 (19.5)
Dermatological	25 (29.8)	47 (19.1)
Head and Neck	7 (8.3)	21 (8.5)
Musculoskeletal	15 (17.9)	48 (19.5)
Neurological	11 (13.1)	38 (15.4)
Respiratory	6 (7.1)	19 (7.7)
Other Body Systems[b]	68 (81.0)	191 (77.6)

- **Numbers analysed**

330 were randomised as ITT and safety populations, as 4 of 334 failed screened

317 were PP population, with 13 excluded due to lack of assessments or protocol deviations

176 were the mITT population, with 154 excluded with lack of moderate/severe GLS score at rest

- **Outcomes and estimation**

Primary endpoint

Study EV001

Study EV001: Responders >/=2point Improvement in GLS at Maximum Frown on Day 30 vs Day 0, ITT

	Investigator Assessment		Subject Assessment		Investigator + Subject[f]	
Responders[a]	**Placebo (N=84)**	**DWP-450 (N=246)**	**Placebo (N=84)**	**DWP-450 (N=246)**	**Placebo (N=84)**	**DWP-450 (N=246)**
ITT Population, All						
Number[b]	1/83	186/240	3/83	184/240	1/83	162/240
Percentage, %	1.2	77.5	3.6	76.7	1.2	67.5
(95% CI)[c]	(0.0, 6.5)	(71.7, 82.6)	(0.8, 10.2)	(70.8, 81.9)	(0.0, 6.5)	(61.2, 73.4)
Absolute Difference, %	76.3		73.1		66.3	
(95% CI)[d]	(69.4, 81.7)		(65.0, 79.3)		(59.0, 72.4)	
P-Value						
Exact Test[d]	<0.001		<0.001		<0.001	
Homogeneity of Sites[e]	0.884		0.253		0.922	

The IA and SA response to DWP-450 varied by site (n=10) from 9/23 (39%) to 19/21 (91%).

Study EV001: Responders >/=2point Improvement in GLS at Maximum Frown by IA & SA on Day 30 vs Day 0, ITT by Age

Responders[a] by Age Group	Subjects <65 Years		Subjects ≥ 65 Years	
	Placebo (N=75)	DWP-450 (N=220)	Placebo (N=9)	DWP-450 (N=26)
ITT Population, < and ≥ 65 yrs				
Number[b]	1/74	150/215	0/9	12/25
Percentage, %	1.4	69.8	0.0	48.0
(95% CI)[c]	(0.0, 7.3)	(63.2, 75.8)	(0.0, 33.6)	(27.8, 68.7)
Absolute Difference, %	68.4		48.0	
(95% CI)[d]	(60.6, 74.8)		(7.0, 70.0)	
P-Value				
Exact Test[d]	<0.001		0.016	
Homogeneity of Sites[e]	0.852		n/a	

Responders were more common in subjects <65 years than those ≥65 years.

Sensitivity analysis

The absolute difference in percent responders overall was 66.3% by ITT and for other populations was

- 58.2% for mITT, i.e. subjects with moderate or severe GLs at rest on day 0
- 66.9% for PP
- 66.2% for ITT excluding 18 subjects treated out of randomised order
- 63.5% - 67.1% depending on which data points were used for missing primary endpoint data; 7 subjects 1 placebo, 6 DWP-450 were missing day 30 data.

Study EV002

A phase III, multi-centre, randomised double-blind placebo controlled, single dose trial to demonstrate safety and efficacy of DWP-450 in adults for treatment of moderate-to-severe glabellar lines.

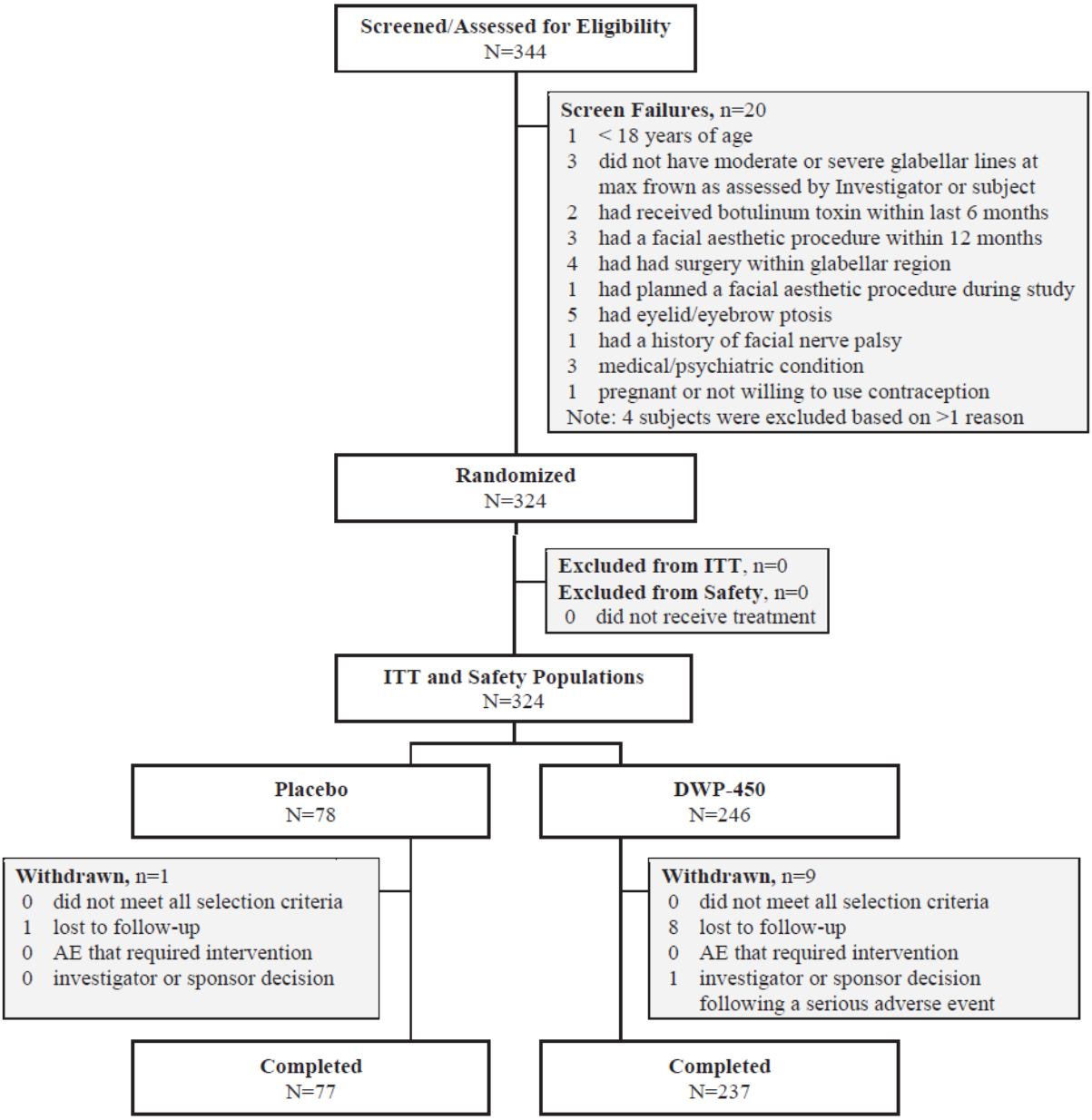
Methods:

The study design, study participants, treatments, objectives, outcomes/endpoints, assessment methods, sample size, randomisation, blinding (masking) and statistical methods were the same as for study EV001.

Results

- **Participant flow**

Study EV002: Summary Disposition of All Subjects and Primary Populations for Analysis



Study EV002: Summary Disposition of Subjects

Site	Treatment	All (N=324)	Completed (N=314)	Discontinued/Withdrawn (N=10)	Subject	A/S/R[a]	Day	Primary Reason for Withdrawal
ALL	Placebo	78	77	1	---	---	---	1 Lost to follow-up
	DWP-450	246	237	9	---	---	---	1 Withdrawn by Investigator, in the subject's best interest
								8 Lost to follow-up

- **Recruitment**

Subjects were recruited from 10 centres in the US

- **Conduct of the study**

The study was performed in accordance with the principles of the Declaration of Helsinki and ICH GCP. All study participants were recruited under EV-002 protocol amendment 1, Oct 27th 2014.

A study vial was selected out of sequential order at 2 sites affecting 13 subjects, 4 Placebo & 9 DWP-450; the influence of this error was assessed in the sensitivity analysis.

Study EV002: Summary of Protocol Deviations

Site	Treatment	All (N=324)	Significant Protocol Deviation (N=60)	Subject	Description of Deviation
ALL	Placebo	78	12	---	6 Visit scheduling (VS) – missed visit
					6 VS – Visit 6 outside ±3 day window
	DWP-450	246	48	---	5 Exclusion criteria (EC)
					2 VS – missed visit
					38 VS – Visit 6 outside ±3 day window
					3 VS – missed visit plus Visit 6 outside window

- **Baseline data**

Study EV002: Demographic characteristics

Characteristic	Placebo (N=78)	DWP-450 (N=246)
Age (years)		
mean ± SD [min, max]	50.4 ± 10.14 [18, 71]	51.5 ± 11.54 [21, 81]
< 65, n (%)	72 (92.3)	219 (89.0)
≥ 65, n (%)	6 (7.7)	27 (11.0)
Sex, n (%)		
Male	8 (10.3)	26 (10.6)
Female	70 (89.7)	220 (89.4)
Fertility Status of Women, n/N (%)		
Post-menopausal	21/70 (30.0)	88/220 (40.0)
Potentially able to bear children	32/70 (45.7)	93/220 (42.3)
Sterile	17/70 (24.3)	39/220 (17.7)
Race, n (%)		
White	69 (88.5)	215 (87.4)
Black or African American	6 (7.7)	19 (7.7)
Asian	2 (2.6)	5 (2.0)
Multiple	1 (1.3)	3 (1.2)
Other	0 (0.0)	4 (1.6)

Treatment groups matched for age (mean age 51 years, ~10% ≥65 years, sex (11% male), race (88% White). Some 43% of women were potentially able to bear children.

Study EV002: Skin Type, Previous Treatment, IA/SA of GL at Maximum Frown and at Rest by ITT

Characteristic	Placebo (N=78)	DWP-450 (N=246)
Fitzpatrick Type,[a] n (%)		
I	4 (5.1)	12 (4.9)
II	19 (24.4)	63 (25.6)
III	31 (39.7)	89 (36.2)
IV	18 (23.1)	55 (22.4)
V	2 (2.6)	19 (7.7)
VI	4 (5.1)	8 (3.3)
Prior History of Botulinum Treatment, n (%)	26 (33.3)	91 (37.0)
Investigator Assessment of Glabellar Lines on the GLS,[b] n (%)		
At Maximum Frown		
Moderate	12 (15.4)	42 (17.1)
Severe	66 (84.6)	204 (82.9)
At Rest		
None	4 (5.1)	17 (6.9)
Mild	20 (25.6)	70 (28.5)
Moderate	35 (44.9)	94 (38.2)
Severe	19 (24.4)	65 (26.4)
Subject Assessment of Glabellar Lines on the GLS,[b] n (%)		
At Maximum Frown		
Moderate	13 (16.7)	46 (18.7)
Severe	65 (83.3)	200 (81.3)
At Rest		
None	1 (1.3)	9 (3.7)
Mild	8 (10.3)	33 (13.4)
Moderate	40 (51.3)	111 (45.1)
Severe	29 (37.2)	93 (37.8)

Subjects were well matched for Fitzpatrick skin type (63% type I or II), whilst 36.3% were previous recipients of botulinum toxin treatment. Investigator assessment noted 17% had moderate GLs at maximum frown, whilst 34% had mild or no GLs at rest. Subject assessment noted 18% had moderate GLS at maximum frown whilst 16% had no or mild GLS at rest.

Study EV002: Medical History

	Placebo (N=78)	DWP-450 (N=246)
Medical History, n (%)		
Any Body System	72 (92.3)	221 (89.8)
Body Systems of Particular Interest[a]		
Cardiovascular	15 (19.2)	64 (26.0)
Dermatological	19 (24.4)	49 (19.9)
Head and Neck	12 (15.4)	56 (22.8)
Musculoskeletal	20 (25.6)	67 (27.2)
Neurological	13 (16.7)	48 (19.5)
Respiratory	6 (7.7)	30 (12.2)
Other Body Systems[b]	65 (83.3)	210 (85.4)
Directed Questions, n[c] (%)	N=76	N=241
Eyebrow Drooping	0 (0.0)	0 (0.0)
Eyelid Drooping	0 (0.0)	0 (0.0)
Headaches	6 (7.9)	12 (5.0)
Problems with Breathing	1 (1.3)	1 (0.4)
Problems with Speaking	0 (0.0)	0 (0.0)
Problems with Swallowing	0 (0.0)	1 (0.4)

- **Numbers analysed**

324 were randomised as ITT and safety populations, as 20 of 344 failed screened

302 were PP population, with 22 excluded due to lack of assessments or protocol deviations

210 were the mITT population, with 114 excluded with lack of moderate/severe GLS score at rest

  - Site 206 excluded no subjects
  - Site 207 excluded few subjects (3/30, 10%)
  - Site 201 excluded 29/34, 85.3% of subjects

- **Outcomes and estimation**

Study EV002: Responders >/=2point Improvement in GLS at Maximum Frown on Day 30 vs Day 0, ITT

	Investigator Assessment		Subject Assessment		Investigator + Subject[f]	
Responders[a]	Placebo (N=78)	DWP-450 (N=246)	Placebo (N=78)	DWP-450 (N=246)	Placebo (N=78)	DWP-450 (N=246)
ITT Population, All						
Number[b]	2/75	198/240	3/75	183/240	1/75	169/240
Percentage, %	2.7	82.5	4.0	76.3	1.3	70.4
(95% CI)[c]	(0.3, 9.3)	(77.1, 87.1)	(0.8, 11.2)	(70.4, 81.5)	(0.0, 7.2)	(64.2, 76.1)
Absolute Difference, %	79.8		72.3		69.1	
(95% CI)[d]	(72.1, 85.2)		(63.7, 78.5)		(61.5, 75.1)	
P-Value						
Exact Test[d]	<0.001		<0.001		<0.001	
Homogeneity of Sites[e]	0.123		0.215		0.130	

The IA + SA response to DWP-450 varied by site (n=10) from 6/27 (22%) to 26/26 (100%).

Responders were more common in subjects <65 years than those ≥65 years.

Study EV002: Responders >/=2point Improvement in GLS at Maximum Frown on Day 30 versus Day 0, ITT by Age

	Subjects <65 Years		Subjects ≥ 65 Years	
Responders[a] by Age Group	Placebo (N=72)	DWP-450 (N=219)	Placebo (N=6)	DWP-450 (N=27)
ITT Population, < and ≥ 65 yrs				
Number[b]	1/70	155/214	0/5	14/26
Percentage, %	1.4	72.4	0.0	53.8
(95% CI)[c]	(0.0, 7.7)	(65.9, 78.3)	(0.0, 52.2)	(33.4, 73.4)
Absolute Difference, %	71.0		53.8	
(95% CI)[d]	(62.9, 77.2)		(-5.5, 74.9)	
P-Value				
Exact Test[d]	<0.001		0.137	
Homogeneity of Sites[e]	0.045		n/a	

Sensitivity analysis

The absolute difference in percent responders overall was 69.1% by ITT and for other populations was

- 66.0% for mITT i.e. subjects with moderate or severe GLs at rest on day 0
- 70.0% for PP

- 69.2% for ITT excluding 13 subjects treated out of randomised order
- 63.6% - 69.9% depending on which data points were used for missing primary endpoint data; 9 subjects 3 placebo, 6 DWP-450 were missing day 30 data.

Summary of main studies

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Summary of efficacy for pivotal study EVB003

<table>
<tr><td colspan="5"><u>Title</u>: A Randomised, DB, active and placebo controlled single dose trial to demonstrate the efficacy and safety of DWP-450 in Adults for the treatment of moderate to severe Glabellar Lines (GLs)</td></tr>
<tr><td>Study identifier</td><td colspan="4">Protocol EVB-003; EudraCT Number 2014-001063-12</td></tr>
<tr><td rowspan="4">Design</td><td colspan="4">Multi-centre (4 Canada, 15 Europe) study with block randomisation in a 1:5:5 ratio to receive Placebo, BOTOX® or DWP-450
Subjects confirmed their GLs had an important psychological impact</td></tr>
<tr><td colspan="2">Duration of main phase:</td><td colspan="2">150 days</td></tr>
<tr><td colspan="2">Duration of Run-in phase:</td><td colspan="2">not applicable</td></tr>
<tr><td colspan="2">Duration of Extension phase:</td><td colspan="2">not applicable</td></tr>
<tr><td>Hypothesis</td><td colspan="4">Primary Non-inferiority of DWP-450 over Placebo and Botox, AND
Primary endpoint tested for superiority of DWP-450 over Placebo & Botoxo</td></tr>
<tr><td rowspan="3">Treatments groups</td><td colspan="2">Placebo</td><td colspan="2">1 treatment of 5x 0.1ml saline
49 randomised</td></tr>
<tr><td colspan="2">BOTOX®</td><td colspan="2">1 treatment of 5x 4U (0.1ml) Botox
246 randomised</td></tr>
<tr><td colspan="2">DWP-450</td><td colspan="2">1 treatment of 5x 4U (0.1ml) DWP-450
246 randomised</td></tr>
<tr><td rowspan="5">Endpoints and definitions</td><td>Primary endpoint</td><td>GLS 0 or 1 at max frown</td><td colspan="2">GLS score 0 or 1 at maximum frown by IA at day 30</td></tr>
<tr><td rowspan="2">Secondary endpoint</td><td>HADS-A</td><td colspan="2">Difference in score: Day 90 vs Baseline</td></tr>
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<tr><td>Exploratory endpoint</td><td>GLS at max frown >/=2 point better</td><td colspan="2">GLS score >/=2point better at max frown by IA at day 30</td></tr>
<tr><td>Exploratory endpoint</td><td>GLS at rest >/=2 point better</td><td colspan="2">GLS score >/=2 point better at rest by IA at day 30</td></tr>
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<tr><td rowspan="3">Descriptive statistics and estimate variability</td><td>Treatment group</td><td>Placebo</td><td>Botox</td><td>DWP-450</td></tr>
<tr><td>Subject Numbers</td><td>48</td><td>244</td><td>235</td></tr>
<tr><td>GLS 0 or 1</td><td>4.2%</td><td>82.8%</td><td>87.2%</td></tr>
</table>

	95% asymptotic CI	0.0, 9.8	78.1, 87.5	83.0, 91.5
	GLS at max frown >/=2point improvement	0	68.9%	77.0%
	95% asymptotic CI	0, 0	63.0, 74.7	71.6, 82.4
	GLS at Rest >/=2point Improvement by IA 95% asymptotic CI	0/27 0% 0, 12.8	36/149 (24.2%) 17.5, 31.8	32/133 (24.1%) 17.1, 32.2
	HADS-A: Day 90 vs Day 0 95% CI	-0.9 -1.7, -0.2	-0.9 -1.3, -0.6	-1.1 -1.4, -0.8
	HADS-D: Day 90 vs Day 0 95% CI	-0.5 -1.1, 0	-0.6 -0.9, -0.3	-0.6 -0.9, -0.3

Effect estimate per comparison	Primary endpoint: GLS 0 or 1	Comparison groups	DWP-450 vs Botox
		Absolute difference > -10.0%	4.4
		2 sided 95% asymptotic CI	-1.9, 10.8
		P-value	nsd
	Primary Endpoint: GLS 0 or 1		DWP-450 vs Placebo
		Absolute difference	83.1
		2 sided 95% asymptotic CI	70.3, 89.4
		P-value	<0.001
	Exploratory endpoint: GLS >/=2point improvement at max frown	Comparison groups	DWP-450 vs Placebo
		Absolute difference	77.0
		variability statistic	69.8, 82.3
		P-value	<0.001
	Exploratory endpoint: GLS >/=2point improvement at Rest	Comparison groups	DWP-450 vs Placebo
		Absolute difference	24.1%
		2 sided 95% asymptotic CI	16.8, 31.3
		P-value	p<0.001
	Secondary endpoint: HADS-A Day 90 vs Baseline	Comparison groups	DWP-450 vs Placebo
		Absolute difference	-0.2
		95% CI from two independent samples t-test	-0.9, 0.6
		P-value	nsd
	Secondary endpoint: HADS-D Day 90 vs Baseline	Comparison groups	DWP-450 vs Placebo
		Absolute difference	-0.1
		95% CI from two independent samples t-test	-0.7, 0.6
		P-value	nsd

Notes	Other endpoints GLS scores by SA; GAIS scores, SSS scores

Summary of efficacy for study EV001

Title: A Randomised, DB, placebo controlled single dose trial to demonstrate the safety and efficacy of DWP-450 in adult subjects for the treatment of moderate to severe Glabellar Lines (GLs)			
Study identifier	Protocol EV001 (Evolus-CLIN001)		
Design	Multi-centre US study with block randomisation in a 3:1 atio to receive DWP-450 or Placebo		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		150 days not applicable not applicable
Hypothesis	Primary superiority of DWP-450 over Placebo		
Treatments groups	Placebo		1 treatment of 5x 0.1ml saline 84 randomised
	DWP-450		1 treatment of 5x 4U (0.1ml) DWP-450 246 randomised
Endpoints and definitions	Primary endpoint	GLS at max frown >/=2 point better	GLS score >/=2point better at max frown by IA at day 30 by IA & SA
	Secondary endpoint	GLS at max frown >/=2 point better (IA)	GLS score >/=2point better at max frown by IA at day 30 by IA
	Secondary endpoint	GLS at max frown >/=2 point better (SA)	GLS score >/=2point better at max frown by IA at day 30 by SA
	Exploratory endpoint	GLS 0 or 1 at max frown	GLS score 0 or 1 at maximum frown by IA at day 30
	Other endpoints	GLS at rest >/=2 point better	GLS score >/=2 point better at rest by IA at day 30
Database lock	March 1, 2016		
Results and Analysis			
Analysis description	**Primary Analysis**		
Analysis population and time point description	ITT: Day 30 by Investigator Assessment		
Descriptive statistics and estimate variability	Treatment group	Placebo	DWP-450
	Number of subject	84	246
	GLS at max frown >/=2point improvement, IA & SA	1/83 (1.2%)	162/240 (67.5%)
	95% exact CI	0, 6.5	61.2, 73.4
	GLS at max frown >/=2point improvement, IA	1/83 (1.2%)	186/240 (77.5%)
	95% exact CI	0, 6.5	71.7, 82.6

