

***Clostridium botulinum* toxin Type A composition, formulation and use thereof**

FIELD

The present invention relates to a protein drug in the aesthetic or pharmaceutical field.

5 Further, it relates to *Clostridium botulinum* toxin composition.

BACKGROUND

Wrinkles, one of the telltale signs of aging, can be caused by biochemical, histological, and physiologic changes that accumulate from environmental damage to the skin. In addition,
10 there are other secondary factors that can cause characteristic folds, furrows, and creases of facial wrinkles (Stegman et al., *The Skin of the Aging Face Cosmetic Dermatological Surgery*, 2nd ed., St. Louis, Mo.: Mosby Year Book: 5-15 (1990)). These secondary factors include the constant pull of gravity, frequent and constant positional pressure on the skin (e.g., during sleep), and repeated facial movements caused by the contraction of facial muscles (Stegman
15 et al., *The Skin of the Aging Face Cosmetic Dermatological Surgery*, 2nd ed., St. Louis, Mo.: Mosby Year Book: 5-15 (1990)).

Different techniques have been utilized in order to potentially mitigate some of the signs of aging. These techniques range from facial moisturizers containing alpha hydroxy acids and retinol to surgical procedures and injections of neurotoxins. For example, in 1986, Jean and
20 Alastair Carruthers, a husband-and-wife team consisting of an oculoplastic surgeon and a dermatologist, developed a method of using the type A form of botulinum toxin for treatment of movement-associated wrinkles in the glabella area (Schantz and Scott, In Lewis G. E. (Ed) *Biomedical Aspects of Botulinum*, New York: Academic Press, 143-150 (1981)). The Carruthers' use of the type A form of botulinum toxin for the treatment of wrinkles led to the
25 seminal publication of this approach in 1992 (Schantz and Scott, in Lewis G. E. (Ed) *Biomedical Aspects of Botulinum*, New York: Academic Press, 143-150 (1981)). By 1994, the same team reported experiences with other movement-associated wrinkles on the face (Scott, *Ophthalmol*, 87:1044-1049 (1980)). This in turn led to the birth of the era of aesthetic treatment using the type A form of botulinum toxin.

30 The type A form of botulinum toxin is reported to be the most lethal natural biological agent known to man. Spores of *C. botulinum* are found in soil and can grow in improperly sterilized and sealed food containers. Botulism, which may be fatal, may be caused by the

ingestion of the bacteria. Botulinum toxin acts to produce paralysis of muscles by preventing synaptic transmission that inhibits the release of acetylcholine across the neuromuscular junction, and is thought to act in other ways as well. Its action essentially blocks signals that normally would cause muscle spasms or contractions, resulting in paralysis. During the last decade, botulinum toxin's muscle paralyzing activity has been harnessed to achieve a variety of therapeutic effects. Controlled administration of botulinum toxin has been used to provide muscle paralysis to treat a variety of medical conditions, for example, neuromuscular disorders characterized by hyperactive skeletal muscles. Conditions that have been treated with botulinum toxin include hemifacial spasm, adult onset spasmodic torticollis, anal fissure, blepharospasm, cerebral palsy, cervical dystonia, migraine headaches, strabismus, temporomandibular joint disorder, and various types of muscle cramping and spasms. More recently, the muscle-paralyzing effects of botulinum toxin have been applied to therapeutic and aesthetic facial applications such as treatment of wrinkles, frown lines, and other results of spasms or contractions of facial muscles.

In addition to the type A form of botulinum toxin, there are seven other serologically distinct forms of botulinum toxin that are also produced by the gram-positive bacteria *Clostridium botulinum*. Of these eight serologically distinct types of botulinum toxin, the seven that can cause paralysis have been designated botulinum toxin serotypes A, B, C, D, E, F and G. Each of these is distinguished by neutralization with type-specific antibodies. The molecular weight of each of the botulinum toxin proteins is about 150 kDa. Due to the molecule size and molecular structure of botulinum toxin, it cannot cross stratum corneum and the multiple layers of the underlying skin architecture. The different serotypes of botulinum toxin vary in the effect and in the severity and duration of the paralysis they evoke in different animal species. For example, in rats, it has been determined that botulinum toxin type A is 500 times more potent than botulinum toxin type B, as measured by the rate of paralysis. Additionally, botulinum toxin type B has been determined to be non-toxic in primates at a dose of 480 U/kg.