	GLS at max frown >/=2point improvement, SA	3/83 (3.6%)	184/240 (76.7%)
	95% exact CI	0.8, 10.2	70.8, 81.9
	GLS 0 or 1 at maximum frown (IA)	2/83 (2.4%)	206/240 (85.8%)
	GLS 0 or 1 at maximum frown (SA)	4/83 (4.8%)	199/240 (82.9%)
	GLS at Rest >/=2point Improvement by IA	0/39	43/135 (31.9%)
	95% exact CI	0, 9.0	24.1, 40.4
	GLS at Rest >/=2point Improvement by SA	0/39	72/135 (53.3%)
	95% exact CI	0, 9.0	44.6, 62.0

Effect estimate per comparison	Primary endpoint: GLS at max frown >/=2point improvement, IA & SA	Comparison groups	DWP-450 vs Placebo
		Absolute difference	66.3
		2 sided 95% exact CI	59.0, 72.4
		P-value	<0.001
	Secondary endpoint: GLS at max frown >/=2 point improvement, IA	Comparison groups	DWP-450 vs Placebo
		Absolute difference	76.3
		2 sided 95% exact CI	69.4, 81.7
		P-value	<0.001
	Secondary endpoint: GLS at max frown >/=2 point improvement, SA	Comparison groups	DWP-450 vs Placebo
		Absolute difference	73.1
		2 sided 95% exact CI	65.0, 79.3
		P-value	<0.001
	Other endpoint: GLS at rest >/=2 point improvement, IA	Comparison groups	DWP-450 vs Placebo
		Absolute difference	31.9
		2 sided 95% exact CI	22.6, 40.6
		P-value	<0.001
	Other endpoint: GLS at rest >/=2 point improvement, SA	Comparison groups	DWP-450 vs Placebo
		Absolute difference	53.3
		2 sided 95% exact CI	43.8, 62.0
		P-value	<0.001
Notes	Other endpoints GAIS scores, SSS scores		

Summary of efficacy for study EV002

<u>Title</u>: A Randomised, DB, placebo controlled single dose trial to demonstrate the safety and efficacy of DWP-450 in adult subjects for the treatment of moderate to severe Glabellar Lines (GLs)		
Study identifier	Protocol EV002 (Evolus-CLIN002)	
Design	Multi-centre US study with block randomisation in a 3:1 atio to receive DWP-450 or Placebo	
	Duration of main phase:	150 days

	Duration of Run-in phase: Duration of Extension phase:		not applicable not applicable
Hypothesis	Primary superiority of DWP-450 over Placebo		
Treatments groups	Placebo		1 treatment of 5x 0.1ml saline 78 randomised
	DWP-450		1 treatment of 5x 4U (0.1ml) DWP-450 246 randomised
Endpoints and definitions	Primary endpoint	GLS at max frown >/=2 point better	GLS score >/=2point better at max frown by IA at day 30 by IA & SA
	Secondary endpoint	GLS at max frown >/=2 point better (IA)	GLS score >/=2point better at max frown by IA at day 30 by IA
	Secondary endpoint	GLS at max frown >/=2 point better (SA)	GLS score >/=2point better at max frown by IA at day 30 by SA
	Exploratory endpoint	GLS 0 or 1 at max frown	GLS score 0 or 1 at maximum frown by IA at day 30
	Other endpoints	GLS at rest >/=2 point better	GLS score >/=2 point better at rest by IA at day 30
Database lock	March 1, 2016		

Results and Analysis

Analysis description	**Primary Analysis**		
Analysis population and time point description	ITT: Day 30 by Investigator Assessment		
Descriptive statistics and estimate variability	Treatment group	Placebo	DWP-450
	Number of subject	78	246
	GLS at max frown >/=2point improvement, IA & SA	1/75 (1.3%)	169/240 (70.4%)
	95% exact CI	0, 7.2	64.2, 76.1
	GLS at max frown >/=2point improvement, IA	2/75 (2.7%)	198/240 (82.5%)
	95% exact CI	0.3, 9.3	77.1, 87.1
	GLS at max frown >/=2point improvement, SA	3/75 (4.0%)	183/240 (76.3%)
	95% exact CI	0.8, 10.2	70.8, 81.9
	GLS 0 or 1 at maximum frown (IA)	2/75 (2.7%)	210/240 (87.5%)
	GLS 0 or 1 at maximum frown (SA)	4/75 (5.3%)	193/240 (80.4%)
	GLS at Rest >/=2point Improvement by IA	0/53	65/153 (42.5%)
	95% exact CI	0, 7.0	34.5, 50.7

	GLS at Rest >/=2point Improvement by SA	1/51	83/153 (54.2%)
	95% exact CI	0, 10.4	46.0, 62.3
Effect estimate per comparison	Primary endpoint: GLS at max frown >/=2point improvement, IA & SA	Comparison groups	DWP-450 vs Placebo
		Absolute difference	69.1
		2 sided 95% exact CI	61.5, 75.1
		P-value	<0.001
	Secondary endpoint: GLS at max frown >/=2 point improvement, IA	Comparison groups	DWP-450 vs Placebo
		Absolute difference	79.8
		2 sided 95% exact CI	72.1, 85.2
		P-value	<0.001
	Secondary endpoint: GLS at max frown >/=2 point improvement, SA	Comparison groups	DWP-450 vs Placebo
		Absolute difference	72.3
		2 sided 95% exact CI	63.7, 78.5
		P-value	<0.001
	Other endpoint: GLS at rest >/=2 point improvement, IA	Comparison groups	DWP-450 vs Placebo
		Absolute difference	42.5
		2 sided 95% exact CI	34.3, 50.7
		P-value	<0.001
	Other endpoint: GLS at rest >/=2 point improvement, SA	Comparison groups	DWP-450 vs Placebo
		Absolute difference	52.3
		2 sided 95% exact CI	41.3, 60.8
		P-value	<0.001
Notes	Other endpoints GAIS scores, SSS scores		

Analysis performed across trials (pooled analyses and meta-analysis)

Analysis of Pooled Data from Randomised Placebo Controlled Trials EV001, EV002, EVB003

The inclusion and exclusion criteria were essentially the same in these healthy volunteer, single dose studies, except for EVB003 which asked subjects to confirm the GLs had a significant psychological impact and specified additional exclusion criteria regarding local or systemic conditions precluding trial entry. Inclusion of EVB003 in pooled analyses of efficacy was therefore not supported.

Studies EV001, EV002: >/=2Point improvement in GLS at Maximum Frown, Day 0 to Day 30 by ITT

	Individual Study Data						POOLED Data		
	EV-001 (N=330)		EV-002 (N=324)		EVB-003 (N=294/540)		001+002+003	001+002	001+002+003
Responders at D30 [a]	Placebo (N=84)	DWP-450 (N=246)	Placebo (N=78)	DWP-450 (N=246)	Placebo (N=49)	DWP-450 (N=245)	Placebo (N=211)	DWP-450 (N=492)	DWP-450 (N=737)
By Both IA and SA									
Number [b]	1/83	162/240	1/75	169/240	0/48	147/241	2/206	331/480	478/721
Percentage, %	1.2	67.5	1.3	70.4	0.0	61.0	1.0	69.0	66.3
(95% CI) [c]	(0.0, 6.5)	(61.2, 73.4)	(0.0, 7.2)	(64.2, 76.1)	(0.0, 0.0)	(54.8, 67.2)	(0.1, 3.5)	(64.6, 73.1)	(62.7, 69.7)
Absolute Difference, %		66.3		69.1		61.0		68.0	65.3
(95% CI) [d]		(59.0, 72.4)		(61.5, 75.1)		(53.6, 67.3)		(63.3, 72.3)	(61.3, 69.0)
P-value [d]		<0.001		<0.001		<0.001		<0.001	<0.001
By IA Only									
Number [b]	1/83	186/240	2/75	198/240	0/48	184/241	3/206	384/480	568/721
Percentage, %	1.2	77.5	2.7	82.5	0.0	76.3	1.5	80.0	78.8
(95% CI) [c]	(0.0, 6.5)	(71.7, 82.6)	(0.3, 9.3)	(77.1, 87.1)	(0.0, 0.0)	(71.0, 81.7)	(0.3, 4.2)	(76.1, 83.5)	(75.6, 81.7)
Absolute Difference, %		76.3		79.8		76.3		78.5	77.3
(95% CI) [d]		(69.4, 81.7)		(72.1, 85.2)		(69.1, 81.6)		(74.2, 82.3)	(73.6, 80.6)
P-value [d]		<0.001		<0.001		<0.001		<0.001	<0.001
By SA Only									
Number [b]	3/83	184/240	3/75	183/240	2/48	170/241	8/206	367/480	537/721
Percentage, %	3.6	76.7	4.0	76.3	4.2	70.5	3.9	76.5	74.5
(95% CI) [c]	(0.8, 10.2)	(70.8, 81.9)	(0.8, 11.2)	(70.4, 81.5)	(0.0, 9.8)	(64.8, 76.3)	(1.7, 7.5)	(72.4, 80.2)	(71.1, 77.6)
Absolute Difference, %		73.1		72.3		66.4		72.6	70.6
(95% CI) [d]		(65.0, 79.3)		(63.7, 78.5)		(55.7, 73.5)		(67.6, 76.9)	(66.0, 74.5)
P-value [d]		<0.001		<0.001		<0.001		<0.001	<0.001

There is a 5% - 10% discordance between investigator and subject assessment at maximum frown.

Studies EV001, EV002: >/=2Point improvement in GLS at Rest by IA and SA, Day 0 to Day 30 by ITT

	Individual Study DWP-450 Data			POOLED Data		
	EV-001	EV-002	EVB-003	001+002+003	001+002	001+002+003
Responders [a]	DWP-450 (N=136)	DWP-450 (N=157)	DWP-450 (N=135)	Placebo (N=121)	DWP-450 (N=293)	DWP-450 (N=428)
Number [b]	35/135	50/153	20/132	0/117	85/288	105/420
Percentage, %	25.9	32.7	15.2	0.0	29.5	25.0
(95% CI) [c]	(18.8, 34.2)	(25.3, 40.7)	(9.5, 22.4)	(0.0, 3.1)	(24.3, 35.1)	(20.9, 29.4)
Absolute Difference, %					29.5	25.0
(95% CI) [d]					(24.3, 35.2)	(20.9, 29.5)
P-value [d]					<0.001	<0.001

Lower rates of improvement in GLs are seen when subjects are assessed at rest.

Studies EV001, EV002: >/=2 Point Improvement in GLS at Maximum Frown from Day 0 to Day 30 by IA & SA by Age – ITT

	Individual Study DWP-450 Data			POOLED Data		
	EV-001	EV-002	EVB-003	001+002+003	001+002	001+002+003
Responders [a] by Age Group	DWP-450 (N=246)	DWP-450 (N=246)	DWP-450 (N=245)	Placebo (N=211)	DWP-450 (N=492)	DWP-450 (N=737)
<65 years						
N	220	219	228	192	439	667
Number [b]	150/215	155/214	137/224	2/188	305/429	442/653
Percentage, %	69.8	72.4	61.2	1.1	71.1	67.7
(95% CI) [c]	(63.2, 75.8)	(65.9, 78.3)	(54.4, 67.6)	(0.1, 3.8)	(66.6, 75.3)	(64.0, 71.3)
Absolute Difference, %					70.0	66.6
(95% CI) [d]					(65.1, 74.5)	(62.4, 70.4)
P-value [d]					<0.001	<0.001
≥65 years						
N	26	27	17	19	53	70
Number [b]	12/25	14/26	10/17	0/18	26/51	36/68
Percentage, %	48.0	53.8	58.8	0.0	51.0	52.9
(95% CI) [c]	(27.8, 68.7)	(33.4, 73.4)	(32.9, 81.6)	(0.0, 18.5)	(36.6, 65.2)	(40.4, 65.2)
Absolute Difference, %					51.0	52.9
(95% CI) [d]					(30.6, 65.5)	(34.0, 65.2)
P-value [d]					<0.001	<0.001

Rates of improvement in GLs at maximum frown are 19 percentage points lower in older subjects for studies EV001, EV002 but little different in study EVB003.

Studies EV001, EV002: >/=2 Point Improvement in GLS at Maximum Frown from Day 0 to Day 30 by IA & SA by Sex – ITT

	Individual Study DWP-450 Data			POOLED Data		
	EV-001	EV-002	EVB-003	001+002+003	001+002	001+002+003
Responders [a] by Sex	DWP-450 (N=246)	DWP-450 (N=246)	DWP-450 (N=245)	Placebo (N=211)	DWP-450 (N=492)	DWP-450 (N=737)
Female						
N	227	220	220	190	447	667
Number [b]	150/221	158/216	134/216	2/185	308/437	442/653
Percentage, %	67.9	73.1	62.0	1.1	70.5	67.7
(95% CI) [c]	(61.3, 74.0)	(66.7, 78.9)	(55.2, 68.5)	(0.1, 3.9)	(66.0, 74.7)	(64.0, 71.3)
Absolute Difference, %					69.4	66.6
(95% CI) [d]					(64.5, 73.8)	(62.4, 70.4)
P-value [d]					<0.001	<0.001
Male						
N	19	26	25	21	45	70
Number [b]	12/19	11/24	13/25	0/21	23/43	36/68
Percentage, %	63.2	45.8	52.0	0.0	53.5	52.9
(95% CI) [c]	(38.4, 83.7)	(25.6, 67.2)	(31.3, 72.2)	(0.0, 16.1)	(37.7, 68.8)	(40.4, 65.2)
Absolute Difference, %					53.5	52.9
(95% CI) [d]					(35.5, 68.8)	(36.0, 65.6)
P-value [d]					<0.001	<0.001

Rates of improvement in GLs at maximum frown are 16 percentage points lower in male subjects for studies EV001, EV002 and 10 percentage points lower in study EVB003.

Studies EV001, EV002: >/=2 Point Improvement in GLS at Maximum Frown from Day 0 to 30 by IA & SA by Skin Type – ITT

Fitzpatrick Skin Type	Placebo (EV001, 002, 003) (n=211)	DWP-450 (EV001, 002) (n=492)	Absolute Difference (95% CI)
I (pale white skin)	0/12	17/21 (81%)	81.0 (51.6, 94.6)
II (Fair skin, blue eyes)	0/62	103/142 (72.5%)	72.5 (64.4, 79.7)
III (Darker white skin)	1/84	121/184 (65.8%)	64.6 (56.7, 71.6)
IV (Light brown skin)	1/35	61/92 (66.3%)	63.4 (49.3, 73.9)
V (brown skin)	0/9	21/29 (72.4%)	72.4 (52.8, 87.3)
VI (dark brown or black)	0/4	8/12 (66.7%)	66.7 (2.0, 90.1))

Rates of improvement in GLs at maximum frown are similar across the main Fitzpatrick skin types, although subtypes I, V and VI are underrepresented.

Studies EV001, EV002: >/=2 Point Improvement in GLS at Maximum Frown from Day 0 to 30 by IA by Baseline GLS – ITT

IA	Placebo (EV001, 002, 003) (n=211)	DWP-450 (EV001, 002) (n=492)	Absolute Difference (95% CI)
Moderate → No GLs	0/53	73/117 (62.4%)	62.4 (53.0, 71.2))
Severe – No/Mild GLs	2/153	258/363 (71.1%)	69.8 (64.4, 75.7)

Rates of improvement in GLs are 7.4 percentage points lower for those assessed as moderate versus severe at baseline assessment.

Studies EV001, EV002: >/=2 Point Improvement in GLS at Maximum Frown by IA and SA, ITT, by Previous Exposure

Responders [a] by Prior History of Botulinum Treatment	Individual Study DWP-450 Data			POOLED Data		
	EV-001 DWP-450 (N=246)	EV-002 DWP-450 (N=246)	EVB-003 DWP-450 (N=245)	001+002 Placebo (N=162)	001+002 DWP-450 (N=492)	001+002+003 DWP-450 (N=737)
Yes						
N	103	91	NA	57	194	NA
Number [b]	72/100	70/89		1/55	142/189	
Percentage, %	72.0	78.7		1.8	75.1	
(95% CI) [c]	(62.1, 80.5)	(68.7, 86.6)		(0.0, 9.7)	(68.3, 81.1)	
Absolute Difference, %					73.3	
(95% CI) [d]					(64.1, 79.9)	
P-value [d]					<0.001	
No						
N	143	155	NA	105	298	NA
Number [b]	90/140	99/151		1/103	189/291	
Percentage, %	64.3	65.6		1.0	64.9	
(95% CI) [c]	(55.8, 72.2)	(57.4, 73.1)		(0.0, 5.3)	(59.2, 70.4)	
Absolute Difference, %					64.0	
(95% CI) [d]					(57.7, 69.6)	
P-value [d]					<0.001	

Rates of improvement in GLs are maximum frown are 9 percentage point slower in subjects with no previous exposure to treatment with botulinum toxin.

Duration of response

EV001, EV002: >/=2 Point Improvement in GLS at Maximum Frown by IA and SA: Time to Response, Duration of Response – ITT

	Placebo (EV001, 002, 003) (n=211)	DWP-450 (EV001, 002) (n=492)
Responders	5/211 (2.4%)	369 (75.0%)
Stopped Responding (*within 150 days to EOS)*	5/5	345/369 (93.5%)
Did not stop Responding	0/5	24/369 (6.5%)
Time to Response (days) 50% (95% CI)	Not evaluable	10 (9, 11)
Duration of Response (days)		
Stopped Responding: 50% (25%, 75%)	13 (11, 92) days	85 (77, 113) days

The treatment response at maximum frown is evident within 2 weeks and in >50% of subjects is no longer evident within 3 months.

Post hoc analyses

Duration of Response: analysis performed using the ITT population to estimate duration of response as the time until 50% (25%, 75%) of responders no longer showed a 1-point improvement in GLS

score at maximum frown compared to baseline. The response rate by IA (SA) for placebo, Botox and DWP-450 was 28.6% (28.6%), 95.9% (96.3%), 96.7% (94.7%) respectively. The duration of response by IA placebo vs Botox vs DWP-450 was 52 (29, 85) vs 132 (99, 154) vs 139 (106, 155) days, and by SA was 65 (32, 85) vs 124 (89, 155) vs 136 (86, 156) days respectively.

Clinical responses of GLS scores of 0 or 1 at maximum frown by IA at day 30 versus baseline variables: baseline GLS =2, or 3 at maximum frown by IA; baseline GLS at Rest; prior exposure to botulinum toxin; age; and gender.

Subjects with **Baseline GLS 2 or 3** versus Day 30 GLS 0 or 1, by IA at maximum frown

	Placebo EV001+002+003 (N=211)			DWP450 EV001+002 (N=492)			DWP450 EV003 (N=245)		
	n'	GLS=0 n (%)	GLS=1 n (%)	n'	GLS=0 n (%)	GLS=1 n (%)	n'	GLS=0 n (%)	GLS=1 n (%)
Baseline GLS at Maximum Frown by Investigator Assessment									
2 (Moderate)	53	0	3 (5.7)	117	79 (67.5)	32 (27.4)	62	35 (56.5)	25 (40.3)
3 (Severe)	153	1 (0.7)	2 (1.3)	363	154 (42.4)	151 (41.6)	179	41 (22.9)	108 (60.3)

Subjects with **Baseline GLS at Rest of** </=1 or >1 vs Day 30 GLS = 0 or 1 at Maximum Frown by IA

	Placebo EV001+002+003 (N=211)			DWP450 EV001+002 (N=492)			DWP450 EV003 (N=245)		
	n'	GLS=0 n (%)	GLS=1 n (%)	n'	GLS=0 n (%)	GLS=1 n (%)	n'	GLS=0 n (%)	GLS=1 n (%)
Baseline GLS at Rest by Investigator Assessment									
<=1	88	0	3 (3.4)	186	126 (67.7)	54 (29.0)	103	61 (59.2)	40 (38.8)
>1	118	1 (0.8)	2 (1.7)	294	107 (36.4)	129 (43.9)	138	15 (10.9)	93 (67.4)

Subjects with **Prior Exposure to Botulinum Toxin** vs Day 30 GLS = 0 or 1 at Maximum Frown by IA

	GLS at Maximum Frown by Investigator Assessment at Day 30					
	Placebo EV001+002 (N=162)			DWP450 EV001+002 (N=492)		
	n'	GLS=0 n (%)	GLS=1 n (%)	n'	GLS=0 n (%)	GLS=1 n (%)
Prior Exposure to Botulinum Toxin						
Yes	55	0	2 (3.6)	188	95 (50.5)	76 (40.4)
No	103	1 (1.0)	1 (1.0)	292	138 (47.3)	107 (36.6)

N.B. Data not collected for EVB003

Subjects **with Age <65 or >/=65 years** versus Day 30 GLS = 0 or 1 at Maximum Frown by IA

	Placebo EV001+002+003 (N=211)			DWP450 EV001+002 (N=492)			DWP450 EV003 (N=245)		
	n'	GLS=0 n (%)	GLS=1 n (%)	n'	GLS=0 n (%)	GLS=1 n (%)	n'	GLS=0 n (%)	GLS=1 n (%)
Age Group									
<65	188	1 (0.5)	5 (2.7)	429	214 (49.9)	165 (38.5)	224	74 (33.0)	121 (54.0)
>=65	18	0	0	51	19 (37.3)	18 (35.3)	17	2 (11.8)	12 (70.6)

Gender versus Day 30 GLS = 0 or 1 at Maximum Frown by IA

	Placebo EV001+002+003 (N=211)			DWP450 EV001+002 (N=492)			DWP450 EV003 (N=245)		
	n'	GLS=0 n (%)	GLS=1 n (%)	n'	GLS=0 n (%)	GLS=1 n (%)	n'	GLS=0 n (%)	GLS=1 n (%)
Sex									
Female	185	1 (0.5)	4 (2.2)	437	222 (50.8)	162 (37.1)	216	73 (33.8)	115 (53.2)
Male	21	0	1 (4.8)	43	11 (25.6)	21 (48.8)	25	3 (12.0)	18 (72.0)

In study EVB003 using GLS scores at maximum frown by IA, subjects with a baseline GLS of 2 or 3 achieved a day 30 GLS score of 0 & 1 in 56.5% & 40.3%, or 22.9% & 60.3% respectively. In subgroup

analysis, subjects with lower baseline GLS at rest in EVB003 were more likely to achieve day 30 GLS score of 0 vs 1 at maximum frown by IA, at 59.2% vs 38.8% for GLS <1 at rest and 10.9% vs 67.4% for GLS>1 at rest. Subjects in EV001 + EV002 with prior exposure to botulinum toxin are more likely to achieve day 30 GLS scores at maximum frown by IA of 0 vs 1 at 50.5% vs 40.4% compared with 47.3% vs 36.6% with no prior exposure. Subjects >65 years old or males were more likely than younger or females to achieve GLS = 1 vs 0 at day 30 on maximum frown by IA.

Day 30 Subject Satisfaction Scale, SSS vs Global Aesthetic Improvement Scale, GAIS, scores

Subject Satisfaction Scale Scores at Day 30 (>0, satisfied or very satisfied) by Day 30 GLS by SA

	Subject Satisfaction Score at Day 30					
	Placebo EV001+002+003 (N=211)		DWP450 EV001+002 (N=492)		DWP450 EV003 (N=245)	
	n'	SSS >0 n (%)	n'	SSS >0 n (%)	n'	SSS >0 n (%)
GLS at Maximum Frown by Subject Assessment at Day 30						
0 (None)	2	0	205	205 (100)	77	77 (100)
1 (Mild)	9	7 (77.8)	186	181 (97.3)	112	109 (97.3)
2 (Moderate)	42	4 (9.5)	63	46 (73.0)	33	29 (87.9)
3 (Severe)	153	2 (1.3)	25	4 (16.0)	18	4 (22.2)

Global Aesthetic Improvement Scale Scores by SA at Day 30 (>0, improved or much improved) by Day 30 GLS scores by SA

	GAIS by Subject Assessment at Day 30					
	Placebo EV001+002+003 (N=211)		DWP450 EV001+002 (N=492)		DWP450 EV003 (N=245)	
	n'	GAIS >0 n (%)	n'	GAIS >0 n (%)	n'	GAIS >0 n (%)
GLS at Maximum Frown by Subject Assessment at Day 30						
0 (None)	2	0	205	203 (99.0)	77	77 (100)
1 (Mild)	9	8 (88.9)	187	179 (95.7)	113	112 (99.1)
2 (Moderate)	42	7 (16.7)	63	52 (82.5)	33	30 (90.9)
3 (Severe)	153	1 (0.7)	25	9 (36.0)	18	8 (44.4)

Neither the Subject Satisfaction Scale (SSS) nor the Global Aesthetic Improvement Scale (GAIS) scores are validated, but with this caveat subjects in study EVB003 with a GLS score of 0 or 1 vs 2 or 3

at maximum frown by IA are more likely to have SSS scores of 1 or 2 186/189 (98.4%) vs 33/51 (64.7%) and GAIS scores of 1 or 2 at 189/190 (99.5) vs 38/51 (74.5%).

Clinical studies in special populations

There were no specific studies in special populations.

Less than 10% of subjects in EVB003 were ≥65 years old.

Supportive studies

Methods

EV004, EV006 were two open-label multi-dose, multi-centre (11, 18), US, studies which investigated the safety of DFWP-450 in adults for the treatment of moderate-to-severe glabellar lines. Subjects were screened and assessed then treated on day 0 with 20U DWO-450 as 5x4U/0.1ml im injections as per studies EV001, EV002, EVB003. Subjects were followed in the office on Days 3, 7, 14, 30. On day 90 subjects were assessed as eligible for repeat treatment if their GLS score was ≥2 at maximum frown by IA else followed monthly until eligible for repeat treatment, or until the study ended on Day 365. After retreatment, follow-up was at the same intervals with day 3, 14 reviews by telephone call. Subjects could receive a maximum of 4 treatments, no treatment allowed after Day 330. Inclusion criteria were like the EV001, EV002 with no assessment of psychological impact. Exclusion criteria included previous treatment with botulinum toxin of any serotype in the forehead area within the last 8 months.

Exploratory objectives were to evaluate the efficacy of DWP-450 to reduce the severity of moderate to severe glabellar lines by IA at maximum frown and at rest by GLS score; SA of aesthetic improvement on the GAIS and SA using the SSS.

Study period was 1 year. First enrolled 15th Sept 2014, last patient completed 23 Nov 2015 in EV004; first patient enrolled 7th May 2015, last patient completed 25th Aug 2016 in study EV006.

Blood samples for immunogenicity assessment were collected prior to and at days 30, 90 of each treatment; and at study end or early termination.

Results: Database lock Sept 12, 2016 (EV-004), Dec 22, 2016 (EV006)

Summary Extent of Exposure, Number of Days Between Treatments in EV004, EV006

Total Number of Days Between Treatments	EV004 Study Completers (N=297) mean ± SD [min, max]	median	EV006 Study Completers (N=487) mean ± SD [min, max]	median
Subjects Who Received Only 1 Treatment	**(n=5)**		**(n=6)**	
From IT to end of study	363.8 ± 3.63 [359, 369]	363	362.8 ± 4.75 [356, 370]	363
Subjects Who Received a Total of 2 Treatments	**(n=43)**		**(n=66)**	
Between IT and RT1	206.7 ± 50.83 [95, 330]	202	199.4 ± 53.02 [82, 330]	190
From RT1 to end of study	161.4 ± 53.34 [28, 272]	172	164.3 ± 55.06 [28, 283]	180
Subjects Who Received a Total of 3 Treatments	**(n=98)**		**(n=203)**	
Between IT and RT1	129.7 ± 28.75 [89, 238]	123	130.6 ± 30.89 [82, 212]	125
Between RT1 and RT2	143.8 ± 31.22 [84, 233]	139.5	137.0 ± 30.38 [84, 217]	132
From RT2 to end of study	94.2 ± 38.83 [28, 169]	95	94.6 ± 42.45 [27, 203]	91
Subjects Who Received a Total of 4 Treatments	**(n=151)**		**(n=212)**	
Between IT and RT1	94.5 ± 10.03 [83, 128]	91	93.9 ± 11.85 [77, 145]	91
Between RT1and RT2	98.3 ± 18.04 [77, 156]	91	96.1 ± 14.38 [82, 160]	91
Between RT2 and RT3	99.8 ± 15.71 [79, 167]	92	99.7 ± 15.44 [65, 154]	92
From RT3 to end of study	73.3 ± 26.75 [21, 127]	83	74.1 ± 23.17 [25, 140]	81.5

(Source: EV-004 CSR Tabel 30, and EV-006 CSR Table 37)

Post hoc analysis

Duration of Response (GLS=0, 1 at maximum frown by IA) after 1st injection by Baseline Score

Duration of Response: GLS=0	**Baseline GLS=2**	**Baseline GLS=3**
(days, Estimate and 95% CI)	EV004+006 (N=253)	EV004+006 (N=669)
Responders: n (%)		
Subjects who stop responding	213 (98.6)	374 (97.4)
Censored	3 (1.4)	10 (2.6)
All	216	384
Time to stop responding(days):		
25% subjects stop responding	66.0 [29.0, 76.0]	29.0 [22.0, 57.0]
50% subjects stop responding	80.0 [79.0, 83.0]	79.0 [78.0, 82.0]
75% subjects stop responding	90.0 [86.0, 93.0]	86.0 [85.0, 88.0]

Duration of Response: GLS=1	**Baseline GLS=2**	**Baseline GLS=3**
(days, Estimate and 95% CI)	EV004+006 (N=253)	EV004+006 (N=669)
Responders: n (%)		
Subjects who stop responding	30 (90.9)	216 (96.9)
Censored	3 (9.1)	7 (3.1)
All	33	223
Time to stop responding(days):		
25% subjects stop responding	78.0 [67.0, 88.0]	72.0 [64.0, 77.0]
50% subjects stop responding	93.0 [80.0, 112.0]	85.0 [82.0, 85.0]
75% subjects stop responding	113.0 [94.0, 150.0]	90.0 [87.0, 93.0]

The estimated median (iqr) duration of response in those achieving a GLS score of 0 vs 1 for baseline GLS = 2 was 80 (66 - 90) days vs 93 (78 - 113) days and for baseline GLS =3 was 79 (29 - 86) days vs 85 (72 - 90) days respectively. These data are comparable with study EVB003 where the duration of response, >/=2-point improvement in GLS score at maximum frown by IA, was a median (25th, 75th centile) of 78 (68, 91) days.

2.5.3. Discussion on clinical efficacy

The proposed therapeutic indication for DWP-450 is '*When the severity of the following facial lines has an important psychological impact in adult patients, Nuceiva is indicated for the temporary improvement in the appearance of moderate to severe vertical lines between the eyebrows seen at maximum frown (glabellar lines)*'. In this regard, a single pivotal European and Canadian multi-centre

study recruited 540 subjects with moderate or severe glabellar lines (GLs) at maximum frown for a randomised single treatment trial of DWP-450 versus Botox versus Placebo. In addition, two confirmatory US placebo-controlled studies recruited 330 and 324 subjects with moderate or severe GLs at maximum frown for a randomised single treatment trial of DWP-450, *without assessing psychological impact*. Additional supportive information is available from two US open label repeat treatment studies of DWP-450 recruiting 352 and 570 subjects with moderate or severe GLs at maximum frown, again *without assessment of psychological impact* in line with the "cosmetic" use of this type of product approved in the US (Botox® Cosmetic).

Design and conduct of clinical studies

In the European trial, EVB003, no upper age limit is applied, in contrast to a restriction to adults below 65 years of age from some botulinum toxin products depending on the data submitted. The number recruited to the European trial was consistent with the revised power calculations which suggested 497 subjects, increased during study monitoring to 540 subjects to allow for 10% missing data at day 30, would provide 80% power to demonstrate the non-inferiority of DWP-450 versus Botox using a responder rate of 0.85, a non-inferiority margin of 0.10, and a one-sided Type I error rate of 2.5%. A block randomisation schedule was used by centre to allocate subjects in a 5:5:1 ratio to receive DWP-450, BOTOX® or placebo, without stratification by centre, in a double-blinded design. Prior history of botulinum toxin treatment was not collected but this potential source of bias should be taken care of by the process of randomisation.

In contrast, the numbers recruited to the single treatment US studies, *EV001, EV002,* were *based on safety considerations*, and subjects randomised 3:1 to DWP-450 or Placebo in a double- blinded design. The numbers recruited to the repeat treatment open label US studies, EV004, EV006, were also based on safety considerations.

Treatment dose

No clinical dose response studies were performed to compare efficacy versus BOTOX®, sex, age ≥65 years, previous exposure to botulinum toxin treatment, or poor response to previous treatment.

The dose of DWP-450 used was matched to the number of toxin units indicated for BOTOX® Cosmetic, a licensed botulinum toxin type A medicinal product; comparability of pharmacology and non-clinical studies. Evidence of comparable clinical efficacy of DWP-450 and BOTOX® Cosmetic is claimed from Korean registration studies demonstrating comparable efficacy and safety in the clinical studies between DWP-450 and Botox over doses of 20U and 360U (lyophilised DWP-450 used; data not presented). There is sufficient evidence of comparable biological activity and potency of the lyophilised and vacuum dried formulations of DWP-450.

A single treatment dose was used. At each treatment, subjects received 5 x4U (0.1ml) of DWP-450 or BOTOX® injection or an equal volume of saline, given as i.m. injections into standard sites of each corrugator muscle and the midline of the procerus muscle.

Single treatment efficacy studies

The placebo-controlled efficacy trials are single treatment only. The three-arm design of EVB003 is the recommended design for demonstrating non-inferiority, allowing for direct proof of efficacy of the new treatment by comparison to Placebo and some within-trial validation of the choice of non-inferiority margin. No active treatment extension was performed.

DWP-450 generates temporary changes requiring repeated treatments *3 – 4 times each year* in >80% of subjects to maintain the clinical effect. Accepting the limitations of open label studies EV004 and EV006, up to 50% of subjects received 4 administrations of DWP-450 over 1 year to maintain a GLS

score <2 at maximum frown. The interval between treatments decreased as the number of treatments increased, with the time to 2nd and 3rd treatments falling from 202 to 91 days and 139.5 to 91 days respectively in study EV004.

Additional analyses provided demonstrate a consistent trend of shorter duration of responses and hence shorter treatment intervals for subjects in the 4 versus 3 versus 2 treatment cohorts, with a similar trend by age, gender, and in subjects with / without treatment-emergent adverse events (TEAEs) or drug related TEAEs. There is a consistent trend within each treatment cohort of a small increase in interval between subsequent treatments. Some 25% of subjects with 3 or 4 treatments had increased gap time between the duration of response and interval to treatment for reasons which are unclear.

Subjects recruited

The subjects recruited were similar in each study, predominantly white females, and 50% of women were potentially able to have children in the European study. The mean age was 49 years with 17 (6.9%) subjects ≥65 years old receiving DWP-450 in the European study EVB003, versus 26 (10.6%) in EV001 and 27 (11.0%) in EV002 – too few to be confident that clinical efficacy can be established in this age group.

A high proportion of subjects were recruited with no or mild GLs at rest by investigator assessment (IA) 40.7% in EVB003, 45.4% EV001, 34.3% EV002.

Prior treatment with botulinum toxin affects response rates and the immunogenicity assessment - these data were not collected in the pivotal study EVB003 but this potential source of bias should have been taken care of by the process of randomisation. The rate of prior treatment with botulinum toxin type A was 134/330 (40.6 %) in EV001, and 115/324 (35.5%) in EV002.

Validation of Assessment tools

The main assessment tool was a 4-point Glabellar Line Scale, **GLS**, score with separate photonumeric scales at maximum frown and at rest, pre-validated for IA, simple kappa coefficients of 0.819, 0.782 for intra-rate variability and Fleiss's generalised kappa coefficient of 0.635, 0.512 respectively for inter-rater variability (<0.60 regarded as inadequate, >0.80 is preferred as the minimum acceptable interrater agreement, *see* McHugh 2012). Investigators were trained and certified when achieving >80% in a grading test, but inter-rater variability was not tested "in the field". GLS scoring was not validated for SA although subjects were provided with onsite training, and a training manual at each visit.

The unvalidated Global Aesthetic Improvement Scale (GAIS) by IA, SA and Subject Satisfaction Scale (SSS) scores deployed were not unique to these studies. Neither the GAIS or the SSS are validated patient-related outcomes (PROs) yet a range of generic validated PROs have been applied for the assessment of aesthetic procedures including the generic tool Freiburg Life Quality Assessment, core version FLQA-c (Sommer 2003) and condition specific PROs such as the Freiburg questionnaire on aesthetic dermatology and cosmetic surgery FQAD (Sommer 2003), Satisfaction with Facial Appearance scale FACE-Q (Panchapakesan 2013, Pusic 2013) and the Facial Line Outcomes Questionnaire FLO-11 (Yaworsky 2014).

Psychological impact was assessed in the pivotal European study only, which had an entry criterion requiring subjects to confirm verbally that the glabellar lines have a significant psychological impact (on mood, anxiety and/or depressive symptoms). Evidence of the psychological impact of treatment was assessed using HADS-Anxiety, HADS-Depression scores for subjects at study entry, day 30 and

day 90, looking for evidence of variation within the "normal range", together with the claim by the Applicant that no minimally important clinical difference could be defined.

Blinding and Centralised Assessment

Botulinum toxin type A treatment has a pronounced effect risking unblinding in subjects who have previously received treatment. There is no independent centralised assessment of the primary endpoints, which might also have improved inter-rater variability, and the applicant regards the photographic images collected inadequate for retrospective cross validation. Randomisation patterns are unbalanced in favour of test treatment at 3:1 for EV001 & EV002 and 10 (DWP-450 or Botox):1 in EVB003. These factors each contribute to the significant risk of unblinding of study treatment and the consequential risk of biased outcome assessments. These concerns are mitigated in the pivotal EVB003 study by the inclusion of an active treatment control and the safeguards of randomisation.

Immunogenicity Assessment

No immunogenicity assessment was carried out in the pivotal European study although it was included in each of the other studies. Minimal validation data are provided for the screening and confirmatory bridging ELISAs for ADAs. The Applicant suggests false positive tests may arise with anti-tetanus Abs but regards this as insignificant although a false positive pre-treatment would prevent the detection of seroconversion after DWP-450.

The screening assay for ADA has poor sensitivity at 3 µg/mL using the CF for NHS (1.048) and is not defined for the assay with the pre-dose patient serum CF (1.204). Assay thresholds were statistically defined for the screening assay, 5% false positives, but statistical analysis to establish the threshold for the confirmatory assay is not described. Neither a more sensitive ELISA nor optimisation of sensitivity is undertaken in contrast to earlier reports (Dressler 2014, An ELISA for detection of or detection of botulinum toxin Abs). Neither linearity of testing nor titering of confirmed ADA positives is described in any of the reports provided.

No validation data are provided for the determination of neutralising Abs (Nabs) by testing the qualitative impact on botulinum toxin in the mouse LD50 assay. It is noted that the selection of the LD50 assay rather than the mouse phrenic nerve hemi-diaphragm assay which is some 25x more sensitive is not justified (reviewed by Naumann 2013).

In summary, the immunogenicity assays are insensitive, poorly validated, risk generating results for binding ADA and NAbs which are substantially lower than reported in the literature and are therefore regarded as not fit for purpose.

Considering the impact of binding ADA and Nabs on loss of treatment efficacy, the Applicant is requested to perform a non-interventional immunogenicity analysis and re-test the previously negative sera from studies EV-001, EV-002, EV-004, EV006 using new binding ADA assays for screening, confirmation and determination of ADA titres, supported by a sensitive assay for neutralising ADA. This commitment is reflected in the RMP as an additional pharmacovigilance activity.

Endpoints

Different primary endpoints were chosen for EVB003 and studies EV001, EV002. The Applicant regarded the targeted optimal level of residual facial expression after a botulinum toxin is a GLS score of 0 (no wrinkles) or 1 (mild) at maximum frown since it:

- Best reflects the mechanism of action of botulinum toxin as it applies to the baseline physical characteristics of the intended patient population
- Reflects the wishes of that population,

and thus, was considered the most clinically relevant GLS endpoint in the DWP-450 clinical development programme. This was agreed by the CHMP.

The primary endpoint in EVB003 was a GLS score of 0 or 1 (none or mild), at day 30 by IA by PP, which will include those with a 1-point improvement in GLS if they score 2 (moderate) at baseline. Despite the fact that in the scientific advice (EMA/CHMP/SAWP/130926/2016) noted that a 1-point change was considered quite a modest level of improvement, the CHMP agreed that the use of 1 point for the definition of the primary endpoint was acceptable.

The EV-001, EV-002 studies used a statistically onerous, rather than clinically relevant, primary endpoint: a two-point composite whereby a subject is judged a responder only if both IA and SA independently and simultaneously agreed a two-point improvement on GLS at maximum frown on Day 30 relative to the baseline score.

The US retreatment studies require a GLS score at maximum frown of <2 by IA before subjects are eligible for retreatment.

A systematic appraisal of whether residual facial expression is desirable is lacking but additional analysis of subject's response by baseline parameters is provided to assist doctors and their patients on the chances of achieving different degrees of therapeutic effect.

Efficacy data and additional analyses

Subjects were reasonably balanced for baseline characteristics in each of the single dose studies across the treatment groups. In the pivotal European study EVB003, subjects confirmed verbally that their glabellar lines had an important psychological impact (on mood, anxiety and/or depressive symptoms).

The non-inferiority margin for absolute difference for the primary endpoint, a GLS score of 0 or 1 at maximum frown at day 30 by IA, was 4.4 (95% CI -1.9, 10.8) for DWP-450 vs Botox, within the -10% non-inferiority margin; it was felt that clinically a difference of 10% would not be noticeable. Similarly, for subjects $\geq$65 years the non-inferiority margin was 14.6 (-14.4, 43.6) for DWP-450 (n=16) versus Botox (n=18).

The number of older subjects studied is too small to address key concerns about the efficacy of botulinum toxin in the elderly where the pathogenesis of wrinkles (including glabellar lines) may reflect gravity-induced tissue sagging due to thinner, less elastic skin and weaker facial muscles rather than muscle contraction. Thus, additional resurfacing procedures may be necessary to achieve visible differences in appearance, although Botulinum toxin may help soften wrinkles that are noticeable even without muscle contraction (reviewed by C M Cheng 2007, Cosmetic use of botulinum toxin type A in the elderly, Clinical Interventions in Aging 2007:2(1) 81–83).