The botulinum toxin, which is released by *Clostridium botulinum* bacteria, is a component of a toxin complex containing the approximately 150 kDa botulinum toxin protein molecule along with associated non-toxin proteins. These endogenous non-toxin proteins are believed to include a family of hemagglutinin proteins, as well as non-hemagglutinin proteins. The non-toxin proteins have been reported to stabilize the botulinum toxin molecule in the

toxin complex and protect it against denaturation by digestive acids when toxin complex is ingested. Thus, the non-toxin proteins of the toxin complex protect the activity of the botulinum toxin and thereby enhance systemic penetration when the toxin complex is administered via the gastrointestinal tract. Additionally, it is believed that some of the non-toxin proteins specifically stabilize the botulinum toxin molecule in blood.

The presence of non-toxin proteins in the toxin complexes typically causes the toxin complexes to have molecular weights that are greater than that of the bare botulinum toxin molecule, which is about 150 kDa, as previously stated. For example, *Clostridium botulinum* bacteria can produce botulinum type A toxin complexes that have molecular weights of about 900 kDa, 500 kDa or 300 kDa. Botulinum toxin types B and C are produced as complexes having a molecular weight of about 700 kDa or about 500 kDa. Botulinum toxin type D is produced as complexes having molecular weights of about 300 kDa or 500 kDa. Botulinum toxin types E and F are only produced as complexes having a molecular weight of about 300 kDa.

To provide additional stability to botulinum toxin, the toxin complexes are conventionally stabilized by combining the complexes with albumin during manufacturing. For example, BOTOX[®] (Allergan, Inc., Irvine, Calif.) is a botulinum toxin-containing formulation that contains 100 U of type A botulinum toxin with ancillary proteins, 0.5 milligrams of human albumin, and 0.9 milligrams of sodium chloride. The albumin serves to bind and to stabilize toxin complexes in disparate environments, including those associated with manufacturing, transportation, storage, and administration.

However, the number of available botulinum toxin type A compositions is limited. There is still a need for new type A botulinum toxin compositions and formulations in this field.

SUMMARY OF THE INVENTION

In view of the need for more type A botulinum toxin complexes in the art, the inventors provide a new *Clostridium botulinum* toxin Type A complex. Compared with existing products (such as Botox[®]), the *Clostridium botulinum* toxin Type A complex of the present invention has a smaller molecular weight, and therefore has a lower production cost; and has increased stability and safety.

In one aspect, the present invention provides a *Clostridium botulinum* toxin Type A complex comprising a HA70 component, a HA17 component, a HA33 component, a NTNH

component, and a BoNT/A1 component, wherein the *Clostridium botulinum* toxin complex has a molecular weight of 740-790 kDa.

In one embodiment, the *Clostridium botulinum* toxin Type A complex consists of the HA70 component, the HA17 component, the HA33 component, the NTNH component, and the BoNT/A1 component, and has a molecular weight of about 760 kDa.

In one embodiment, the HA70 component comprises any of:

- (1) the amino acid sequence of SEQ ID NO. 1; or
- (2) the amino acid sequence obtained by adding, deleting, substituting or inserting one or more amino acids in SEQ ID NO. 1, with retaining the function of the HA70 component.

In one embodiment, the HA17 component comprises any of:

- (1) the amino acid sequence of SEQ ID NO. 2; or
- (2) the amino acid sequence obtained by adding, deleting, substituting or inserting one or more amino acids in SEQ ID NO. 2, with retaining the function of the HA17 component.

In one embodiment, the HA33 component comprises any of:

- (1) the amino acid sequence of SEQ ID NO. 3; or
- (2) the amino acid sequence obtained by adding, deleting, substituting or inserting one or more amino acids in SEQ ID NO. 3, with retaining the function of the HA33 component.

In one embodiment, the NTNH component comprises any of:

- (1) the amino acid sequence of SEQ ID NO. 4; or
- (2) the amino acid sequence obtained by adding, deleting, substituting or inserting one or more amino acids in SEQ ID NO. 4, with retaining the function of the NTNH component.

In one embodiment, the BoNT/A1 component comprises any of:

- (1) the amino acid sequence of SEQ ID NO. 5; or
- (2) the amino acid sequence obtained by adding, deleting, substituting or inserting one or more amino acids in SEQ ID NO. 5, with retaining the function of the BoNT/A1 component.