Superiority of DWP-450 over Botox was not formally tested for any endpoint. Responses are substantially higher than placebo irrespective of the assessment threshold applied for both DWP-450 and Botox, with the absolute difference from placebo depending on the assessment performed. Assessments by subjects appeared supportive. Neither the Subject Satisfaction Scale (SSS) nor the Global Aesthetic Improvement Scale (GAIS) scores were validated, but with this caveat subjects in study EVB003 with a GLS score of 0 or 1 vs 2 or 3 at maximum frown by IA are more likely to have SSS scores of 1 or 2 186/189 (98.4%) vs 33/51 (64.7%) and GAIS scores of 1 or 2 at 189/190 (99.5) vs 38/51 (74.5%).

Fitzpatrick skin types were not included in the sensitivity analysis for EVB003 and DWP-450 treated subjects were limited to 4 with type V (brown skin) and 1 with type VI (dark brown or black skin).

Evidence of a significant response across skin types was presented from pooled data from EV001, EV002 (with placebo values for EV001, EV002, EVB003) using a ≥2point improvement in GLS score at maximum frown by IA and SA at day 30 to show an absolute difference from placebo for the most common skin types III of 64.6 (95% CI 56.7, 71.6) and IV 63.4 (49.3, 73.9) versus type V 72.4 (29.9, 87.4), and type VI 66.7 (2.0, 90.1). Similar pooled analysis showed treatment responses were lower in males than females 53.5 (35.5, 68.8) versus 69.4 (64.5, 73.8), and in subjects with no prior history of botulinum toxin use at 64.0 (57.7, 69.6) versus 73.3 (64.1, 79.9).

The duration of response in the placebo-controlled trials was assessed by the maintenance of a >/=2point improvement in GLS score at maximum frown by IA, with a median (iqr) of 78 (68, 91) days in EVB003, 85 (76, 113) days in EV001, and 86 (78, 113) days in EV002; with 3.5%, 6.5%, 6.5% respectively remaining as responders through day 150. In the open label studies EV004, EV006, the estimated median (iqr) duration of response in those achieving a GLS score of 0 vs 1 for baseline GLS = 2 was 80 (66 - 90) days vs 93 (78 - 113) days and for baseline GLS =3 was 79 (29 - 86) days vs 85 (72 - 90) days respectively.

In post hoc subgroup analysis, subjects with lower baseline GLS at rest in EVB003 were more likely to achieve day 30 GLS score of 0 vs 1 at maximum frown by IA, at 59.2% vs 38.8% for GLS <1 at rest and 10.9% vs 67.4% for GLS>1 at rest. Subjects in EV001 + EV002 with prior exposure to botulinum toxin are more likely to achieve day 30 GLS scores at maximum frown by IA of 0 vs 1 at 50.5% vs 40.4% compared with 47.3% vs 36.6% with no prior exposure. Subjects >65 years old or males were more likely than younger or females to achieve GLS = 1 vs 0 at day 30 on maximum frown by IA.

2.5.4. Conclusions on the clinical efficacy

The proposed indication for Nuceiva is for the temporary improvement in the appearance of moderate to severe vertical lines between the eyebrows seen at maximum frown (glabellar lines), when the severity of the above facial lines has an important psychological impact in adult patients. The clinical studies demonstrated a clear improvement in the appearance of glabellar lines which was non-inferior to the improvement observed with active comparator.

For the claimed indication, evidence that the study population had psychological impact was dependent on a subject's simple verbal response at baseline was confirmed. This is in line with clinical practice where practitioners consult with patients and assess the psychological impact of the glabellar lines prior to administering botulinum toxin A, to ensure it is an appropriate treatment for their patient.

Patients were also assessed using the Hospital Anxiety and Depression scale, which is not validated for this specific patient population. Most patients were in the normal range of this scale at baseline and both Nuceiva and the active comparator Botox used in the study did not demonstrate a beneficial effect as compared to placebo on this scale. In a subset of patients who had higher Hospital Anxiety and Depression scores at baseline demonstrated beneficial effects following treatment compared to placebo.

Whilst the treatment had no beneficial effect on psychological impact given that the change in HADS scores was overall no different from placebo, the results on other patient-related outcome scales (Subject Satisfaction Score (SSS) and Global Aesthetic Improvement Scale (GAIS)) demonstrated significant benefit from treatment with Nuceiva.

Although the use of validated PROs would have been preferred, the CHMP was satisfied that the psychological wellbeing improvement for the treated patients was sufficiently demonstrated as similar to the active comparator Botox and it is therefore acceptable in relation to the agreed indication.

Despite several minor other concerns since the pivotal study EVB003 lacks an active controlled extension to assess clinical efficacy of multiple doses typical of clinical practice and relies on unvalidated PROs to claim high rates of subject satisfaction based on the GAIS score by SA and the SSS, instead of a selection from a range of validated PROs, the CHMP agrees that the data provided to support efficacy of the product were considered sufficient.

Data on previous exposure to botulinum toxin is not collected, relying on randomisation to balance this important variable. The two-fold variation in treatment response rates between study sites, with a range of risk difference between Botox and DWO-450 of -11.9% to 20.0% points, is potentially attributable to random variation and inter-rater variability of IA.

The efficacy data from the single dose RCTs EV001, EV002 are influenced by a strong treatment effect, the lack of independent centralised assessment, and unbalanced randomisation, in the context of high rates of previous exposure. These factors each contribute to the significant risk of unblinding of study treatment and the consequential risk of biased outcome assessments. These concerns being mitigated in the pivotal EVB003 study by the inclusion of an active treatment control and the safeguards of randomisation, the RCTs EV001 and EV002 are therefore considered as supportive studies only.

The open label studies EV004, EV006 are not designed to collect efficacy data and factors affecting subject dropout versus progression to retreatment are poorly characterised. Additional analyses demonstrate a consistent trend of shorter duration of responses and hence shorter treatment intervals for subjects in the 4 versus 3 versus 2 treatment cohorts, with a similar trend by age, gender, and in subjects with / without TEAEs or drug related TEAEs. There is a consistent trend within each treatment cohort of a small increase in interval between subsequent treatments. Some 25% of subjects with 3 or 4 treatments had increased gap time between the duration of response and interval to treatment for reasons which are unclear.

The CHMP considers the following measures necessary to address issues related to efficacy:

- Non-interventional immunogenicity analysis, with re-test of previously negative sera from studies EV-001, EV-002, EV-004, EV006 using new binding ADA assays for screening, confirmation and determination of ADA titres, supported by a sensitive assay for neutralising ADA. This commitment is reflected in the RMP as an additional pharmacovigilance activity.

2.6. Clinical safety

Patient exposure

Study EV001 and Study EV002

Each subject, EV001 = 246, EV002 = 246, randomised to DWP-450 received 5x4U DWP-450 i.e. 20U in 0.5ml saline im, with Placebo subjects, n = 84 and 78 respectively, receiving the same volumes as saline.

Study EVB003

Each subject randomised to DWP-450, n=245, received 5x4U DWP-450 i.e. 20U in 0.5ml saline im, to Botox, n=246, received 5x4U Botox 20U in 0.5ml saline im similarly, with Placebo subjects, n = 49 receiving the same volumes as saline.

Study EV004

The median dose administered was 60 units of DWP-450, and 262/353 (74.5%) of subjects received 3 or 4 treatments. N.B. The effective dose of toxin received by patients in this study was standardised using the LD50 assay, although the amount of botulinum toxin protein differed since the lyophilised

product used contained 50% overage of toxin versus the 5% overage of toxin in the vacuum dried product used in the each of the other clinical studies.

Study EV004: Extent of Exposure

	DWP-450	
Total Drug Administered	**Study Completers (N=297)**	**All Subjects (N=352)**
Total Dose Injected (U), mean ± SD [min, max]	66.6 ± 15.67 [20, 80]	61.8 ± 19.69 [20, 80]
median	80	60
Total Treatments Administered, n (%)		
1 treatment (IT only)	5 (1.7)	33 (9.4)
2 treatments (IT + RT1)	43 (14.5)	57 (16.2)
3 treatments (IT + RT1 + RT2)	98 (33.0)	108 (30.7)
4 treatments (IT + RT1 + RT2 + RT3)	151 (50.8)	154 (43.8)
Treatment Interrupted, n (%)[a]	1 (0.3)	1 (0.3)

IT=initial treatment; RT=repeat treatment

Study EV006

The median dose administered was 60 units of DWP-450, and 431/570 (75.6%) of subjects received 3 or 4 treatments.

Study EV006: Extent of Exposure

	DWP-450	
Total Drug Administered	**Study Completers (N=487)**	**All Subjects (N=570)**
Total Dose Injected (U), mean ± SD [min, max]	65.5 ± 14.76 [20, 80]	61.0 ± 18.53 [20, 80]
median	60	60
Total Treatments Administered, n (%)		
1 treatment (IT only)	6 (1.2)	46 (8.1)
2 treatments (IT + RT1)	66 (13.6)	93 (16.3)
3 treatments (IT + RT1 + RT2)	203 (41.7)	217 (38.1)
4 treatments (IT + RT1 + RT2 + RT3)	212 (43.5)	214 (37.5)
Dose Interrupted, n (%)	0 (0.0)	0 (0.0)

IT=initial treatment; RT=repeat treatment

The interval between the initial treatment and first retreatment declined as the number of treatments given increased from 2 – 4, in both studies EV004, EV006.

Adverse events

Study EV001: Summary of Adverse Events

	Study: EV001	
N (%) Subjects or Total Events	Placebo (n=84)	DWP-450 (n=246)
Subjects with ≥1 TEAE	27 (32%)	94 (38%)
TEAEs	42	144
Subjects with ≥1 Drug related TEAEs	11 (13%)	38 (15%)
Subjects with ≥1 Treatment Emergent Serious AEs	0	3 (1.2%)
TEAEs leading to Discontinuation	0	0
Malignancy	0	2 (1%)
Deaths	0	0
Possible Hypersensitivity Reaction # (*None drug related)*	2 (2%)	5 (2%)
TEAEs ≥5%: Nervous system disorder, headache	14 (17%)	36 (17%)
Injection site bruising, pain or swelling related to study drug	0	1

Key: # Placebo: 1 hypersensitivity, 1 angioedema; DWP-450: 1 eye allergy, 1 pyrexia, 2 with headache due to seasonal allergy, 3 seasonal allergies.

More subjects had TEAEs after a single administration of DWP-450 than after placebo, incident difference, % (95% CI) 6.1 (-6.3, 18.3). The most common TEAE was headache. .

Study EV002: Summary of Adverse Events

	Study: EV002	
N (%) Subjects or Total Events	Placebo (n=78)	DWP-450 (n=246)
Subjects with ≥1 TEAE	21 (27%)	70 (29%)
TEAEs	36	130
Subjects with ≥1 Drug related TEAEs	6 (8%)	24 (10%)
Subjects with ≥1 Treatment Emergent Serious AEs	0	4 (1.6%)
TEAEs leading to Discontinuation	0	1 #
Malignancy	0	0
Deaths	0	0
Possible Hypersensitivity Reaction ## (*None drug related)*	1 (1%)	3 (1%)
TEAEs ≥5%: Nervous system disorder, headache	7 (9%)	21 (9%)
Injection site bruising, pain or swelling related to study drug	0	3 (1%)

TIA 13 days after DWP-3450, judged as unrelated to study drug

Placebo: 1 eye pruritus, possibly related to study drug. DWP-450: 2 eyelid oedema – 1 possibly study drug related; 1 wheezing – not related to study drug.

More subjects had TEAEs after a single administration of DWP-450 than after placebo, incident difference, % (95% CI) 1.5 (-11.2, 14.2). The most common TEAE was headache.

Study EVB-003: Summary of Adverse Events

	Study EVB-003		
N (%) Subjects or Total Events	Placebo (n=49)	Botox (n=246)	DWP-450 (n=245)
Subjects with ≥1 TEAE	16 (32.7%)	103 (41.9%)	92 (37.6%)
TEAEs	27	165	152
Subjects with ≥1 Drug related TEAEs	2 (4.1%)	36 (14.6%)	38 (15.4%)
Subjects with ≥1 Treatment Emergent Serious AEs (*None regarded as study drug related)*	1 (2%)	1 (0.4%)	3 (1.2%)
Severe AEs	2 (4.1%)	5 (2.0%)	8 (3.3%)
TEAEs leading to Discontinuation	0	1	0
Malignancy	1	0	0
Deaths	0	0	0
Possible Hypersensitivity Reaction	0	5 (2.0%)	4 (1.6%)
TEAEs ≥5%: Nervous system disorder, headache	7 (14.3%)	25 (10.2%)	34 (13.9%)
Injection site bruising, pain or swelling related to study drug	Not available	4 (1.6%)	6 (2.4%)

In EVB003, more subjects had TEAEs after a single administration of DWP-450 or Botox than after placebo at 37.6%, 41.9% and 32.7% respectively, with an incidence difference for DWP-450 versus Botox of -4.3 (-13.3, 4.4). Similarly, more subjects had ≥1 Drug related TEAEs at 15.4%, 14.6%, and 4.1% respectively. The most common TEAE was headache. No long-term extension of study EVB003 was performed

Study EV001 and Study EV002: Summary of Adverse Events by System Organ Class

	Study EV001		**Study EV002**	
	Placebo (n=84)	DWP-450 (n=246)	Placebo (n=78)	DWP-450 (n=246)
System Organ Class	Subjects n (%)			
All TEAEs	27 (32.1)	94 (38.2)	21 (26.9)	70 (28.5)
incident difference, % (95% CI)	*6.1 (-6.3, 18.3)*		*1.5 (-11.2, 14.2)*	
Blood and lymphatic system disorders	1 (1.2)	1 (0.4)	0	0
Cardiac disorders	0	1 (0.4)	0	4 (1.6)
Endocrine disorders	0	0	1 (1.3)	1 (0.4)
Eye Disorders	3 (3.6)	8 (3.3)	1 (1.3)	10 (4.1)
Gastrointestinal disorders	0	4 (1.6)	3 (3.8)	2 (0.8)
General disorders & administration site conditions	1 (1.2)	2 (0.8)	0	5 (2.0)
Immune system disorders *including drug hypersensitivity*	1 (1.2) *0*	3 (1.2) *0*	0	0
Infections and infestations	9 (10.7)	26 (10.6)	8 (10.3)	24 (9.8)
Injury, poisoning, & procedural complications	1 (1.2)	7 (2.8)	4 (5.1)	7 (2.8)
Investigations	1 (1.2)	7 (2.8)	0	1(0.4)
Metabolism and Nutrition Disorders	0	1 (0.4)	0	1 (0.4)
Musculoskeletal and connective tissue disorders	0	6 (2.4)	3 (3.8)	5 (2.0)
Neoplasms, Benign, malignant & unspecified	0	3 (1.2)	0	2 (0.8)
Nervous system disorders *including headache*	15 (17.9) *14 (16.7)*	41 (16.7) *36 (14.6)*	7 (9.0) *7 (9.0)*	25 (10.2) *21 (8.5)*
Psychiatric Disorders	0	3 (1.2)	0	0

Renal and Urinary Disorders	0	0	1 (1.3)	1 (0.4)
Reproductive System and Breast disorders	0	0	0	3 (1.2)
Respiratory, thoracic and mediastinal disorders	2 (2.4)	3 (1.2)	1 (1.3)	6 (2.4)
Skin and subcutaneous tissue disorders	3 (3.6)	4 (1.6)	2 (2.6)	5 (2.0)
Surgical and Medical Procedures	0	0	0	1(0.4)
Vascular Disorders	1 (1.2)	4 (1.6)	0	2 (0.8)

Study EVB003: Summary of Adverse events by System Organ Class

	Study EVB003		
	Placebo (n=49)	Botox (n=246)	DWP-450 (n=246)
System Organ Class	Subjects n (%)		
All TEAEs	16 (32.7)	103 (41.9)	92 (37.6)
incident difference DWP-450 vs Botox, % (95% CI)	*-4.3 (-13.3, 4.4)*		
Blood and lymphatic system disorders	0	0	0
Cardiac disorders	0	0	0
Ear and labyrinth disorders	0	2 (0.8)	0
Eye Disorders	0	12 (4.9)	11 (4.5)
Gastrointestinal disorders	1 (2.0)	4 (1.6)	1 (0.4)
General disorders & administration site conditions	0	12 (4.9)	9 (3.7)
Immune system disorders *including drug hypersensitivity*	0	2 (0.8) *0*	0
Infections and infestations	5 (10.2)	46 (18.7)	38 (15.5)
Injury, poisoning, & procedural complications	0	7 (2.8)	5 (2.0)
Investigations	0	2 (0.8)	0
Metabolism and Nutrition Disorders	0	1 (0.4)	0
Musculoskeletal and connective tissue disorders	2 (4.1)	6 (2.4)	8 (3.3)
Neoplasms, Benign, malignant & unspecified	1 (2.0)	1 (0.4)	0
Nervous system disorders *including headache*	7 (14.3) *7 (14.3)*	33 (13.4) *25 (10.2)*	43 (17.6) *34 (13.9)*
Pregnancy, puerperium and perinatal conditions	0	0	1 (0.4)
Psychiatric Disorders	0	0	1 (0.4)
Reproductive System and Breast disorders	1 (2.0)	1 (0.4)	1 (0.4)
Respiratory, thoracic and mediastinal disorders	1 (2.0)	6 (2.4)	7 (2.9)
Skin and subcutaneous tissue disorders	0	7 (2.8)	5 (2.0)
Social circumstances	0	1 (0.4)	0
Surgical and Medical Procedures	1 (2.0)	0	0
Vascular Disorders	1 (2.0)	4 (1.6)	2 (0.8)

Studies EV004, EV006: Summary of Adverse Events

	Study	
N (%) Subjects or Total Events	**EV004 (n=352)**	**EV006 (n=570)**
Subjects with ≥1 TEAE	148 (42%)	235 (41.2%)
TEAEs	265	475
Subjects with ≥1 Drug related TEAEs	51 (14.5%)	61 (10.7%)
Subjects with ≥1 Treatment Emergent Serious AEs	7 (2.0%)	7 (1.2%)
TEAEs of special interest	11 (3.1%)	16 (2.8%)
TEAEs leading to Discontinuation	2 (0.6%)	1 (0.2%)
Malignancy	8 (2.3%)	10 (1.8%)
Deaths	0	1 (0.2%)
Possible Hypersensitivity Reaction	6 (1.7%)	12 (2.1%)
TEAEs ≥5%: Nervous system disorder, headache	54 (15.3%)	75 (13.2%)
Injection site bruising, pain or swelling related to study drug	5 (1.4%)	5 (0.9%)
Time course: Last treatment before onset (% receiving drug)		
Initial treatment	104/352 (29.5%)	144/570 (25.3%)
Retreatment 1	49/319 (15.4%)	101/524 (19.3%)
Retreatment 2	33/262 (12.6%)	67/431 (15.5%
Retreatment 3	16/154 (10.4%)	19/214 (8.9%)

Summary of Adverse Events by System Organ Class (for AEs reported by >/=0.3% of Patients

	Study EV004 (n=352)		**Study EV006 (n=570)**	
System Organ Class	Subjects (%)	Events	Subjects (%)	Events
All TEAEs	148 (42%)	265	235 (41.2)	475
Blood and lymphatic system disorders	0	0	5 (0.9)	5
Cardiac disorders	2 (0.6)	2	12 (2.1)	13
Ear and Labyrinth Disorders	1 (0.3)	1	6 (1.1)	6
Eye Disorders	14 (4.0)	15	17 (3.0)	23
Gastrointestinal disorders	8 (2.3)	11	17 (3.0)	21
General disorders & administration site conditions	15 (4.3)	16	14 (2.5)	19
Hepatobiliary disorders	0	0	1 (0.2)	1
Immune system disorders *including drug hypersensitivity*	2 (0.6) *0*	2 *0*	6 (1.1) *1*	6 *1*
Infections and infestations	54 (15.3)	66	80 (14.0)	98
Injury, poisoning, & procedural complications	11 (3.1)	14	39 (6.8)	44
Investigations	1 (0.3)	1	6 (1.1)	6
Metabolism and Nutrition Disorders	5 (1.4)	5	3 (0.5)	3
Musculoskeletal and connective tissue disorders	12 (3.4)	12	24 (4.2)	34
Neoplasms, Benign, malignant & unspecified	8 (2.3)	8	10 (1.8)	10
Nervous system disorders *including headache*	64 (18.2) *54 (15.3)*	80 *65*	81 (14.2) *75 (13.2)*	108 *94*
Psychiatric Disorders: Anxiety Depression Insomnia	6 (1.7) 1 2 2	6	10 (1.8) 3 4 1	10

ADHD Other	1 0		1 1	
Renal and Urinary Disorders	1 (0.3)	2	2 (0.4)	2
Reproductive System and Breast disorders	3 (0.9)	4	3 (0.5)	3
Respiratory, thoracic and mediastinal disorders	5 (1.4)	6	14 (2.5)	15
Skin and subcutaneous tissue disorders	8 (2.3)	10	26 (4.6)	32
Social Circumstances	0	0	2 (0.4)	2
Surgical and Medical Procedures	0	0	0	0
Vascular Disorders	4 (1.1)	4	11 (1.9)	11

One subject had injection site pruritus following the 1st treatment and persisting for 12 days but not recurring with a repeat treatment, assessed as a possible hypersensitivity reaction possibly related to study drug, (1/570, 0.2%).