In another aspect, the present invention provides a nucleic acid encoding the *Clostridium botulinum* toxin Type A complex of the present invention.

In another aspect, the present invention provides a pharmaceutical composition comprising the *Clostridium botulinum* toxin Type A complex of the present invention and a pharmaceutically or dermatologically acceptable carrier.

In yet another aspect, the present invention provides a liquid formulation comprising the *Clostridium botulinum* toxin Type A complex of any one of the claims 1-6, which comprises

human serum albumin, methionine, arginine, sodium chloride, Tween 80, citrate buffer, and/or phosphate buffer.

In yet another aspect, the present invention provides an aesthetic method comprising administering to a subject in need thereof an effective amount of the *Clostridium botulinum* toxin Type A complex, the pharmaceutical composition or the liquid formulation of the present invention.

In one embodiment, the aesthetic method comprises reducing the appearance of fine lines and/or wrinkles, particularly on the face, or enlarging eyes, lifting the corners of mouth, or smoothing lines that appear on the upper lip, or generally relieving the muscle tone.

In yet another aspect, the present invention provides a method for treating or preventing a disease caused by synaptic transmission or release of acetylcholine in a subject in need thereof, comprising administering to the subject an effective amount of the *Clostridium botulinum* toxin Type A complex, the pharmaceutical composition or the liquid formulation of the present invention.

In one embodiment, the disease is selected from the group consisting of neuromuscular disorders characterized by skeletal muscle hyperactivity, neuropathic pain, migraine, overactive bladder, rhinitis, sinusitis, acne, dystonia, dystonic contraction, hyperhidrosis, hypersecretion of one or more glands controlled by the cholinergic nervous system, hemifacial spasm, adult-onset spastic torticollis, anal fissure, blepharospasm, cerebral palsy, cervical dystonia, strabismus, temporomandibular joint disorders and various types of muscle convulsions and spasms.

In yet another aspect, the present invention provides use of the *Clostridium botulinum* toxin Type A complex, the pharmaceutical composition or the liquid formulation of the present invention in the preparation of an aesthetic product or a medicament, wherein the aesthetic product is used to reduce fine lines and/or the appearance of wrinkles, especially on the face, or enlarge eyes, lift the corners of mouth, or smooth lines that appear on the upper lip, or generally relieve muscle tone; wherein the medicament is used to treat or prevent a disease caused by synaptic transmission or release of acetylcholine .

In one embodiment, the disease is selected from the group consisting of neuromuscular disorders characterized by skeletal muscle hyperactivity, neuropathic pain, migraine, overactive bladder, rhinitis, sinusitis, acne, dystonia, dystonic contraction, hyperhidrosis, hypersecretion of one or more glands controlled by the cholinergic nervous system, hemifacial

spasm, adult-onset spastic torticollis, anal fissure, blepharospasm, cerebral palsy, cervical dystonia, strabismus, temporomandibular joint disorders and various types of muscle convulsions and spasms.

In yet another aspect, the present invention provides a *Clostridium botulinum* toxin Type A complex, pharmaceutical composition or liquid formulation for use in reducing the appearance of fine lines and/or wrinkles, especially on the face, or enlarging eyes and lifting the corners of mouth, or smoothing lines appearing on the upper lip, or generally relieving muscle tone; wherein the medicament is used to treat or prevent a disease caused by synaptic transmission or release of acetylcholine.

In one embodiment, the disease is selected from the group consisting of neuromuscular disorders characterized by skeletal muscle hyperactivity, neuropathic pain, migraine, overactive bladder, rhinitis, sinusitis, acne, dystonia, dystonic contraction, hyperhidrosis, hypersecretion of one or more glands controlled by the cholinergic nervous system, hemifacial spasm, adult-onset spastic torticollis, anal fissure, blepharospasm, cerebral palsy, cervical dystonia, strabismus, temporomandibular joint disorders and various types of muscle convulsions and spasms.

The *Clostridium botulinum* toxin Type A complex of the present invention (also referred to as "OBI-858" herein) has the following advantages compared with the commercial product Botox®:

(1) its therapeutic effect (Effective Dose: ED₅₀) is comparable to that of Botox® in a mouse animal experiment, but its lethal dose (Lethal Dose: LD₅₀) is higher than that of Botox® in a mouse animal experiment, and its margin of safety (MOS) is also higher than that of Botox® in a mouse animal experiment, indicating that the *Clostridium botulinum* toxin preparation of the present invention has a higher safety than the commercial product Botox®;

(2) the *Clostridium botulinum* toxin Type A complex of the present invention has a total protein molecular weight of 760 kDa, which is different from Botox® with a molecular weight of 900 kDa, and is a new *Clostridium botulinum* toxin Type A complex;

(3) the formulation of the *Clostridium botulinum* toxin Type A complex of the present invention has an improved stability.

BRIEF DESCRIPTION IF THE FIGURES

Figure 1: Product purity measurement by using Size exclusion chromatography-High

Performance Liquid Chromatography (SEC-HPLC).

Figure 2: Product molecular weight measurement by using multi-angle light scattering (MALS).

Figure 3 : Product total protein molecular weight measurement by using electron microscopy. (a) OBI-858, the arrow indicated the toxin particles; and (b) Reference studies of 760 kDa L-PTC/A, the arrow indicated the four different orientations.

Figure 4: The scoring picture of Digit Abduction Score (DAS) assay.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a *Clostridium botulinum* toxin Type A complex and its liquid formulation. As described herein, the composition of the present invention can be used as an injectable application for providing *botulinum* toxin to a subject for various therapeutic and/or aesthetic purposes. Compared with existing *Clostridium botulinum* toxin Type A complex products, the *Clostridium botulinum* toxin Type A complex of the present invention has better safety and enhanced or comparable efficacy. Regarding the *Clostridium botulinum* toxin Type A complex of the present invention, the present invention provides a liquid formulation for the *Clostridium botulinum* toxin Type A complex, which has excellent stability.

The term "*Clostridium botulinum* toxin Type A complex " as used herein refers to a type A *Clostridium botulinum* toxin protein molecule of about 150 kD and related endogenous non-toxin proteins, *i.e.* a complex formed by hemagglutinin (HA) and non-toxin non-hemagglutinin (NTNH). The hemagglutinin protein is composed of subcomponents of 70, 33, and 17 kDa, namely HA70, HA33, and HA17. The *Clostridium botulinum* toxin complex does not need to be derived from *Clostridium botulinum* as a single toxin. For example, *Clostridium botulinum* toxin or modified *Clostridium botulinum* toxin can be prepared recombinantly, and then combined with non-toxin proteins. Recombinant *Clostridium botulinum* toxin can also be purchased and combined with non-toxic proteins.

The *Clostridium botulinum* toxin Type A complex herein may also contain HA70, HA33, HA17, NTNH or BoNT/A1 as described herein, or a variant thereof. A variant has an amino acid sequence obtained by adding, deleting, substituting or inserting one or more amino acids in the sequence described herein (e.g., any one of SEQ ID Nos. 1-5).

The term "one or more" refers to the number of regions that do not impair protein activity to a large extent. The number referred to by the term "one or more" is, for example, 1 to 100,

preferably 1 to 80, more preferably 1 to 50, still more preferably 1 to 40, and particularly preferably 1 to 30, 1 to 20, 1 to 10 or 1 to 5 (e.g. 1, 2, 3, 4, or 5).

The sequences of each component of the *Clostridium botulinum* toxin Type A complex in the examples are provided below.

5 HA70 (SEQ ID NO. 1)
MNSSIKKIYNDIQEKVINYSDTIDLADGNYVVRRGDWILSRQNQILGGSVISNGSTG
IVGDLRVNDNAIPYYYPTPSFNEEYIKNNIQTVFTNFTEANQPIGFEFSKTAPSNKNLY
MYLQYTYIRYEIIKVLQHEIIRAVLYVPSLGYVKSIEFNPGEKINKDFYFLTNDKCILN
EQFLYKKILETTKNIPTNNIFNSKVSSTQRVLPYSNGLYVINKGDGYIRTNDKDLIGTL
10 LIEAGSSGSIIQPRLRNTTRPLFTTSNDTKFSQQYTEERLKDAFNVQLFNTSTSLFKFV
EEAPSDKNICIKAYNTYEKYEYELIDYQNGSIVNKAEEYLPYSLGYCEVTNAPSESEVVK
MQVAEDGFIQNGPEEEIVVGVIDPSENIQEINTAISDNYTYNIPGIVNNNPFYILFTVNT
TGIYKINAQNNLPSLKIYEAIGSGNRNFQSGNLCDDDIKAINYITGFDSPNAKSYLVVL
LNKDKNYYIRVPQTSSNIENQIQFKREEGDLRNLNMNSSVNIIDNLNSTGAHYTRQSP
15 DVHDYISYEFTIPGNFNNKDTSNIRLYTSYNQGIGTLFRVTETIDGYNLINIQQNLHLL
NNTNSIRLLNGAIYILKVEVTELNNNYIIRLHIDITN