Summary of Treatment Emergent Adverse Events: Single vs Multiple Dose Studies

	Pooled Single Dose Studies – DWP-450 Subjects Only (N=737)		Pooled Multiple Dose Studies – All DWP-450 Subjects (n=922)	
	n/N	(%)	n/N	(%)
All TEAEs	254/737	(34.5)	383/922	(41.5)
By Study Period				
IT	254/737	(34.5)	248/922	(26.9)
RT1	--	--	150/843	(17.8)
RT2	--	--	100/693	(14.4)
RT3	--	--	35/368	(9.5)
All Study Drug Related TEAEs	100/737	(13.6)	112/922	(12.1)
By Study Period				
IT	100/737	(13.6)	76/922	(8.2)
RT1	--	--	30/843	(3.6)
RT2	--	--	18/693	(2.6)
RT3	--	--	4/368	(1.1)
All TEAEs of Special Interest	20/737	(2.7)	27/922	(2.9)
By Study Period				
IT	20/737	(2.7)	15/922	(1.6)
RT1	--	--	9/843	(1.1)
RT2	--	--	2/693	(0.3)
RT3	--	--	3/368	(0.8)
All Study Drug Related TEAEs of Special Interest	12/737	(1.6)	14/922	(1.5)
By Study Period				
IT	12/737	(1.6)	9/922	(1.0)
RT1	--	--	3/843	(0.4)
RT2	--	--	1/693	(0.1)
RT3	--	--	2/368	(0.5)

IT = initial treatment; RT = repeat treatment
Source: ISS Tables 26, 32, 47 and 48.

Serious adverse event/deaths/other significant events

Study EV001: Summary of All Serious Adverse Events

System Organ Class and Preferred Term	Placebo (N=84)			DWP-450 (N=246)		
	n	(%)	Events	n	(%)	Events
All Serious Adverse Events	0	(0.0)	0	3	(1.2)	3
Neoplasms Benign, Malignant and Unspecified	0	(0.0)	0	2	(0.8)	2
Malignant melanoma	0	(0.0)	0	1	(0.4)	1
Uterine cancer	0	(0.0)	0	1	(0.4)	1
Nervous System Disorders	0	(0.0)	0	1	(0.4)	1
Intracranial aneurysm	0	(0.0)	0	1	(0.4)	1

None were attributed to the study drug.

Study EV002: Summary of All Serious Adverse Events

System Organ Class and Preferred Term	Placebo (N=78)			DWP-450 (N=246)		
	n	(%)	Events	n	(%)	Events
All Serious Adverse Events	0	(0.0)	0	4	(1.6)	4
Cardiac Disorders	0	(0.0)	0	1	(0.4)	1
Stress cardiomyopathy	0	(0.0)	0	1	(0.4)	1
Injury, Poisoning and Procedural Complications	0	(0.0)	0	1	(0.4)	1
Femur fracture	0	(0.0)	0	1	(0.4)	1
Neoplasms Benign, Malignant and Unspecified	0	(0.0)	0	1	(0.4)	1
Breast cancer	0	(0.0)	0	1	(0.4)	1
Nervous System Disorders	0	(0.0)	0	1	(0.4)	1
Transient ischemic attack	0	(0.0)	0	1	(0.4)	1

None were regarded as study drug related.

Study EVB-003: Summary of All Serious Adverse Events

System Organ Class and Preferred Term	Placebo (N=49)			BOTOX (N=246)			DWP-450 (N=245)		
	n	(%)	Events	n	(%)	Events	n	(%)	Events
All Serious Adverse Events	1	(2.0)	3	1	(0.4)	2	3	(1.2)	6

Placebo: A 53-year-old black female subject with breast cancer 100 days after treatment

Botox: 52-year old white female with a fibroelastoma of Ao valve 68 & 117 days after treatment

DWP-450:

- Spasms of facial muscles with severe pain 151 & 164 days after treatment in 37-year white female
- Worsening of conjunctival cyst 84 days after treatment in 75-year old female
- Spontaneous abortion 114 days after treatment in 43-year old white female.

None were regarded as study drug related.

Study EV004: Summary of All Serious Adverse Events

System Organ Class and Preferred Term	DWP-450 (N=352)		
	n	(%)	No. of Events
All Serious Adverse Events	7	(2.0)	9
Neoplasms Benign, Malignant & Unspecified	5	(1.4)	5
Basal cell carcinoma (406011, 414008)	2	(0.6)	2
Breast cancer (404040)	1	(0.3)	1
Malignant anorectal neoplasm (403029)	1	(0.3)	1
Ovarian adenoma (404020)	1	(0.3)	1
Reproductive System & Breast Disorders	1	(0.3)	2
Dysfunctional uterine bleeding (415006)	1	(0.3)	2
Gastrointestinal Disorders	1	(0.3)	1
Pancreatitis (404040)	1	(0.3)	1
General Disorders & Administration Site Conditions	1	(0.3)	1
Device failure – failure of pacemaker/defibrillator (410016)	1	(0.3)	1

None were regarded as study drug related.

Study EV006: Summary of All Serious Adverse Events

System Organ Class and Preferred Term (Subject Number)	DWP-450 (N=570)		
	n	(%)	No. of Events
All Serious Adverse Events	7	(1.2)	8
Neoplasms Benign, Malignant & Unspecified	3	(0.5)	3
Breast cancer (611011)	1	(0.2)	1
Squamous cell carcinoma (613006)	1	(0.2)	1
Uterine leiomyoma (607012)	1	(0.2)	1
Gastrointestinal Disorders	2	(0.4)	2
Colitis (607004)	1	(0.2)	1
Small intestinal obstruction (603045)	1	(0.2)	1
Injury, Poisoning and Procedural Complications	1	(0.2)	1
Overdose (611011)	1	(0.2)	1
Nervous System Disorders	1	(0.2)	1
Carotid artery stenosis (613020)	1	(0.2)	1
Psychiatric Disorders	1	(0.2)	1
Anxiety (610014)	1	(0.2)	1

None were regarded as study drug related.

Overall Summary of TEAS – Pooled Analysis of the Safety Population

	Controls						DWP-450											
	Pooled Placebo (N=211)			BOTOX (N=246)			US Pooled Single Dose (N=492)			Pooled Single Dose (N=737)			Pooled Multiple Dose (N=922)			Pooled All (N=1659)		
Type of Adverse Event [a]	n	(%)	[E]	n	(%)	[E]	n	(%)	[E]	n	(%)	[E]	n	(%)	[E]	n	(%)	[E]
All Adverse Events	64	(30.3)	[104]	103	(41.9)	[165]	162	(32.9)	[272]	254	(34.5)	[424]	383	(41.5)	[740]	637	(38.4)	[1164]
Study Drug Related	19	(9.0)	[24]	36	(14.6)	[45]	62	(12.6)	[89]	100	(13.6)	[135]	112	(12.1)	[150]	212	(12.8)	[285]
AEs Leading to Death	0	(0.0)	[0]	0	(0.0)	[0]	0	(0.0)	[0]	0	(0.0)	[0]	1	(0.1)	[1]	1	(<0.1)	[1]
Study Drug Related	0	(0.0)	[0]	0	(0.0)	[0]	0	(0.0)	[0]	0	(0.0)	[0]	0	(0.0)	[0]	0	(0.0)	[0]
AEs Leading to Study Discontinuation	0	(0.0)	[0]	1	(0.4)	[1]	1	(0.2)	[1]	1	(0.1)	[1]	3	(0.3)	[3]	4	(0.2)	[4]
Study Drug Related [b]	0	(0.0)	[0]	0	(0.0)	[0]	0	(0.0)	[0]	0	(0.0)	[0]	2	(0.2)	[2]	2	(0.1)	[2]
Serious Adverse Events	1	(0.5)	[3]	1	(0.4)	[2]	7	(1.4)	[7]	10	(1.4)	[13]	14	(1.5)	[17]	24	(1.4)	[30]
Study Drug Related	0	(0.0)	[0]	0	(0.0)	[0]	0	(0.0)	[0]	0	(0.0)	[0]	0	(0.0)	[0]	0	(0.0)	[0]
AEs of Special Interest [c]	1	(0.5)	[1]	4	(1.6)	[4]	13	(2.6)	[17]	20	(2.7)	[24]	27	(2.9)	[32]	47	(2.8)	[56]
Study Drug Related	0	(0.0)	[0]	3	(1.2)	[3]	7	(1.4)	[11]	12	(1.6)	[16]	14	(1.5)	[17]	26	(1.6)	[33]
Possible Hypersensitivity AEs [d]	3	(1.4)	[3]	5	(2.0)	[6]	8	(1.6)	[10]	12	(1.6)	[14]	18	(2.0)	[20]	30	(1.8)	[34]
Study Drug Related	1	(0.5)	[1]	1	(0.4)	[1]	2	(0.4)	[2]	3	(0.4)	[3]	3	(0.3)	[3]	6	(0.4)	[6]

Adverse events of special interest

Summary Table of Pooled Safety Analysis by Severity and TEAEs of Special Interest

	Pooled placebo EV001, EV002, EVB003	**Botox single treatment EVB003**	**Pooled single treatment EV001, EV002, EVB003**	**Pooled multiple treatments EV004, EV006**
N (%) Subjects or Total Events	**211**	**246**	**737**	**922**
Subjects with ≥1 TEAE	64 (30.3)	103 (41.9)	254 (34.5)	383 (41.5)
TEAEs	104	165	424	740
Mild	51 (24.2)	79 (32.1)	199 (27.0)	306 (33.2)
Moderate	16 (7.6)	30 (12.2)	83 (11.3)	146 (15.6)
Severe	2 (0.9)	5 (2.0)	12 (1.6)	20 (2.2)
Subjects with ≥1 Drug related TEAEs	19 (9.0)	36 (14.6)	100 (13.4)	112 (12.1)
Subjects with ≥1 Treatment Emergent Serious AEs	1 (0.5)	1 (0.4)	10 (1.4)	14 (1.5)
TEAEs of special interest	1 (0.5)	4 (1.6)	20 (2.7)	27 (2.9)
Eye disorders	0	4 (1.6)	15 (2.0)	17 (1.8)
Cardiac disorders (*all bradycardias)*	0	0	0	4 (0.4)
Respiratory: Dysphonia	0	0	1	0
Respiratory: Dyspnoea	1 (0.5)	0	1 (0.1)	2 (0.2)
Gastrointestinal: Dysphagia	0	0	1 (0.1)	2 (0.2)
Neurological: Speech disorder	0	0	0	2 (0.2)
TEAEs leading to Discontinuation	0	1	1	1
Deaths	0	0	0	1
Possible Hypersensitivity Reaction	3 (1.4)	5 (2.0)	12 (1.6)	14 (1.5)

More subjects had any TEAE and TEAE of special interest after multiple versus single administrations of DWP-450.

Summary Table of TEAEs of Special Interest Investigator Assessed as Related to Study Drug by Preferred Term

Study	Treatment	Event(s)	Preferred Term(s)
EV-001	DWP-450	AESI (x2): R (x2)	Eyelid ptosis and vision blurred
	DWP-450	AESI	Brow ptosis
	DWP-450	AESI	Eyelid ptosis
EV-002	DWP-450	AESI	Eyelid ptosis
	DWP-450	AESI (x2): R (x2)	Brow ptosis (x2)
	DWP-450	AESI (x2): R (x2)	Eyelid ptosis and diplopia
	DWP-450	AESI (x2): R (x2)	Vision blurred and eyelid ptosis
EVB-003	DWP-450	AESI	Muscle twitching
	DWP-450	AESI	Eyelid ptosis
	DWP-450	AESI	Eyelid ptosis
	BOTOX	AESI	Brow ptosis
	DWP-450	AESI	Eyelid ptosis
	DWP-450	AESI	Eyelid ptosis
	BOTOX	AESI	Blepharospasm
	BOTOX	AESI	Blepharospasm
EV-004	DWP-450	AESI	Eyelid ptosis
	DWP-450	AESI	Eyelid ptosis
	DWP-450	AESI	Eyelid ptosis
	DWP-450	AESI	Speech disorder
	DWP-450	AESI	Speech disorder
	DWP-450	AESI	Blepharospasm
EV-006	DWP-450	AESI	Eyelid ptosis
	DWP-450	AESI (x4): R (x4)	Eyelid ptosis (x2), brow ptosis, and blepharospasm
	DWP-450	AESI	Eyelid ptosis
	DWP-450	AESI	Eyelid ptosis
	DWP-450	AESI	Eyelid ptosis
	DWP-450	AESI (x2): R (x1) NR (x1)	Brow ptosis and (blepharospasm, NR)
	DWP-450	AESI	Vision blurred
	DWP-450	AESI	Brow ptosis

Eye disorders were the main adverse event of special interest affecting 27/1659 (1.6%) of subjects including 17 with eyelid ptosis (complicated by blurred vision or diplopia in 3); 5 subjects had brow ptosis, 3 had blepharospasm, 1 had intermittent twitching of the left eye, 1 had blurred vision alone.

Two subjects had speech disorder:

- a subject reported seeming tongue tied and stumbled over words the evening after the 1st retreatment, resolving after a couple of hours; no further treatment was given.

- a subject reported mild intermittent difficulty speaking multiple syllable words with onset 4 days after the IT visit, resolving the same day

Summary of Hypersensitivity Reactions Investigator Assessed as Related to DWP-450

Study	001	002	003	004	006	Total
Eyelid oedema		2	1			
Influenza-like illness				2		

Injection-site pruritus					1	
All						6 (0.4%)

Summary of Adverse Events of Special Interest Investigator Assessed as Unrelated to Study Drug

Study	Treatment	Event(s)	Preferred Term(s)
EV-001	DWP-450	AESI	Eyelid ptosis
	Placebo	AESI	Dyspnea
	DWP-450	AESI	Eyelid ptosis
EV-002	DWP-450	SAE + Discon. + AESI	Transient ischaemic attack
	DWP-450	AESI	Eyelid ptosis
	DWP-450	AESI	Dyspnea
	DWP-450	AESI	Blepharospasm
EVB-003	DWP-450	AESI	Dysphagia
	BOTOX	AESI	Strabismus
	DWP-450	AESI	Dysphonia
EV-004	DWP-450	AESI	Dyspnea
	DWP-450	AESI	Presbyopia
	DWP-450	AESI	Eyelid ptosis
	DWP-450	AESI	Dyspnea
	DWP-450	AESI	Eyelid ptosis
EV-006	DWP-450	AESI	Sinus bradycardia
	DWP-450	AESI	Bradycardia
	DWP-450	AESI	Bradycardia
	DWP-450	AESI	Sinus bradycardia
	DWP-450	AESI	Dysphagia
	DWP-450	AESI (x2): NR (x2)	Eyelid ptosis and vision blurred
	DWP-450	AESI (x2): R (x1) NR (x1)	Blepharospasm and (brow ptosis, R)
	DWP-450	AESI	Eyelid ptosis
	DWP-450	AESI	Dysphagia

Eye disorders

Attribution of events of special interest is difficult and it is not possible to completely exclude the involvement of study drug in local events including eyelid ptosis in 7 subjects (1 with blurred vision) and 2 with blepharospasm.

Two other eye disorders might be indicative of a change in muscle strength after DWP-450:

- a subject with moderate sudden strabismus 14 days after DWP-450 lasting approximately 10 minutes.

- a patient had presbyopia on day 5 which responded to a change in prescription for lenses and further treatment was given without problems.

The 27 cases investigator assessed as related to study drug together with 11 similar cases of eye disorders investigator assessed as not related to study drug suggest a total rate for adverse events of special interest: eye disorders of 38/1659 (2.3%).

(Distant) Events of Special Interest Investigator Assessed as not due to the study drug

Key details from the case records are provided for the following events of special interest investigator assessed as not due to the study drug.

One patient had dyspnoea resolving after 2 hours on day 6, concurrent with gastroenteritis.

A patient had multiple symptoms including eye drooping, vision loss and weakness of lateral gaze on left side, mild in severity 13 days after treatment, was seen by his GP, attended the Emergency Department later that evening where a CT scan prompted admission which was declined. The episode was updated as a transient ischemic attack (TIA), the Investigator assessed the event as not related to study treatment later indirect follow up recorded the adverse event as not recovered/not resolved. The subject declined to provide his medical records.

A patient with mild-to moderate mitral valve regurgitation reported mild shortness of breath on day 125 reported as resolving at EOS.

A patient had dysphagia from day 144 which was resolving at EOS 2 days later

A patient had dysphonia onset day 25 which had resolved by day 42

A patient had dyspnoea after smoke inhalation on day 112, further treatment given without problems

A patient reported mild dyspnoea on day 2 resolving within 2 days

A patient reported mild dysphagia on day 3 after retreatment 1, resolving within 10 minutes, and two further treatments were given without problems

A patient reported difficulty in swallowing on two separate occasions 4 days after re-treatment 3 which resolved by day 8.

The case reports give no reasonable cause for concern that any of these (distant) events of special interest were attributable to the study drug.

Cardiac events of Special Interest Investigator Assessed as not due to the study drug

A patient had a sinus bradycardia, HR 58 bpm day 25 after treatment, considered resolved at EOS when HR 60 bpm, having received 2 retreatments without problems

A patient had a sinus bradycardia, HR 56 bpm day 36 after treatment versus baseline 68 bpm, with no additional follow-up available

A patient had a bradycardia at the radial pulse, HR 57 bpm day 7 after treatment although 12 lead ECGs were normal at baseline, on day 7 and at EOS having received 2 retreatments without problems

A patient had a sinus bradycardia HR 59 bpm 106 days (EOS) after retreatment 2, versus 60 bpm on 12 lead ECG at baseline, and 61 bpm on day 30 IT ECG.

The case reports give no reasonable cause for concern that any of these cardiac events were attributable to the study drug.

Cardiovascular Safety Report

Review of the centrally reported ECG findings for studies EV001, EV002, EV004, EV006 revealed minimal notable interval findings for prolonged or short PR, however higher changes in QT interval were seen in some incidences QTcB - Bazett's Correction ≥30 msec with DWP-450 versus placebo. One patient had a > 60 m sec increase. Subject #205026 had a QTcB value of 517 (i.e., >500 msec) at EOS but using the recommended Friderica fixed exponent correction method whilst the QTc was >480msec and increased by ≥60msec over baseline on day 30 it returned to normal by EOS day 150.

The risk of prolongation of the QTc was therefore low at 1/1333 (≤0.1%), transient, and without a mechanistic basis.

In addition, diastolic blood pressure, pulse rate and respiratory rate appeared to be higher following DWP 450 compared with placebo. It is unclear whether any patients were symptomatic, whether any persistent changes (i.e. following EOS) were clinically relevant and related to study drug.

In Study EV-002, one subject who was randomized to treatment with DWP-450 and treated on February 26, 2015. On March 11, 2015 (13 days after treatment) he experienced multiple symptoms including eye drooping, vision loss and weakness of lateral gaze on left side, mild in severity. The event was classified as a transient ischemic attack (TIA) even though the event was reported as not resolved /not recovered 5 months after the initial event. The subject was terminated from the study at this point. The subject had no relevant risk factors in his past medical history. Case details are unclear including why it was not considered causally related to DWP 450.

Laboratory findings

There are no relevant laboratory safety issues to report.

Safety in special populations

Study EV001: Summary of Adverse events by Age Group

	Subjects <65 Years				Subjects ≥ 65 Years			
	Placebo (N=75)		DWP-450 (N=220)		Placebo (N=9)		DWP-450 (N=26)	
Adverse Event Parameter	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**
Any Adverse Event	25	(33.3)	80	(36.4)	2	(22.2)	14	(53.8)
Any Serious AE	0	(0.0)	1	(0.5)	0	(0.0)	2	(7.7)
Any AE of Special Interest [a]	1	(1.3)	4	(1.8)	0	(0.0)	1	(3.8)
Any AE Identified as a Hypersensitivity Reaction [b]	2	(2.7)	5	(2.3)	0	(0.0)	0	(0.0)
Any Study Drug-Related [c] AE	9	(12.0)	32	(14.5)	2	(22.2)	6	(23.1)
Any AE Leading to Discon.	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Any AE Leading to Death	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)

Rates of any adverse event, serious AE, AE of special interest and Study-drug related AEs are higher in older subjects.

Two of the 3 serious AEs after DWP-450 occurred in the older age group – 1 malignant melanoma, 1 uterine cancer, neither attributable to the study drug_

Study EV002: Summary of Adverse events by Age Group

	Subjects <65 Years				Subjects ≥ 65 Years			
	Placebo (N=72)		DWP-450 (N=219)		Placebo (N=6)		DWP-450 (N=27)	
Adverse Event Parameter	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**
Any Adverse Event	21	(29.2)	61	(27.9)	0	(0.0)	9	(33.3)
Any Serious AE	0	(0.0)	2	(0.9)	0	(0.0)	2	(7.4)
Any AE of Special Interest [a]	0	(0.0)	7	(3.2)	0	(0.0)	1	(3.7)
Any AE Identified as a Hypersensitivity Reaction [b]	1	(1.4)	2	(0.9)	0	(0.0)	1	(3.7)
Any Study Drug-Related [c] AE	6	(8.3)	22	(10.0)	0	(0.0)	2	(7.4)
Any AE Leading to Discon.	0	(0.0)	1	(0.5)	0	(0.0)	0	(0.0)
Any AE Leading to Death	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)

Rates of any adverse event, serious AEs, and AEs of special interest, but not Study-drug related AEs, are higher in older subjects.

Two of the 4 serious AEs after DWP-450 occurred in the older age group – 1 stress-induced cardiomyopathy on a patient; 1 femur fracture in an patient, neither attributable to the study drug.

Study EVB003: Summary of Adverse events by Age Group

	Placebo (N=49)		BOTOX (N=246)		DWP-450 (N=245)	
Adverse Event Parameter	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**
Subjects < 65 Years	N=45		N=227		N=228	
All Adverse Events	15	(33.3)	95	(41.9)	88	(38.6)
Any Serious AE	1	(2.2)	1	(0.4)	2	(0.9)
Any AE of Special Interest [a]	0	(0.0)	4	(1.8)	7	(3.1)
Any AE Identified as a Possible Hypersensitivity Reaction [b]	0	(0.0)	5	(2.2)	4	(1.8)
Any Study Drug-Related [c] AE	2	(4.4)	35	(15.4)	38	(16.7)
Any AE Leading to Study Discontinuation	0	(0.0)	1	(0.4)	0	(0.0)
Any AE Leading to Death	0	(0.0)	0	(0.0)	0	(0.0)
Subjects ≥65 Years	N=4		N=19		N=17	
All Adverse Events	1	(25.0)	8	(42.1)	4	(23.5)
Any Serious AE	0	(0.0)	0	(0.0)	1	(5.9)
Any AE of Special Interest [a]	0	(0.0)	0	(0.0)	0	(0.0)
Any AE Identified as a Possible Hypersensitivity Reaction [b]	0	(0.0)	0	(0.0)	0	(0.0)
Any Study Drug-Related [c] AE	0	(0.0)	1	(5.3)	0	(0.0)
Any AE Leading to Study Discontinuation	0	(0.0)	0	(0.0)	0	(0.0)
Any AE Leading to Death	0	(0.0)	0	(0.0)	0	(0.0)

Rates of AEs of special interest are higher in older subjects.