HA17 (SEQ ID NO.2)
MSVERTFLPNGNYNIKSIFSGSLYLNPVSKSLTFSNESSANNQKWNVEYMAENRCFKI
20 SNVAEPNKYLSYDNFGFISLDSLSNRCYWFPKIAVNTYIMLSLNKVNELDYAWDIYD
TNENILSQPLLLLPNFDIYNSNQMFKLEKI

HA33 (SEQ ID NO. 3)
MEHYSVIQNSLNDKIVTISCKADTNLFFYQVAGNVSLFQQTRNYLERWRLIYDSNKA
25 AYKIKSMDIHNTNLVLTWNAPTHNISTQQDSNADNQYWLLKDIGNNSFIIASYKNP
NLVLYADTVARNLKLSTLNNSNYIKFIIEDYIISDLNNTFTCKISPILDLNKVVQQVDVT
NLNVNLYTWDYGRNQKWTIRYNEEKAAYQFFNTILSNGVLTWIFSNGNTVRVSSSN
DQNNDAQYWLINPVSDTDETYTITNLRDITTKALDLYGGQTANGTAIQVFNYHGDDN
QKWNIRNP

30 NTNH (SEQ ID NO. 4)
MNINDNLSINSPVDNKNVVRARKTDTVFKAFKVAPNIWVAPERYYGESLSIDEEY
KVDGGIYDSNFLSQDSEKDKFLQAIITLLKRINSTNAGEKLLSLISTAIPFPYGYIGGGY
YAPNMITFGSAPKSNKKLNSLISSTIPFPYAGYRETNYLSSDNKSFYASNIVIFGPGAN
35 IVENNTVFYKKEDAENGMGTMTEIWFQPFITYKYDEFYIDPAIELIKCLIKSLYFLYGI
KPSDDLVIPIYRLRSELENIEYSQLNIVDLLVSGGIDPKFINTDPYWFTDNYFSNAKKVF
EDHRNIYETEIEGNNAIGNDIKRLRLKQKFRININDIWELNLNYFSKEFSIMMPDRFNN
ALKHFYRKQYYKIDYPENYSINGFVNGQINAQLSLSDRNQDIINKPEEIIINLLNGNNV
SLMRSNIYGDGLKSTVDDFYSNYKIPYNRAYEYHFNNNSNDSSLDNVNIGVIDNIPEIID
40 VNPYKENCDFKSPVQKITSTREINTNIPWPINYLQAQNTNNEKFSLSDFVEVVSSKD
KSLVYSFLSNVMFYLDSEKIDNSPIDTDKKYYLWLREIFRNYSFDITATQEINTNCGINK
VVTWFGKALNLTSDSFVEEFQNLGAISLINKKENLSMPIIESYEIPNDMLGLPLNDL
NEKLFNIYSKNTAYFKKIYYNFLDQWWTQYYSQYFDLICMAKRSVLAQETLIKRIIQ
KKLSYLIGNSNISSDNLALMNLTTTNTLRDISNESQIAMNNVDSFLNNAICVFESNIY

PKFISFMEQCINNINIKTKEFIQKCTNINEDEKLQLINQNVFNSLDFEFLNIQNMKSLFS
SETALLIKEETWPYELVLYAFKEPGNNVIGDASGKNTSIEYSKDIGLVYGINSDALYLN
GSNQSISFSNDFEFENGLTNSFSIYFWLRNLGKDTIKSKLIGSKEDNCGWEIYFQDTGLV
FNMIDSNGNEKNIYLSDVSNNSWHYITISVDRLKEQLLIFIDDLNLANESIKEILNIYSS
5 NIISLLSENNPSYIEGLTILNKPTTSQEVLSNYFEVLNNSYIRDSNEERLEYNKTYQLYN
YVFSDKPICEVKQNNNIYLTINNTNNLNLQASKFKLLSINPNKQYVQKLDEVIISVLD
NMEKYIDISEDNRLQLIDNKNNAKKMIISNDIFISNCLTLSYNGKYICLSMKDENHNW
MICNNDMSKYLYLWSFK