Study EV004: Summary of Adverse events by Age Group

Adverse Event Parameter	Subjects <65 Years (N=319)	Subjects ≥ 65 Years (N=33)
Any Adverse Event, n (%)	132 (41.4)	16 (48.5)
Any Serious AE	6 (1.9)	1 (3.0)
Any AE of Special Interest [a]	9 (2.8)	2 (6.1)
Any AE Identified as a Possible Hypersensitivity Reaction [b]	6 (1.9)	0 (0.0)
Any Study Drug-Related [c] AE	46 (14.4)	5 (15.2)
Any AE Leading to Discon.	2 (0.6)	0 (0.0)
Any AE Leading to Death	0 (0.0)	0 (0.0)

Discon.=discontinuation

Rates of any adverse event, serious AEs, AEs of special interest and Study-drug related AEs are higher in older subjects.

Study EV006: Summary of Adverse events by Age Group

Adverse Event Parameter	Subjects <65 Years (N=519)	Subjects ≥ 65 Years (N=51)
Any Adverse Event, n (%)	215 (41.4)	20 (39.2)
Any Serious AE	6 (1.2)	1 (2.0)
Any AE of Special Interest[a]	14 (2.7)	2 (3.9)
Any AE Identified as a Possible Hypersensitivity Reaction[b]	9 (1.7)	3 (5.9)
Any Study Drug Related[c] AE	59 (11.4)	2 (3.9)
Any AE Leading to Discontinuation	1 (0.2)	0 (0.0)
Any AE Leading to Death	1 (0.2)	0 (0.0)

n= the number of subjects at each level of summarization.

Rates of serious AEs and AE of special interest are higher in in older subjects.

Summary: Pooled Analysis of TEAEs by Age, Sex, Race

	Controls				DWP-450							
	Pooled Placebo (N=211)		BOTOX (N=246)		US Pooled Single Dose (N=492)		Pooled Single Dose (N=737)		Pooled Multiple Dose (n=922)		Pooled All (N=1659)	
Subgroup Analysis	**n/N**	**(%)**	**n/N**	**(%)**	**n/N**	**(%)**	**n/N**	**(%)**	**n/N**	**(%)**	**n/N**	**(%)**
All Adverse Events	64/211	(30.3)	103/246	(41.9)	162/492	(32.9)	254/737	(34.5)	383/922	(41.5)	637/1659	(38.4)
By Age Group												
<65 years	61/192	(31.8)	95/227	(41.9)	139/439	(31.7)	227/667	(34.0)	347/838	(41.4)	574/1505	(38.1)
≥65 years	3/19	(15.8)	8/19	(42.1)	23/53	(43.4)	27/70	(38.6)	36/84	(42.9)	63/154	(40.9)
By Sex												
Female	59/190	(31.1)	92/215	(42.8)	151/447	(33.8)	229/667	(34.3)	364/841	(43.3)	593/1508	(39.3)
Male	5/21	(23.8)	11/31	(35.5)	11/45	(24.4)	25/70	(35.7)	19/81	(23.5)	44/151	(29.1)
By Race												
White	51/168	(30.4)	90/183	(49.2)	138/420	(32.9)	217/585	(37.1)	327/756	(43.3)	544/1341	(40.6)
Black [a]	3/14	(21.4)	0/1	(0.0)	14/37	(37.8)	14/40	(35.0)	16/47	(34.0)	30/87	(34.5)
Asian	3/7	(42.9)	1/5	(20.0)	2/7	(28.6)	6/13	(46.2)	3/9	(33.3)	9/22	(40.9)
Other	4/9	(44.4)	2/2	(100.0)	6/18	(33.3)	8/26	(30.8)	35/103	(34.0)	43/129	(33.3)
Multiple	0/3	(0.0)	0/1	(0.0)	2/10	(20.0)	2/11	(18.2)	2/7	(28.6)	4/18	(22.2)
Missing	3/10	(30.0)	10/54	(18.5)	0/0	(0.0)	7/62	(11.3)	0/0	(0.0)	7/62	(11.3)

TEAEs are more common in older subjects for each pooled analysis, overall 40.9% vs 38.1%.

TEAEs are more common in females than males for all the US studies, particularly after multiple doses 43.3% vs 23.5% and overall 39.3% versus 29.1%.

Potential differences between racial groups in rates of TEAEs are not assessable because of the small number of non-white subjects.

Summary of Treatment-Emergent Adverse Events of Special Interest by Subgroup – Safety Population

	Controls				DWP-450					
	Pooled Placebo (N=211)		BOTOX (N=246)		US Pooled Single Dose (N=492)		Pooled Single Dose (N=737)		Pooled Multiple Dose (N=922)	
Subgroup Analysis	n/N	(%)	n/N	(%)	n/N	(%)	n/N	(%)	n/N	(%)
All AESIs	1/211	(0.5)	4/246	(1.6)	13/492	(2.6)	20/737	(2.7)	27/922	(2.9)
By Age Group										
<65 years	1/192	(0.5)	4/227	(1.8)	11/439	(2.5)	18/667	(2.7)	23/838	(2.7)
≥65 years	0/19	(0.0)	0/19	(0.0)	2/53	(3.8)	2/70	(2.9)	4/84	(4.8)
By Sex										
Female	0/190	(0.0)	3/215	(1.4)	11/447	(2.5)	15/667	(2.2)	24/841	(2.9)
Male	1/21	(4.8)	1/31	(3.2)	2/45	(4.4)	5/70	(7.1)	3/81	(3.7)
By Fitzpatrick Skin Type										
I	0/12	(0.0)	0/4	(0.0)	0/21	(0.0)	0/30	(0.0)	0/57	(0.0)
II	0/64	(0.0)	1/84	(1.2)	3/144	(2.1)	6/228	(2.6)	12/273	(4.4)
III	1/86	(1.2)	2/118	(1.7)	7/187	(3.7)	9/303	(3.0)	9/320	(2.8)
IV	0/35	(0.0)	0/36	(0.0)	1/96	(1.0)	3/127	(2.4)	3/211	(1.4)
V	0/10	(0.0)	1/2	(50.0)	2/32	(6.3)	2/36	(5.6)	3/39	(7.7)
VI	0/4	(0.0)	0/2	(0.0)	0/12	(0.0)	0/13	(0.0)	0/22	(0.0)
By Baseline GLS Score [b]										
Moderate	0/53	(0.0)	1/70	(1.4)	0/120	(0.0)	0/182	(0.0)	2/253	(0.8)
Severe	1/158	(0.6)	3/176	(1.7)	13/372	(3.5)	20/555	(3.6)	25/669	(3.7)

Key b = at maximum frown by IA.

TEAEs of Special interest are more common in older than younger subjects and in males than females in each pooled analysis, overall 6/154 (3.9%) vs 41/1505 (2.7%) and 8/151 (5.3%) vs 39/1508 (2.6%) respectively. Racial differences are not evaluable due to the predominance of white subjects. Subjects with fair skin (II) may have higher rates of TEAEs of special interest than those with light brown skin (IV).

TEAEs of Special interest are more common in subjects with severe versus moderate GLs at maximum frown by IA at baseline in each study and overall the rate is 7-fold higher at 45/1224 (3.7%) vs 2/435 (0.5%).

Safety in special populations: Pregnancy

Summary of Subjects with a Pregnancy During the Study

Study	Treatment	Event(s)
EV-001	DWP-450	Pregnancy
EVB-003	DWP-450	Pregnancy and SAE
EVB-003	DWP-450	Pregnancy
EV-004	DWP-450	Pregnancy
EV-004	DWP-450	Pregnancy
EV-006	DWP-450	Pregnancy
EV-006	DWP-450	Pregnancy
EV-006	DWP-450	Pregnancy

Each subject had a negative pregnancy test at study entry.

All except one subject delivered healthy infants.

A subject had a spontaneous abortion 114 days after study treatment, which was not regarded as attributable to the study drug.

No further treatment was given, and all were withdrawn from the study except two subjects.

Immunological events

Study EV001: Anti-Botulinum toxin A Abs (Confirmed positive)

Botulinum toxin type A Ab testing (ADA) was performed on 1294 samples of which 4 were positive, all from a subject with previous exposure, lastly in 2013 with a forehead injection, treated with DWP-450 on 20/02/2015 when the GLS score at maximum frown was 3 (severe) by IA. There was no response to treatment.

Study EV002: Anti-Botulinum toxin A Abs (Confirmed positive)

Botulinum toxin type A Ab testing was performed on 1258 samples of which none were positive.

Study EVB003: Anti-Botulinum toxin A Abs (Confirmed positive)

No Botulinum toxin type A Ab testing reported in study EVB003.

Study EV004: Summary of Anti-Botulinum toxin A Antibody testing over Time

One of two positive ADA subjects was a non-responder – prior history of botulinum toxin exposure unknown.

In study EV004 2393 unique samples were received and tested. Specifically, 426 visits were duplicated when a subject who qualified for a repeat treatment at a Day 90 visit had data for both a Day 90 and a Repeat Treatment Day 0 at the same office visit entered by the study site, which resulted in the inflated subtotal of 2819 negative sera.

Study EV006

Botulinum toxin type A Ab testing was performed on 4060 sera of which one sample from one subject was positive at baseline at 1 in 50, whilst 5 subsequent samples tested negative, was a treatment responder.

Summary of Subjects Positive for ADA in the Screening, Confirmatory, and Neutralising assays

Study	General Antibodies		Neutralizing Antibody	Clinical Response	Prior Toxin Exposure
	Sensitive Screening	Confirmatory Assay			
EV001	1294 Samples 59 (4.6%) positives	Subject [redacted] - 4 positive samples (positive at screening)	Positive	Non-Responder	2013 botulinum toxin treatment, forehead
EV002	1259 samples 54 (4.3%) positives	No Positives	-	-	-
EV004	2393 samples 108 (4.5%) positives	Subject [redacted] - 1 positive sample (seroconversion)	Negative	Responder	No
		Subject [redacted] - 1 positive sample (seroconversion)	Negative	Responder	3 separate Botox Treatments Last treatment, one year prior to screening
EV006	4060 samples 440 (10.8%) positives	Subject [redacted] - 1 positive sample (positive at screening)	Negative	Responder	2012 Botox facial area 2014 botulinum toxin, facial area

Safety related to drug-drug interactions and other interactions

No drug-drug interactions were explored.

Discontinuation due to adverse events

4 DWP-450 subjects (4/1659, 0.2%), 1 Botox subject (1/246, 0.4%) and no Placebo subjects discontinued the study due to an adverse event.

Post marketing experience

Not applicable.

2.6.1. Discussion on clinical safety

The active controlled single dose study EVB003, the single dose placebo-controlled studies EV001, EV002; and the open label studies EV004, EV006 with $\leq$3 repeat treatments over 1 year each contribute to the safety assessment. The exposure of some 1800 subjects to one or more doses of DWP-450 in the five clinical trials reported here should enable ADRs with a frequency of at least <0.01% - 0.1% to be detected if there was no background incidence.

Exposure

Each subject in studies EV001, EV002, EVB003 received a single administration of 20 units of DWP-450. No long-term extension of study EVB003 was performed.

The effective dose of toxin received by patients in study EV004 was standardised using the LD50 assay, although subjects received significantly higher amounts of botulinum toxin protein since the lyophilised product used contained 50% overage of toxin versus the 5% overage of toxin in the vacuum dried product used in the each of the other clinical studies.

Otherwise in studies EV004 and EV006, the median dose administered was 60 units of DWP-450 where 262/353 (74.5%) and 431/570 (75.6%) of subjects respectively received 3 or 4 treatments. As the number of treatments given increased from 2 – 4 the interval between the initial treatment and first retreatment declined, with a median interval of 202, 123, 91 days and 190, 125, 91 days respectively.

Treatment-emergent adverse events (TEAEs)

In EVB003, TEAEs were more common than placebo for DWP-450 and Botox for subjects with ≥1 TEAEs at 32.7%, 37.6% & 41.9% (incidence difference DWP-450 versus Botox -4.3 (-13.3, 4.4)) and ≥1 Drug related TEAEs at 4.1%, 15.4% & 14.6%, respectively. TEAEs occurred at similar rates after Botox and DW-450 in study EVB003 and were in the same range in studies EV001, EV002. The most common TEAE was headache for placebo, Botox, and DWP-450 at 14.3%, 10.2%, 13.9% respectively. No long-term extension of study EVB003 was performed.

The rate of subjects with ≥1 Treatment Emergent Serious AEs after ≤4 treatments at 1% - 2% is like the rate after a single treatment. The most frequent TEAE is headache at 13% - 15%, a similar rate to the placebo controlled single dose studies.

Some 4 – 5% of subjects had administration site TEAEs.

No serious adverse events were regarded as related to the study drug, including a single death due to an unrelated drug overdose. The overall rate of TEAEs leading to study discontinuation is low at <0.5% and none attributable to the study drug.

TEAEs of Special Interest

Six (6/1659, 0.4%) of subjects had *hypersensitivity* reactions including eyelid oedema, injection site pruritus, and influenza like illness, assessed as related to study drug.

The 27 cases with **eye disorders** investigator assessed as related to study drug together with 11 similar cases of eye disorders investigator assessed as not related to study drug suggest a total rate for adverse events of special interest: eye disorders of 38/1659 (2.3%).

Two (2/1659 0.1%) of subjects, study EV004, had **short-lived dysarthria** investigator assessed as related to the study drug. Case reports give no reasonable cause for concern that any of the other (distant) events of special interest, including cardiac events, were attributable to the study drug. The risk of prolongation of the QTc was low at 1/1333 (<0.1%), transient, not seen after repeat administration and without a mechanistic basis.

Muscle volume loss

The expectation that muscle volume loss will occur after botulinum toxin treatment has been addressed in the Product Information.

TEAEs after Multiple versus Single Administration of DWP-450

No direct comparison between multiple vs single administration of DWP-450 is available in the absence of an active controlled extension of the pivotal study EVB003. Whilst collecting safety data in an open label design is acceptable, the issue is the extent to which the population in the single arm open label study is representative of the population studied in the pivotal study. Data from the open label studies relating to repeated dosing may not be sufficiently robust given the concern about bias both in assessment and patient selection. This risk is evident from the unexplained difference in TEAEs after the first dose, where TEAEs and study drug related TEAEs after the initial treatment were substantially lower in the multiple dose open label studies (EV004, EV006) versus the single dose randomised clinical trials. Considering each treatment dose, The rate of TEAEs, study drug related TEAEs, TEAEs of special interest, and study drug related TEAEs of special interest are lower at subsequent treatment intervals in the open label studies EV004, EV006 for subjects who undergo retreatment, but the factors influencing the decision to undergo retreatment are unclear in these open label uncontrolled studies.

In pooled analysis, the overall increase in TEAEs after multiple administrations of DWP-450 (34.5% vs 41.5%) is evident for mild, moderate, severe TEAEs (1.6% vs 2.2%); serious TEAEs (1.4% vs 1.5%) and TEAEs of special interest (2.7% vs 2.9%), but not study drug related TEAEs at 13.6% vs 12.1%.

Although the trend of adverse events in study EVB-003 is consistently in favour of Botox over DWP-450, it is accepted that conclusions should be guarded when they are based on single placebo-controlled RCT. This uncertainty places further importance on a clear safety signal from the repeat dose studies. Repeated administration of Botulinum toxin by i.m. injection is typically performed and therefore the rate of frequency of TEAEs both after each administration and over the whole treatment course are relevant to the safety assessment. Overall, rates of TEAEs and TEAEs of special interest are increased after repeated versus single doses of DWP-450. Direct comparison of multiple vs single administration of DWP-450 is precluded by the absence of an active controlled extension of the pivotal study EVB003. Indirect comparison of multiple vs single administration of DWP-450 is limited on several grounds. There is a risk that patient characteristics and patient expectations will differ between RCTs and single arm open-label studies, and indeed the studies are poorly matched for Fitzpatrick skin types. The open-label studies are confounded by much higher rates of significant protocol deviations (up to 40% in study EV004) and ~10fold excess of subject withdrawals compared with the single dose RCT EVB-003. Many of the subject withdrawals are unexplained. Data on the subjects' previous exposure to botulinum toxin injections was not collected systematically. These multiple confounders significantly limit confidence these open label studies accurately reflect real-world experience or support the claim that the rate of adverse events per administration decrease with successive administrations of botulinum toxin.

TEAEs in Special Populations and Subgroups

Overall, TEAEs were more common in females than males, particularly after multiple doses 43.3% vs 23.5%, and overall 39.3% versus 29.1%. In contrast, TEAEs of Special interest are more common in males than females in each pooled analysis, at 8/151 (5.3%) vs 39/1508 (2.6%) overall respectively.

There was an excess of TEAEs in **subjects ≥65 years** than <65 years in some clinical studies (EV001 53.8% vs 36.4%, EV002 33.3 vs 27.9%; EVB003 23.5% vs 38.6%; EV004 48.5% vs 41.4%, EV006 39.2%, 41.4% respectively), and overall at 40.9% vs 38.1%. TEAEs of Special interest are **more common in older** than younger subjects overall 6/154 (3.9%) vs 41/1505 (2.7%). Too few older subjects were studied to address key concerns about the safety of botulinum toxin in the elderly:

- Increased risk of eyelid ptosis
    - Many use the frontalis muscle to raise their eyebrows and eyelids to see
    - May have extra skin under the brow (pseudoptosis)
    - May have a reduced or absent orbital septum
- Increased susceptibility to bruising after botulinum toxin injections
    - Delicate skin
    - Interaction with prescribed or OTC medications such as anti-inflammatory agents, anti-coagulants, anti-platelet agents and aspirin

These additional risks in the elderly might be mitigated by conservative dosing, low volume injections, electromyographic guidance to ensure proper placement of injections, and reviewing the options for

temporary cessation of interacting medications. (See review by C M Cheng 2007, Cosmetic use of botulinum toxin type A in the elderly, Clinical Interventions in Aging 2007:2(1) 81–83).

Subjects with fair skin (II) may have higher rates of TEAEs of special interest than those with light brown skin (IV). Potential differences between racial groups in rates of TEAEs and TEAEs of special interest are not assessable because of the small number of non-white subjects.

TEAEs of Special interest rates are higher in subjects with severe versus moderate GLs at maximum frown by IA at baseline in each study and 7-fold higher overall, 45/1224 (3.7%) vs 2/435 (0.5%).

Eight subjects had a pregnancy during the study despite a negative pregnancy test at study entry and after deploying a range of strategies to avoid becoming pregnant. Seven of eight delivered healthy infants whilst one subject had a spontaneous abortion assessed as unrelated to the study drug. No further treatment was given.

The risk of facial asymmetry is not discussed. No drug-drug interactions were explored

Immunogenicity

The screening assay for Anti-Botulinum toxin A Antibodies (ADA) has poor sensitivity at 3 µg/mL using the CF for NHS (1.048) and is not defined for the assay with the pre-dose patient serum CF (1.204). Assay thresholds were statistically defined for the screening assay, 5% false positives, but statistical analysis to establish the threshold for the confirmatory assay is not described. Neither a more sensitive ELISA nor optimisation of sensitivity is undertaken in contrast to earlier reports (Dressler 2014, An ELISA for detection of or detection of botulinum toxin Abs). Neither linearity of testing nor titering of confirmed ADA positives is described in any of the reports provided. It is unclear whether the sample analysis is within the defined stability of samples since long term stability data are not provided. Neutralisation assays are typically qualitative but selection of the LD50 assay rather than the mouse diaphragm assay which is some 25x more sensitive is not justified. The neutralisation assay has no initial validation documented and the clinical conditions of use differ since the clinical report provided states "Due to the limited volume for the Human Serum Samples, a lower volume than previously validated was used to conduct this study".

The immunogenicity assessment of DWP-450 is solely dependent on studies without an active control, on the basis that the low rate of ADA detected renders comparative studies irrelevant, hence the absence of assessment within the pivotal study. Few ADA subjects are identified by the insensitive assay, whilst 7/761 (0.9%) of screen positive samples are confirmed positive. The rate of neutralisation Abs of 1 in 1414 (0.07%) subjects is substantially lower than reported in a systematic review and meta-analysis by Fabbri 2016 who cites an overall prevalence of 1.1% (95% CI 0.1% - 7.5%) for hyperhidrosis, glabellar line, and hypersalivation, versus 0.4% (0.1% - 0.7%) in clinically responding glabellar line patients. No studies are reported which examine neutralising Abs in secondary non-responder patients treated for glabellar lines although in a retrospective series of secondary non-responders, Lange 2009 reports NAbs in 16% - 26% of patients treated with botulinum toxin for blepharospasm.

In summary, the immunogenicity assessment is regarded as not fit for purpose since the assays are insensitive, poorly validated, generate results for NAbs which are substantially lower than reported in the literature, and provide no realistic estimation of ADA rates for DWP-450 alone or in comparison with Botox.