10 BoNT/A1 (SEQ ID NO. 5)

MPFVNKQFNYKDPVNGVDIAYIKIPNAGQMOPVKAFKIHNIWVIPERDTFTNPEEG
DLNPPPEAKQVPVSYDSTYLSTDNEKDNLYLKGVTCLFERIYSTDLGRMLLTSIVRGI
PFWGGSTIDTELKVIDTNCINVIQPDGSYRSEELNLVIIGPSADIQFECKSFGHEVLNL
TRNGYGSTQYIRFSPDFTFGFEESLEVDTNPLLGAAGKATDPAVTLAHELIHAGHRLY
15 GIAINPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAAKFDLSQENEFRLYYYNKF
KDIASLTNKAASIVGTTASLQYMKNVFEKEYLLSEDTSGKFSVDKLFKFDKLYKMLTE
IYTEDNFVKFFKVLNRKTYLNFDAVFKINIVPKVNYTIYDGFNLRNTNLAANFNGQ
NTEINNMNFTKLKNFTGLFEFYKLLCVRGIITSKTKSLDKGYNKALNDLCIKVNNWD
LFFSPSEDNFTNDLNKGEEITSDTNIEAAEENISLDLIQQYYLTNFNFDNEPENISIEENLSS
20 DIIGQLELMPNIEFPPNGKKYELDKYTMFHYLRAQEFEGHKSRIALTNSVNEALLNPS
RVYTFFSSDYVKKVNKATEAAMFLGWVEQLVYDFTDETSEVSTTDKIADITIIIPYIGP
ALNIGNMLYKDDFVGALIFSGAVILLEFIPEIAIPVLGTFALVSYIANKVLTQVQIDNAL
SKRNEKWDEVYKYIVTNWLAKVNTQIDLIRKKMKEALENQAEATKAIINYQYNQY
TEEEKNNINFNIDDLSSKLNESINKAMININKFLNQCSVSYLMNSMIPYGVKRLEDFFD
25 ASLKDALLKYIYDNRGTLIGQVDRLKDKVNNTLSTDIPFQLSKYVDNQRLSTFTEYI
KNIINTSILNLRYESNHLIDLSRYASKINIGSKVNFDPIDKNQIQLFNLESSKIEVILKNAI
VYNMYENFSTSFWRIPKYFNSISLNNEYTIINCMENNSGWKVSLSNYGEIHWTLQDT
QEIQRVVFYKYSQMINISDYINRWIFVTITNNRLNNSKIYINGRLIDQKPISNLGNIHAS
NNIMFKLDGCRDTHRYIWIYFNLFDKELNEKEIKDLYDNQSNISGILKDFWGDYDQ
30 YDKPYYMLNLYDPNKYVDVNNVGIRGYMYLKGPRGSVMTTNIYLNSSLYRGTKFII
KKYASGNKDNIVRNNDRVYINVVVKNKEYRLATNASQAGVEKILSALEIPDVGNLS
QVVVMKSKNDQGITNKCKMNLQDNNGNDIGFIGFHQFNNAIKLVASNWNRYNRIERS
SRTLGCSEWEIFVDDGWGERPL

The present invention also relates to a method for producing a biological effect by injecting
35 an effective amount of the composition of the present invention into a subject or patient in
need thereof. The biological effect can include, for example, muscle paralysis, reduction of
excessive secretion or sweating, treatment of neuropathic pain or migraine, control of rhinitis
or sinusitis, treatment of overactive bladder, reduction of muscle spasms, prevention or
reduction of acne, reduction or enhancement of immune response, reduction of wrinkles, or
40 prevention or treatment of various other diseases.

The composition of the present invention is in a form that allows injection into the skin or
epithelium of a subject or patient (i.e., a human or other mammal in need of specific treatment).