2.6.2. Conclusions on the clinical safety

Short-term safety profile

The type and incidence of ADRs to the test and reference products were broadly comparable and in-line with those expected from the Botox SmPC: Thus, for the granted indication, the short-term safety profile is of concern given the increased rate of drug related TEAEs, serious TEAEs, and TEAEs of special interest including a 2% rate of eye disorders. The uncertainty about the rate of TEAE based on a small single placebo-controlled RCT is accepted. In order to further quantify the safety concerns, and in particular the eye disorders including eyelid ptosis, a non-interventional PASS is required (see also below, safety of multiple vs single administration and long-term data).

Safety of multiple vs single administration and long-term safety data

In the absence of an active controlled extension of the pivotal study EVB003, there is no direct comparison between multiple vs single administration of DWP-450.

In addition, whilst collecting safety data in an open label design is acceptable, the issue is the extent to which the population in the single arm open label study is representative of the population studied in the pivotal study. Data from the open label studies relating to repeated dosing may not be sufficiently robust given the concern about bias both in assessment and patient selection. The risk that subjects recruited to Randomised Clinical Trials differ significantly from those recruited to open-label uncontrolled studies is evident from the unexplained difference in TEAEs and study drug related TEAEs after the initial treatment, where the rate of these TEAEs is substantially lower in the multiple dose open label studies (EV004, EV006) versus the single dose RCTs. Considering each treatment dose, the rate of TEAEs, study drug related TEAEs, TEAEs of special interest, and study drug related TEAEs of special interest are lower at subsequent treatment intervals in the open label studies EV004, EV006 for subjects who undergo retreatment, but the factors influencing the decision to undergo retreatment are unclear in these open label uncontrolled studies. These limited open label studies indicate increased rates of TEAEs after multiple administrations of DWP-450 is evident for mild, moderate, severe TEAEs; a similar trend is seen for serious TEAEs and TEAEs of special interest; and there is an unexplained trend in interval shortening. In addition to local spread of toxin effect as shown by eye disorders, there is limited evidence of distant effects as shown by reports of difficulties with speech, albeit short-lived.

Whilst it was agreed that the long-term data available did not raise any major concerns, it was felt important to have more robust data. Therefore, in order to quantify the safety concerns, including eye disorders, and provide additional characterisation of the long-term safety of Nuceiva, a non-interventional PASS is required. The commitment to perform a non-interventional PASS is appropriately reflected as an additional pharmacovigilance activity in the RMP, including agreement of the protocol, annual study progress report and final study report submission.

Muscle volume loss

New literature reveals muscle volume loss after single and repeat injections of botulinum toxin.

A statement that muscle atrophy is expected after repeated of botulinum treatment is therefore included in the SmPC.

Immunogenicity assessment not fit for purpose

Overall the immunogenicity risk with botulinum toxin A administration is regarded as low but not insignificant. In the absence of an immunogenicity assessment within the pivotal study, there are no reliable comparative ADA data for DWP-450 versus Botox. Nor are there reliable immunogenicity data for DWP-450 per se since the assays are insensitive, poorly validated, generate low rates of binding Abs and NAbs which are substantially lower than reported in the literature. The clinical relevance of NAbs is underlined by high rates reported in secondary non-responders.

As a post-authorisation commitment**,** the applicant is to re-test previously negative sera from studies EV-001, EV-002, EV-004, EV006 using new binding ADA assays for screening, confirmation and determination of ADA titres, supported by a sensitive assay for neutralising ADA (see also above, Conclusions on the Clinical Efficacy).

In addition, the applicant will, when possible, ask the subjects who are non-responders to have a blood sample tested for NAb using the mouse hemi-diaphragm model. The neutralising antibody analysis for non-responders who volunteer blood samples will be reported via routine pharmacovigilance and is reflected in the RMP as an additional pharmacovigilance activity.

In summary, the uncertainty about the rate of adverse events of special interest including eye disorders based on a single small RPCT is accepted. The open label repeat dose studies did not collect exposure history systematically and are subject to multiple confounders which significantly limit confidence they accurately reflect real-world experience or support the claim that the rate of adverse events per administration decrease with successive administrations of botulinum toxin. Accordingly, a **non-interventional PASS** is required to quantify safety concerns and to provide additional characterisation of the long-term safety of Nuceiva. The study synopsis was provided and agreed. The study protocol will be submitted for agreement

The CHMP considers the following measures necessary to address issues related to safety:

- Non-interventional PASS is required to quantify safety concerns, including eye disorders, and to provide additional characterisation of the long-term safety of Nuceiva.

- Re-test previously negative sera from studies EV-001, EV-002, EV-004, EV006 using new binding ADA assays for screening, confirmation and determination of ADA titres, supported by a sensitive assay for neutralising ADA. These studies will be supported by post-marketing surveillance to monitor for cases of hypersensitivity that may be caused by ADA formation**.**

- When possible, subjects who are non-responders will also be asked to have a blood sample tested for NAb using the mouse hemi-diaphragm model.

2.7. Risk Management Plan

Safety concerns

Important identified risks	• Eyelid ptosis • Immunogenicity • Distant spread of toxin • Development of or exacerbation of neuromuscular disorders • Hypersensitivity
Important potential risks	• Incorrect drug administration due to 100U vial • Long-term use
Missing information	• Use during pregnancy and lactation

Pharmacovigilance plan

Table of On-going and planned additional pharmacovigilance activities				
Study **Status**	**Summary of objectives**	**Safety concerns addressed**	**Milestones**	**Due dates**
Category 1 - Imposed mandatory additional pharmacovigilance activities which are conditions of the marketing authorisation - **None**				
Category 2 - Imposed mandatory additional pharmacovigilance activities which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances - **None**				
Category 3 - Required additional pharmacovigilance activities				
Non-interventional PASS Planned	To provide additional characterisation of the long-term safety of Nuceiva. The study protocol will include the systematic recording of previous exposure to botulinum toxin A	Eyelid Ptosis Long-term use	Protocol submission Study progress report Final study report submission	3 months after EC decision Annually Mar 2022
Development and replacement of 100U vial by 50U vial size. Planned	To reduce the potential for misuse with Nuceiva, as per Incorrect drug administration due to 100U vial	Incorrect drug administration (Nuceiva misuse) linked to 100U vial size	Line extension application for a 50U vial size filing	within 3 months of the Commission Decision
Non-interventional immunogenicity analysis Planned	Retesting of previously negative sera from studies EV-001, EV-002, EV-004, EV006 using new binding ADA assays for screening, confirmation and determination of ADA titres, supported by a sensitive assay for neutralising ADA.	Immunogenicity	Protocol submission Final report submission	6 months after EC decision *(provisional due date)* Mar 2021*
Neutralising antibody analysis for non-responders who volunteer blood samples	Test volunteered non-responder blood samples for neutralising antibodies	Immunogenicity	Reporting via routine pharmacovigilance	Reporting via routine pharmacovigilance

Risk minimisation measures

Safety Concern	Routine Risk Minimisation Measure	Pharmacovigilance activities
Important identified risks		
Eyelid ptosis	Routine risk minimisation measures: DWP-450 will be a prescription only medicine administered by clinicians. Text in DWP-450 EU SmPC and EU PL: EU SmPC: • Information and diagrams in section 4.2 of the EU SmPC regarding the administration steps to be taken in order to reduce the complication of eyelid ptosis. • Information in section 4.4 of the EU SmPC that the relevant anatomy, and any alterations to the anatomy due to prior surgical procedures, must be understood prior to administering DWP-450 and injection into vulnerable anatomic structures must be avoided. • Information in section 4.4 of the Eu SmPC communicates a specific warning regarding the risk of eyelid ptosis • Information in section 4.8 of the EU SmPC that eyelid ptosis is a common adverse drug reaction EU PL: • Information in section 2 of the EU PL that drooping of the eyelid may occur after treatment • Information in section 4 of the EU PL that drooping eyelid is a common adverse drug reaction. Additional risk minimisation measures: *None*	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: *None* Additional pharmacovigilance activities: Non-interventional PASS
Immunogenicity	Routine risk minimisation measures: DWP-450 will be a prescription only medicine administered by clinicians. Text in DWP-450 EU SmPC and EU PL: EU SmPC: • Information in section 4.2 of the EU SmPC that DWP-450	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: *None* Additional pharmacovigilance activities: Non-interventional immunogenicity

	treatment intervals should not be more frequent than every three months. • Information in section 4.4 of the EU SmPC communicates a specific warning regarding the risk of immunogenicity • Information in section 4.8 of the EU SmPC communicates immune response as undesirable effect EU PL: • Guidance in section 2 of the EU PL to limit the risk of antibody formation, the interval between two treatments must not be less than three months. Additional risk minimisation measures: *None*	analysis; neutralising antibody analysis for non-responders who volunteer blood samples
Distant spread of toxin	Routine risk minimisation measures: DWP-450 will be a prescription only medicine administered by clinicians. Text in DWP-450 EU SmPC and EU PL: EU SmPC: • Information in section 4.2 of the EU SmPC that care must be taken to ensure that DWP-450 is not injected into a blood vessel and that physical manipulation (such as rubbing) of the injection site in the immediate post-administration period should be avoided. • Information in section 4.4 of the EU SmPC communicates a specific warning regarding the risk of local and distant spread of toxin effect • Adverse reactions possibly related to the spread of toxin distant from the site of administration listed as class effect in section 4.8 of the EU SmPC EU PL: • Information in section 2 of the EU PL communicates a specific warning regarding the risk of local and distant spread of toxin effect. • Guidance in section 2 of the EU PL that patient should visit their doctor immediately if they find it difficult to swallow, to speak or to breathe after treatment • Information in section 4 of the EU PL that patient should visit their doctor immediately if they	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: *None* Additional pharmacovigilance activities: *None*

	have any difficulty in breathing, swallowing or speaking after receiving DWP-450 (in upper case text). Additional risk minimisation measures: *None*	
Development of or exacerbation of neuromuscular disorders	Routine risk minimisation measures: DWP-450 will be a prescription only medicine administered by clinicians. Text in DWP-450 EU SmPC and EU PIL: EU SmPC: • Information in section 4.3 of the EU SmPC that DWP-450 is contraindicated in the presence of myasthenia gravis or Eaton Lambert Syndrome. • Information in section 4.4 of the EU SmPC communicates a specific warning regarding use in patients with pre-existing neuromuscular disorders. • Information in section 4.4 of the EU SmPC that patients or caregivers should be advised to seek immediate medical care if swallowing, speech or respiratory disorders arise. EU PL: • Information in section 2 of the EU PL that DWP-450 must not be used if patient has a pre-existing myasthenia gravis or Eaton-Lambert syndrome and is not recommended in patients with dysphagia or breathing problems • Information in section 2 of the EU PL that patient should visit their doctor immediately if they find it difficult to swallow, to speak or to breathe after treatment. • Information in section 4 of the EU PL that patient should visit their doctor immediately if they have any difficulty in breathing, swallowing or speaking after receiving DWP-450 (in upper case text). Additional risk minimisation measures: *None*	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: *None* Additional pharmacovigilance activities: *None*
Hypersensitivity	Routine risk minimisation measures: DWP-450 will be a prescription only medicine administered by clinicians. Text in DWP-450 EU SmPC and EU	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: *None*

	PIL: EU SmPC: • Information in section 4.3 of the EU SmPC that DWP-450 is contraindicated in patients with known hypersensitivity to active substance or to any of the excipients of the formulation • Information in section 4.4 of the EU SmPC communicates a specific warning regarding the risk of hypersensitivity. EU PL: • Information in section 2 of the EU PL that DWP-450 must not be used if patient has a known allergy to botulinum toxin type A or any other ingredient • Information in section 2 of the EU PL communicates allergic reaction as a potential adverse drug reaction • Information in section 2 of the EU PL that patient should visit their doctor immediately if they find it difficult to swallow, to speak or to breathe after treatment. • Information in section 4 of the EU PL that patient should visit their doctor immediately if they have any difficulty in breathing, swallowing or speaking after receiving DWP-450 (in upper case text). Additional risk minimisation measures: *None*	Additional pharmacovigilance activities: *None*
Important potential risks		
Incorrect drug administration due to 100U vial	Routine risk minimisation measures: Text in DWP-450 EU SmPC and EU PIL: EU SmPC: • Information in section 4.2 of the EU SmPC that DWP-450 is for single use and after reconstitution, must be used only for one session of injection(s) per patient. • Information in section 6.3 of the EU SmPC that from a microbiological point of view, the product should be used immediately. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: *None* Additional pharmacovigilance activities: Development and replacement of 100U vial by 50U vial size

	user and would normally not be longer than 24 hours at 2° to 8°C, unless reconstitution/dilution has taken place in controlled and validated aseptic conditions. • Information in section 6.6 of the EU SmPC that it is mandatory that DWP-450 is used for one single patient treatment only during a single session. EU PL: • *None* Additional risk minimisation measures: *None*	
Long-term use	Routine risk communication: EU SmPC: • Information in section 4.2 of the EU SmPC that the efficacy and safety of repeat injections beyond 12 months has not been evaluated. EU PL: • *None* Additional risk minimisation measures: *None*	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: *None* Additional pharmacovigilance activities: Non-interventional PASS
Missing information		
Use during pregnancy and lactation	Routine risk minimisation measures: DWP-450 will be a prescription only medicine administered by clinicians. Text in DWP-450 EU SmPC and EU PL: EU SmPC: • Information in section 4.6 of the EU SmPC that DWP-450 is not recommended during pregnancy and in women of childbearing potential not using contraception. • Information in section 4.6 of the EU SmPC that DWP-450 should not be used during breast-feeding. EU PL: • Guidance in section 2 of the EU PL that patients should contact their doctor if they are pregnant, planning pregnancy or become pregnant while being treated. • Guidance in section 2 of the EU PL that the use of DWP-450 is	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: *None* Additional pharmacovigilance activities: *None*

	not recommended during pregnancy or breastfeeding. Additional risk minimisation measures: *None*	

Conclusion

The CHMP and PRAC considered that the risk management plan version 2.0 is acceptable.

2.8. Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

Periodic Safety Update Reports submission requirements

Based on indication, the CHMP is of the opinion that a separate entry in the EURD list for Nuceiva is needed, as it cannot follow the already existing entry for botulinum toxin type A. The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did request the alignment of the new PSUR cycle with the international birth date (IBD). The IBD is 16.08.2018. The new EURD list entry will therefore use the IBD to determine the forthcoming Data Lock Points.

2.9. New Active Substance

The applicant declared that botulinum toxin type A has been previously authorised in a medicinal product in the European Union but differs significantly with regard to safety and/or efficacy due to differences in the manufacturing process as it is significantly purer than other medicinal products that are available in the European Union. Botulinum toxin Type A is an active substance previously authorised in the European Union and contained in medicinal products as Botox/Vistabel, Dysport/Azzalure and Xeomin/Bocouture.

The differences in manufacturing processes alone are not sufficient to establish new active substance status and are only relevant when it can be demonstrated that they are linked to differences in safety or efficacy profile for the resulting product.

Significantly different properties in terms of safety/efficacy have not been demonstrated and no data has been provided to support that the manufacturing process differences described in the submission result in a product with a significantly different safety/efficacy profile.

The applicant acknowledged the CHMP position and has withdrawn the New Active Substance claim during the procedure.

Based on the available quality and clinical data, the CHMP considers, that botulinum toxin type A, which have some differences in molecular structure, nature of the source material or manufacturing process, does not differ significantly in properties with regard to safety and/or efficacy from botulinum toxin type A contained in medicinal product(s) previously authorised within the European Union and therefore is not considered to be a new active substance

2.10. Product information

2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.10.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Nuceiva (botulinum toxin type A) is included in the additional monitoring list as it is a biological product and authorised after 1 January 2011.

Therefore the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

All the available clinical studies are in healthy adults >18 years old. A single clinical study examines psychological impact in otherwise healthy adults with moderate to severe glabellar lines.

In other clinical context, not the subject of this application, botulinum toxin type A injections are used to induce cholinergic blockade at the neuromuscular junction for temporary therapeutic flaccid muscle paralysis in focal muscle spasticity and striated muscle related pain syndromes; afferent parasympathetic cholinergic blockade inducing detrusor muscle relaxation in bladder dysfunction; cholinergic blockade for relief of autonomic syndromes such as axillary hyperhidrosis and excessive salivation.

3.1.1. Disease or condition

The claimed indication is: *NUCEIVA is indicated for the temporary improvement in the appearance of moderate to severe vertical lines between the eyebrows seen at maximum frown (glabellar lines), when the severity of the above facial lines has an important psychological impact in adult patients.*

For the claimed indication, evidence of psychological impact was addressed in the pivotal study (EVB003) at the baseline assessment using a subject's simple verbal response. This is in line with clinical practice where practitioners consult with patients and assess the psychological impact of the glabellar lines prior to administering botulinum toxin A, to ensure it is an appropriate treatment for their patient. There are no validated scales to assess the psychological impact in this specific patient population.

Although use of scales / patient-reported outcomes that have been specifically validated in the target population are generally preferred, it is acknowledged that such scales are not available in this case. However, the GASI and SSS scores used in the submitted studies are considered sufficiently applicable to investigate patient benefit in the proposed target population.

Different efficacy endpoints were used in the European and US studies to favour clinical and statistical perspectives without addressing the limitations of assessment methods, and whether a smaller or larger effect is required to achieve the optimal level of residual facial expression at maximum frown or at rest, and for standard variables such as age, gender, or race.

3.1.2. Available therapies and unmet medical need

Three botulinum toxin A preparations are licensed for use in the EU for treatment of glabellar lines (Vistabel, Bocouture, Azzalure). All three are licensed for use using mutual recognition procedures, and the indication for use in each case refers to the treatment of glabellar lines that have an important psychological impact for the patient.

3.1.3. Main clinical studies

Each study recruited healthy adults ≥18 years old with moderate or severe GLs at maximum frown. The European pivotal multi-centre study EVB003 was a non-inferiority, active treatment and placebo randomised controlled single treatment trial of DWP-450 versus Botox versus Placebo for the temporary improvement of moderate or severe glabellar lines (GLs) at maximum frown where the severity has an important psychological impact in adults; 540 subjects recruited. In addition, two confirmatory US placebo-controlled studies recruited 330 and 324 subjects for a randomised single treatment trial of DWP-450, without assessing psychological impact.

Information on multiple administration is dependent on two uncontrolled open label repeat treatment safety studies of DWP-450 in the US recruiting 352 and 570 subjects respectively, without assessment of psychological impact in line with the "cosmetic" use of this type of product approved in the US (Botox® Cosmetic).

3.2. Favourable effects

The collected data from the pivotal and supportive clinical studies confirmed the efficacy of the product in the intended indication. They demonstrated the ability of the product to not only exert a physiological activity on the appearance of moderate to severe vertical lines between the eyebrows, but also induce a beneficial psychological impact in adults below 65 years of age, with this condition. The main data supporting these benefits are listed below:

- The non-inferiority margin for absolute difference for the primary endpoint, a GLS score of 0 or 1 at maximum frown at day 30 by IA, was 4.4 (95% CI -1.9, 10.8) for DWP-450 vs Botox, within the -10% non-inferiority margin.
- The absolute differences between Botox and Placebo, DWP-450 and Placebo for the primary endpoint, were 78.6% and 83.1%, respectively (both $p<0.001$).
- A ≥2 point improvement in the GLS score at maximum frown at day 30 by IA was seen in 0/48 placebo, 168/244 (68.9%) Botox and 181/235 (77%) DWP-450 treated subjects. Assessment by subjects was supportive.
- The mean change (95% CI) from baseline in day 90 HADS-A scores was -0.9 (-1.7, -0.2) for Placebo vs -0.9 (-1.3, -0.6) for Botox vs -1.1 (-1.4, -0.8) for DWP-450, and for HADS-D scores was -0.5 (-1.1, 0.0) vs -0.6 (-0.9, -0.3) vs -0.6 (-0.9, -0.3) respectively. The absolute difference from placebo for the change in HADS-A scores from baseline to day 90 after DWP-450 was -0.2 (-0.9, 0.6), and for HADS-D scores was -0.1 (-0.7, 0.6), no significant difference.
- The mean absolute difference in subjects with a ≥1 point improvement in the Subject Satisfaction Scale score was 85.0 $p<0.001$ for DWP-450 versus placebo and 4.7 (-0.9, 10.2) for DWP-450 versus Botox.
- The percentages of positive responders on the GAIS and SSS scores support the analyses based on the GLS scores.

In addition, the pooled data from EV001, EV002 (with placebo values for EV001, EV002, EVB003), where a ≥2point improvement in GLS score at maximum frown by IA and SA at day 30 was the primary endpoint, showed an absolute difference from placebo that was significant across skin types e.g. type III of 64.6 (95% CI 56.7, 71.6), type IV 63.4 (49.3, 73.9), type V 72.4 (29.9, 87.4), and type VI 66.7 (2.0, 90.1).

There were high rates of subject satisfaction using PROs (GAIS score by SA and the SSS), which although not validated, were considered relevant for Nuceiva treatment as a large difference from placebo was demonstrated and similar effect was seen compared to Botox, which is considered to adequately reflect the expected benefit.

In the open label studies EV004 and EV006, up to 50% of subjects required 4 administrations of DWP-450 over 1 year to maintain a GLS score <2 at maximum frown and the interval between treatments decreased as the number of treatments increased.

3.3. Uncertainties and limitations about favourable effects

Multiple administration

- There are insufficient data to robustly conclude on the expected clinical efficacy of multiple doses application (a situation that will be frequent in clinical practice). This is due to the fact that the uncontrolled open label studies EV004, EV006 were primarily safety studies not designed to assess long-term and multiple-dose efficacy, and that there was no active controlled extension of study EVB003.

Validation of Assessment Tools

- Validity of the key endpoints, GLS scores by IA, are limited by suboptimal kappa coefficients of interrater agreement for assessment at maximum frown (0.635) and unreliable values at rest (0.512) pre-trial, together with the lack of validation "in the field"
- Photographic images were recorded but no independent centralised assessment was performed, and the applicant regards the images as inadequate for retrospective cross-validation
- Neither GLS score by SA or GAIS score by IA were validated for the intended use.
- No validated PROs were used from the range of generic and specific clinical tools available.