The term "need" is meant to include needs related to drugs or health (e.g., to treat conditions involving undesirable facial muscle spasms), as well as aesthetic and subjective needs (e.g., to change or improve the appearance of facial tissues). In their simplest form, the composition may comprise an aqueous pharmaceutically acceptable diluent, such as buffered saline (e.g., phosphate buffered saline). However, the composition may comprise other ingredients commonly present in injectable pharmaceutical or aesthetic compositions, including a dermatologically or pharmaceutically acceptable carrier that is compatible with the tissue to be applied. As used herein, the term "dermatologically or pharmaceutically acceptable" means that the compositions or its components described herein are suitable for contact with tissues or for use in general patients without undue toxicity, incompatibility, instability, allergic reactions, etc. When appropriate, the composition of the present invention may comprise any components conventionally used in the field under consideration, especially in aesthetics and dermatology.

The *Clostridium botulinum* toxin complex according to the present invention can be delivered to the muscles under the skin by injection (usually using a syringe), or applied to the glandular structure in the skin in an effective amount to produce paralysis, produce relaxation, relieve contraction, prevent or relieve spasticity, reduce glandular output or produce other required effects. Local delivery of *botulinum* toxin in this manner allows dose reduction, toxicity reduction, and allow more precise dose optimization relative to injectable or implantable materials to achieve the desired effect.

The *Clostridium botulinum* toxin complex of the present invention is administered to deliver an effective amount of *botulinum* toxin. The term "effective amount" as used herein refers to such amount that is sufficient to produce the desired muscle paralysis or other biological or aesthetic effects, but it is implicitly a safe amount, that is, it is low enough to avoid serious side effects. The desired effect includes relaxing certain muscles, for example, reducing the appearance of fine lines and/or wrinkles, especially on the face, or adjusting the appearance of the face in other ways, such as enlarging eyes, raising the corners of mouth, or relieving muscle tone. Muscle tone can be relieved on the face or elsewhere. The *Clostridium botulinum* toxin complex of the present invention can be administered to patients by injection for the treatment of, e.g., wrinkles, undesirable facial muscle or other muscle spasms, hyperhidrosis, acne or other diseases of the body that require relief of muscle pain or spasms. The *Clostridium botulinum* toxin complex is administered to muscle or other skin-related or

other target tissue structures by injection. For example, the *Clostridium botulinum* toxin complex can be administered to legs, shoulders, backs (including lower backs), armpits, palms, feet, necks, faces, groins, back of hands or feet, elbows, upper arms, knees, upper legs, buttocks, torsos, pelvis, or any other part of the body in need thereof.

5 The administration of the *Clostridium botulinum* toxin complex of the present invention can also be used to treat other conditions, including any conditions that would benefit from the prevention of synaptic transmission or release of acetylcholine. For example, conditions that can be treated by the complex of the present invention include, but are not limited to, neuropathic pain, migraine or other headache pain, overactive bladder, rhinitis, sinusitis, acne, dystonia, dystonic contraction, hyperhidrosis, and hypersecretion of one or more glands
10 controlled by the cholinergic nervous system, neuromuscular disorders characterized by skeletal muscle hyperactivity. Conditions treated with *botulinum* toxin include half-face spasm, hemifacial spasm, adult-onset spasmodic torticollis, anal fissure, blepharospasm, cerebral palsy, cervical dystonia, strabismus, temporomandibular joint disorders and various types of muscle convulsions and spasms. The complex of the present invention can also be used to
15 reduce or enhance immune response, or to treat other conditions for which injection of *Clostridium botulinum* toxin complex has been suggested or administered. The *Clostridium botulinum* toxin complex of the present invention can be prepared in solid form, such as lyophilized powder, tablets, etc., and reconstituted in water for injection before use.

20 The *Clostridium botulinum* toxin complex, composition or formulation of the present invention is administered by or under the guidance of a physician or other health care professional. They can be administered in one treatment or in a series of treatments over time. In a preferred embodiment, the *Clostridium botulinum* toxin complex, composition or formulation according to the present invention is injected at one or more locations where
25 *Clostridium botulinum* toxin-related effects are required. Due to the nature of the *Clostridium botulinum* toxin complex, the *botulinum* toxin is preferably administered in an amount, rate, and frequency that produces the desired result without producing any adverse or undesirable results.