Blinding

- High risk of unblinding was detected due to a strong treatment effect, unbalanced randomisation, and the absence of independent centralised assessment, in the context of high rates of previous exposure to botulinum toxin.
- Treatment response rates for the primary endpoint in EV001 & EV002 (a >2point improvement in GLS score at maximum frown by IA and SA at day 30) were higher in subjects with vs without prior history of botulinum toxin use, absolute difference from placebo 73.3% (95% CI 64.1, 79.9) vs 64.0% (57.7, 69.6).
- Concerns about the risk of unblinding are supported by large variation between study sites in the response rate by primary endpoint, 60% vs 100% in study EVB003, whilst studies EV001, EV002 exhibit 5-fold variations in responses rates by study site and 5-fold variations in differences in response by gender between these otherwise identical studies.

Immunogenicity

- There are no immunogenicity data from the pivotal study EVB003, so there are no contemporary comparative data with an established product.
- The immunogenicity assessment is reliant on data from the other randomised clinical trials and open label studies, compromised by insensitive, poorly validated assays which generate rates of binding Abs and NAbs that are substantially lower than in the literature.
- Therefore the strong link between ADA and secondary non-response, as reported in a systematic review and meta-analysis, is not adequately assessed.
- The immunogenicity assessment is inadequate to compare freeze-dried product with 5% overage with the lyophilised product with 50% overage of botulinum toxin protein but LD50 matched levels of toxin activity associated with seroconversion in study EV004.

Psychological impact

Changes in HADS scores assessed at day 90 showed no benefit over placebo for the psychological impact of DWP-450 treatment, but the failure to show a clear benefit on this rating scale can be explained by the fact that, at baseline, the majority of patients were within normal HADS A and D scores. In addition, the HADS tool has not been validated for the target population of this application and may be insensitive to detect changes following treatment. However, for the intended context of use it was considered that the demonstrated favourable results on patient reported outcomes were sufficient. Reassuringly similar results were seen compared to Botox.

Dose response

- No clinical dose response studies were performed. The single 20U dose used is based on a claim of matching dose comparability with Botox, in preclinical studies and Korean clinical studies, which used a different, lyophilised preparation which has a 50%.
- The single 20U dose regimen is based on dose comparability with Botox, but expert medical consensus recommends doses of Botox for GLs as low as 8U for some patients, and clinical safety data with DWP-450 suggests dose adjustment by sex, age $\geq$65 years, and GLS score at baseline may be appropriate.

Age

- Few subjects $\geq$65 years were included in study EVB003, where the non-inferiority margin for absolute difference for the primary endpoint, a GLS score of 0 or 1 at maximum frown at day 30 by IA was 14.6 (-14.4, 43.6) for DWP-450 (n=16) versus Botox (n=18).
- The number of older subjects studied was too small to address concerns about the efficacy of botulinum toxin in this group where the pathogenesis of wrinkles reflects gravity-induced tissue sagging due to thinner, less elastic skin, weaker facial muscles as well as muscle contraction.
- Similarly, it is unknown whether dose adjustment is required to achieve the optimal level of residual facial expression including in subjects $\geq$65 years.

Gender

- Pooled data from EV001, EV002 (with placebo values for EV001, EV002, EVB003), where a $\geq$2point improvement in GLS score at maximum frown by IA and SA at day 30 was the primary endpoint showed treatment responses were lower in males than females 53.5 (35.5, 68.8) versus 69.4 (64.5, 73.8).

Duration of Response

- The median time to response, using ≥2point improvement in GLS at maximum frown by IA and SA, was 10 days in studies EV001, EV002.
- Median (iqr) duration of treatment response (maintenance of a ≥2point improvement in GLS score at maximum frown by IA) was 78 (68, 91) days in EVB003, vs 85 (76, 113) days in EV001 vs 86 (78, 113) days in EV002 the other RCTs.

Optimal level of residual facial expression after botulinum toxin treatment

- Post- hoc analysis of treatment response, according to GLS at maximum frown by IA, indicates
- Subjects with a baseline score of 2 achieved a day 30 score of 0 & 1 in 56.5% & 40.3%
- Subjects with a baseline score of 3 achieved a day 30 score of 0 & 1 in 22.9% & 60.3% respectively.

Race

- The limitations of unvalidated PROs are exacerbated by the absence of subjects from Southern and Eastern Europe in the European EVB003 study, precluding investigation of any differences in treatment effects and expectation.
- The pivotal study EVB003 included only 3 Black/African American and 6 Asian subjects, and overall racial groups other than white are poorly represented precluding investigation of TEAEs of special interest

3.4. Unfavourable effects

The side effect profile of the active substance Botulinum toxin is well known and includes headache, local muscle weakness, muscle twitching and eyelid ptosis. Adverse events directly related to administration can include injection site haematoma, haemorrhage and pain or paraesthesia at the injection site. Local spread of toxin effects is reported with eye disorders including blepharospasm, eyelid ptosis, eyebrow drooping and diplopia. Distant spread of toxin effects is uncommon, is more likely at higher doses and at other injection sites, but includes dysphonia, dysphagia and dyspnoea.

In study EVB003, the rate of TEAEs is higher than placebo (32.7%) for both Botox (41.9%) and DWP-450 (37.6%), and similarly for Drug related TEAEs at 4.1% vs 14.6% vs 15.4 % respectively. The most common TEAE was headache at 14.3% for placebo, 10.2% Botox, and 13.9% for DWP-450.

The rate of TEAEs is similar between placebo and DWP-450 in studies EV001 and EV002 at 32.1% versus 38.2%, incident difference 6.1 (-6.3, 18.3) and 26.9% versus 28.5%, 1.5 (-11.2, 14.2) respectively. There was a small excess of Drug related TEAEs at 13% vs 15% in study EV001 and 8% versus 10% in study EV002. The most common TEAE is headache after both placebo and DWP-450.

Injection site bruising, pain or swelling related to study drug occurred in 1.6% after placebo versus 2.4% after DWP-450 injections in study EVB003.

TEAEs leading to Discontinuation were uncommon at ≤0.1% after single or multiple treatments with DWP-450.

Excess TEAEs after multiple versus single administration of DWP-450

Repeated administration of Botulinum toxin by i.m. injection is typically performed and therefore the rate of frequency of TEAEs both after each administration and over the whole treatment course are relevant to the safety assessment. Overall, rates of TEAEs and TEAEs of special interest are increased after repeated versus single doses of DWP-450.

Pooled analysis of studies of TEAEs after a single treatment (EV001, EV002, EVB003) versus multiple treatments (EV004, EV006) for placebo vs single vs multiple administrations showed TEAE rates of 30.3% vs 34.5% vs 41.5%; serious TEAEs 0.5% vs 1.4% vs 1.5%, and TEAEs of special interest 0.5% vs 2.7% vs 2.9%; whilst Drug related TEAEs were 9% vs 13.4% vs 12.1%.

Local Spread of Toxin Effects: Eye disorders

Within TEAEs of special interest, eye disorders including blepharospasm, eyelid ptosis, eyebrow drooping and diplopia are common at 0% after placebo, 1.6% Botox, 2.0% single treatment DWP450, 1.8% multiple treatments of DWP-450.

Distant Spread of Toxin Effects

Distant effects are uncommon but increased after multiple administrations:

- Dysphonia 0% placebo, 0.1% single, 0% multiple administrations of DWP-450
- Dyspnoea 0.5% placebo, 0.1% single, 0.2% multiple administrations of DWP-450
- Dysphagia 0% placebo, 0.1% single, 0.2% multiple administrations of DWP-450
- Speech disorder 0% placebo, 0.1% single, 0.2%* multiple administrations of DWP-450.

*Two subjects (0.12%, 2/1659) had transient disorder of speech regarded as related to study drug

Hypersensitivity

Possible hypersensitivity reactions occurred in 1.4% after placebo, 1.6% single, 1.5% after multiple administration of DWP-450. Hypersensitivity reactions assessed as *related to study drug* occurred in 0.4% (6/1659) of subjects who had eyelid oedema, injection site pruritus, and influenza like illness.

Immunogenicity

ADA were uncommon, detected pre-trial in 1 of 654 subjects in EV001, EV002; 2 of 352 subjects seroconverted after 2xDWP-450 administrations in E004 and 1 of 560 subjects pre-trial in study EV006. One of the 4 ADA positive subjects were non-responders to DWP-450 treatment.

Excess TEAEs in older subjects

There is an inconsistent excess of all TEAEs in subjects $\geq$65 years than <65 years in the clinical studies, EVB003 23.5% vs 38.6%; EV001 53.8% vs 36.4%, EV002 33.3 vs 27.9%; EV004 48.5% vs 41.4%, EV006 39.2%, 41.4% respectively.

3.5. Uncertainties and limitations about unfavourable effects

Multiple administration

- No direct comparison is available between multiple vs single administration of DWP-450 in the absence of an active controlled extension of the pivotal study EVB003.

- Subjects in the uncontrolled open-label multiple dose studies EV004, EV006 have different characteristics to the subjects in the single dose RCTs and subject dropout is poorly characterised which limits the value of indirect comparison.

Immunogenicity

- The sensitivity and validation of the Immunogenicity assays was inadequate
- No immunogenicity assessment was performed in the pivotal study EVB003
- The contribution of ADA to secondary non-response is not adequately addressed

Muscle atrophy

- Muscle wasting is presented as an inevitable part of treatment, representing disease atrophy after the induction of flaccid paralysis by botulinum toxin.
- Muscle wasting is consistent with recent data showing loss of muscle volume persisting 12 months after a single administration of botulinum toxin type A, and muscle volume loss with fat accumulation after multiple administrations
- The clinical significance of this muscle atrophy is unclear

Age

The small number of older subjects studied has not addressed key safety concerns

- Increased risk of eye disorders in older subjects given many use the frontalis muscle to raise their eyebrows and eyelids to see, have extra skin under the brow (pseudoptosis), or have a reduced or absent orbital septum
- Increased susceptibility to bruising due to delicate skin
- Increased susceptibility to bruising due to common prescribed or over the counter medications such as anti-inflammatory agents, anti-coagulants, anti-platelet agents and aspirin.

Race

- Potential differences between racial groups in rates of TEAEs and TEAEs of special interest are not assessable because of the small number of non-white subjects.

Skin type

- Case numbers are too small to confirm the trend of higher rates of TEAEs of special interest in subjects with fair skin (II) versus light brown skin (IV).

Dose

- The absence of dose response studies precludes comparison of dose versus safety.

Concomitant therapies

- Interaction with concomitant therapies was not investigated although expert medical consensus note co-treatment with dermal fillers would be used in 21% of subjects with GLs.

3.6. Effects Table

Effects Table for Nuceiva (fka DWP-450) for the temporary improvement in the appearance of moderate to severe glabellar lines, when the severity of these facial lines has an important psychological impact in adults below 65 years of age (data cut-off: Dec 22, 2016).

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
			Mean (95% CI)		Absolute difference Mean (95% CI)	
GLS score 0 or 1 day 30	At maximum frown by IA, PP	Single admin	87.2% DWP-450, (82.8% Botox)	4.2%	83.1 (70.3, 89.4) p<0.001 vs Botox 4.4 (-1.9, 10.8)	Study EVB003
GLS score 0 or 1 day 30	At maximum frown by IA, PP	Single admin	Baseline GLS=2		56.5% GLS=0 40.3% GLS=1	Study EVB003
GLS score 0 or 1 day 30	At maximum frown by IA, PP: Baseline 3	Single admin	Baseline GLS=3		22.9% GLS=0 60.3% GLS=1 in	Study EVB003
GLS score ≥2point better at day 30	**At Rest** by IA, ITT: Baseline score = 2	Single admin	24.1% DWP-450, (24.2% Botox)	0	24.1% (16.8, 31.3) p<0.001 vs Botox 0.1 (-10.3, 10)	Study EVB003
Patient reported Outcomes	Psychological impact		Psychological impact of glabellar lines			Study EVB003
Patient reported Outcomes	Psychological impact		Similar to Botox, the active comparator used in the pivotal trial, a beneficial effect on psychological wellbeing could not be shown as compared to placebo, but significant effects on patient reported outcomes were demonstrated.			Study EVB003
Unfavourable Effects						
Headache	Overall *Botox*	Pooled data	9% *6.9%*	7.6%		
TEAEs of special interest	Eye disorders	Single admin	2.0%	0	1.8% *multiple* DWP-450 1.6% single Botox	EV001, EV002 & EVB003
TEAEs of special interest	Cardiac disorders	Single admin	0	0	0.4% *multiple* DWP-450 = bradycardias *1 transient long QTc*	EV004, EV006
TEAEs of special interest	Dysphonia	Single admin	1 (0.1%)	0	0 *multiple* DWP-450 0 after Botox	EV001, EV002 & EVB003 EV004, EV006
TEAEs of special interest	Dyspnoea	Single admin	0.1%	0.5%	0.2% *multiple* DWP-450 0 Botox	
TEAEs of special interest	Dysphagia	Single admin	0.1%	0	0.2% *multiple* DWP-450 single 0 Botox	
TEAEs of special interest	Speech disorder	Single admin	0.1%	0	0.2% *multiple* DWP-450 *transient,* ***drug related*** 0 Botox	EV004, EV006

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
TEAEs of special interest	Anti-drug Abs	Single admin	0.1% *	0	0.3%* *multiple* DWP-450 *1 of 4 subjects with ADA are non-responders *Assays unreliable. Repeat Immunogenicity assessment with new assays*	EV001, EV002, EV004, EV006 #

Abbreviations:
Notes: # Two subjects in EV004 seroconverted after lyophilised product

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

A single treatment with DWP-450 induces temporary improvement in moderate to severe glabellar lines at maximum frown in most cases, similarly to Botox and unlike placebo which has little effect. For the claimed indication, evidence of psychological impact with a subject's simple verbal confirmation of psychological impact at baseline was obtained. This is in line with the approach taken in clinical practice. The day 90 change in HADS scores versus baseline is no different from placebo, but failure to show a clear benefit on this rating scale may be explained as this scale is not validated for this specific patients population and the baseline scores at enrolment were for the majority in the normal range. In a subset of patients who had higher scores demonstrated a favourable effect similar to Botox. Similar to Botox, the active comparator used in the pivotal trial, a beneficial effect on psychological wellbeing could not be shown as compared to placebo, but significant effects on patient reported outcomes were demonstrated. Overall, efficacy of Nuceiva can be considered non-inferior to Botox.

There are uncertainties around efficacy of DWP-450 in the pivotal study EVB003 due to the lack of an active controlled extension to assess the clinical efficacy of multiple doses typical of clinical practice, and the use of unvalidated PROs. The supportive open-label studies are not designed as efficacy studies and they do not examine the drivers to repeat administration from either the physician or subject perspective, or account adequately for subject dropout. The efficacy data from the supporting single dose RCTs EV001, EV002 are limited by a strong treatment effect, the lack of independent centralised assessment, and unbalanced randomisation, in the context of high rates of previous exposure. These factors generate concerns about the robustness of blinding, which are potentially reflected in the 5-fold variations in responses rates by study site and 5-fold variation in the rate difference between genders in otherwise identical studies.

The immunogenicity data are poor and unreliable with no data from the pivotal study EVB003, whilst data from the other RCTs and open label studies are compromised by insensitive, poorly validated assays which generate rates of binding Abs and NAbs which are substantially lower than in the literature. The clinical relevance of NAbs is underlined by high rates in secondary non-responders reported in systematic review and meta-analysis.
Despite the uncertainties listed above, the CHMP is of the opinion that the totality of the gathered efficacy data is sufficient to provide reassurance for a consistent and well-demonstrated effect, in the intended indication.

No new side-effects are reported and the type and incidence of ADRs are broadly comparable and in line with those expected from the active substance. Due to the RCTs and open label studies design, there are some uncertainties about the short term safety, including a 2% rate of eye disorders, and

the long term safety of DWP-450. The CHMP agreed that the safety profile of DWP-450 did not raise any major concerns and is sufficiently established at this stage. In order to have more robust safety, better quantify the safety concerns and provide additional characterisation of the long-term safety of Nuceiva, a non-interventional PASS is required.

The treatment response is seen in males and females; across Fitzpatrick skin types when pooled analyses are considered, is temporary and reproducible for up to 4 treatments. Too few subjects ≥65 years old were studied to provide confidence in either efficacy or safety in this age group. Post hoc analysis provides rates for a range of treatment responses in subjects grouped by the baseline severity glabellar lines at maximum frown by IA.

Matching dose comparability to Botox is claimed based on Korean clinical studies not presented here that used a different lyophilised preparation, together with preclinical data which do not allow for any differences in diffusion of drug within the tissues. Dose response studies were omitted from the Korean clinical studies and the Evolus programme, and a fixed dose of 20U was used throughout. This contrasts with expert medical consensus on the use of Botox for glabellar lines, which recommends tailoring the dose to the individual down to 8U in some cases. Thus, there is no experience with DWP-450 to guide tailoring the dose to the benefit/risk balance as prompted by differences in treatment response by age, sex, and prior treatment with botulinum toxin. Persistence of effect after repeat treatment was consistent with that seen in the placebo controlled setting. Reduced efficacy in the elderly and in males could have implications for dosing and treatment strategies. This has been identified with other botulinum toxin products and has been addressed in section 5.1 of the SmPC.

3.7.2. Balance of benefits and risks

The review of the data shows that Nuceiva significantly reduces the severity of glabellar lines at maximum frown. The temporary changes last for up to 139 days. In order to maintain an effect, it is expected that Nuceiva is administered repeatedly.

The psychological impact of glabellar lines was confirmed at study entry in the pivotal trial. While a positive psychological impact was not demonstrated using the HADS score, this instrument may not be appropriate to reflect the situation in the intended population of use. The data have demonstrated favourable patient reported outcomes, which were statistically significantly higher for Nuceiva compared to placebo and similar to the active comparator.

Considering the totality of efficacy evidence, it is considered that sufficient supportive evidence of efficacy for the type of medicinal product and the intended context of use in clinical practice has been provided.

The safety profile is in line with those of other similar products. In view of the available data, there are some uncertainties regarding repeated administration and long-term safety, as well as immunogenicity. There is also a high rate of eye disorders which deserves further investigation. In the view of these uncertainties, appropriate measures have been put in place to gather and assess the relevant data in the post-marketing setting.

Overall, the benefit/risk of Nuceiva is positive.

3.7.3. Additional considerations on the benefit-risk balance

This product is restricted to use by physicians with appropriate qualifications and expertise in this treatment and use of the required equipment.

The claim to new active substance status is not supported on quality or efficacy or safety grounds, has been withdrawn by the applicant, and this MAA is now for a known active substance.

Nuceiva is available in a 100U vial and this may present a risk of incorrect drug administration due to the recommended dose of 20U. In order to minimize the risk, appropriate wording is implemented in the Product Information so Nuceiva is only used for a single patient during a single session. The applicant also committed to develop and replace the 100U vial by a 50U vial within 3 months of the Commission Decision.

The routine control of biological activity of the finished product of Nuceiva is performed by means of an LD50 method currently as per the current standards of the Ph. Eur. Taking into account the requirements of Directive 2010/63/EU and the technical feasibility to develop an alternative method, the CHMP believes that it is important to request the conduct the development of an in *vitro* alternative method to replace the LD50 method in routine control of the finished product by September 2020. An Annex II condition to the Marketing Authorisation has been requested accordingly.

3.8. Conclusions

The overall B/R of Nuceiva is positive for the temporary improvement of the glabellar lines when the severity of these has an important psychological impact.

Divergent position is appended to this report.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by majority decision that the risk-benefit balance of Nuceiva is favourable in the following indication:

NUCEIVA is indicated for the temporary improvement in the appearance of moderate to severe vertical lines between the eyebrows seen at maximum frown (glabellar lines), when the severity of the above facial lines has an important psychological impact in adults below 65 years of age.

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Other conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Risk Management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Obligation to conduct post-authorisation measures

The MAH shall complete, within the stated timeframe, the below measures:

Description	**Due date**
Introduction of an *in vitro* method as a replacement for the potency assay of the Nuceiva finished product.	September 2020

New Active Substance Status

Based on the review of the available data, the CHMP considers that botulinum toxin type A, which have some differences in molecular structure, nature of the source material or manufacturing process, does not differ significantly in properties with regard to safety and/or efficacy from botulinum toxin type A contained in medicinal product(s) previously authorised within the European Union and therefore is not considered to be a new active substance.

Appendix

1. Divergent position to the majority recommendation

DIVERGENT POSITION DATED 25 July 2019

Nuceiva EMEA/H/C/004587/0000

The undersigned members of the CHMP did not agree with the CHMP's positive opinion recommending the granting of the marketing authorisation of Nuceiva indicated for the "temporary improvement in the appearance of moderate to severe vertical lines between the eyebrows seen at maximum frown (glabellar lines), when the severity of the above facial lines has an important psychological impact in adults below 65 years of age".

The reason for divergent opinion was the following:

The proposed use of an *in vivo* LD50 potency assay for routine batch release violates current principles and standards on the protection of animals used for scientific purposes, as set out in EC Directive 2010/63, and is considered unacceptable.

- There is no medical need that would justify the use of the LD50 animal assay as other similar Botulinum toxin products are available on the market for the same indication. All of these products employ *in vitro* cell based potency assays for routine batch release. There is no valid scientific reason for not having developed an alternative in-vitro assay for routine release of the new product.
- A commitment to develop a cell based potency assay post approval cannot be accepted; this should have been done during the pharmaceutical development of the product. The timelines proposed by the applicant are merely assumptions and thus the transition period where animal are still used might be exceeded significantly.

For the aforementioned reasons the marketing authorisation application is considered to be not approvable.

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