30 The formulation herein comprises human serum albumin, methionine, arginine, sodium chloride, Tween 80, citrate buffer, and/or phosphate buffer. Preferably, the formulation comprises human serum albumin, sodium chloride and Tween 80 dissolved in 50 mM citrate buffer. In the formulation, the concentration of human serum albumin may be 0.1-1, such as

0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, or 0.9 mg/mL. The concentration of sodium chloride may be 5-20, such as 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 mg/mL, more preferably 11.25 mg/mL. The concentration of Tween 80 dissolved in 50 mM citrate buffer may be 0.1-0.3, such as 0.2 mg/mL, more preferably 0.188 mg/mL. The formulation has a certain pH value, such as 4-8, preferably pH 5.6.

Hereinafter, the inventive concept will be described in more detail by referring to the following examples. However, these examples are for illustrative purposes only, and are not intended to limit the scope of the invention.

10 EXAMPLE

Example 1: The manufacture process of OBI-858

(1) The manufacture process of OBI-858 drug substance (DS):

Frozen OBI-858 bacteria was reconstituted from cell-bank and cultivated within medium. Then the bacteria was transferred to fermenter. After cultivation, cell and its fragment was removed by filtration. The purification procedure included: two-steps precipitation, buffer-exchange, and column-chromatography. After column-chromatography, DS solution was filtered by 0.22 μ m filter and directly transferred into an isolator and lyophilized.

(2) The manufacture process of OBI-858 drug product (DP):

OBI-858 DS powder was reconstituted within formulation buffer. The reconstituted solution was aseptic filtered by 0.22 μ m filters and directly transferred into a sterile bag located in isolator. Then the solution was lyophilized to obtain OBI-858 DP,

Example 2: The identification of OBI-858

(1) Genome sequence alignment

The production strain of OBI-858 was developed independently and confirmed 98.28% sequence identity compared to *Clostridium botulinum* A str. ATCC 3502, 89.69% sequence identity compared to *Clostridium botulinum* A str. Hall through whole genome sequencing (Table 1). Therefore, it differs to the marketing products under bacteria genome analysis.

Table 1: Genome sequence alignment of *Clostridium botulinum* strains

<i>Clostridium botulinum</i> strain	Genome sequence identity (%)
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<i>Clostridium botulinum</i> A str. ATCC 3502	98.28
<i>Clostridium botulinum</i> A str. ATCC 19397	90.29
<i>Clostridium botulinum</i> A str. Hall	89.69
<i>Clostridium botulinum</i> H04402 065(A5)	68.98
<i>Clostridium botulinum</i> A2 str. Kyoto	68.39
<i>Clostridium botulinum</i> A3 str. Loch Maree	48.84

(2) Protein composition analysis

Experimental materials/method:

In order to achieve the highest sequence coverage, OBI-858 protein sample was digested with different enzymes followed by LC-MS/MS analysis. OBI-858 protein was first denatured with 6 M urea, reduced with 10 mM DTT at 37 °C for 1 hour, and alkylated with 50 mM IAM in dark at room temperature for 30 min. The resulting proteins were digested in 50 mM ammonium bicarbonate with the following digestion conditions:

- Trypsin digestion at 37 °C for 18 hours;
- Glu-C digestion at 37 °C for 18 hours;
- Trypsin digestion at 37 °C for 18 hours + Glu-C digestion at 37 °C for 18 hours;
- Lys-C digestion at 37 °C for 18 hours;
- Chymotrypsin digestion at 37 °C for 18 hours;
- Thermolysin digestion at 37 °C for 18 hours.

After digestion, the protein sample was diluted and acidified with 0.1% FA for MS analysis.

Samples were analyzed with Q Exactive mass spectrometer (Thermo Scientific) coupled with Ultimate 3000 RSLC system (Dionex). The LC separation was performed using the C18 column (Acclaim PepMap RSLC, 75 µm x 150 mm, 2 µm, 100 Å) with the gradient shown below:

Table 2: LC-MS/MS analysis program

Time (min)	Mobile phase A (%)	Mobile phase B (%)	Flow rate (µL/min)
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0	99	1	0.25
5.5	99	1	0.25
45	75	25	0.25
48	40	60	0.25
50	20	80	0.25
60	20	80	0.25
65	99	1	0.25
70	99	1	0.25

Mobile phase A: 0.1% FA

Mobile phase B: 95% ACN/0.1% FA

Full MS scan was performed with the range of m/z 300-2000, and the ten most intense ions from MS scan were subjected to fragmentation for MS/MS spectra. Raw data was processed into peak lists by Proteome Discoverer 1.4 for Mascot database search.

Experimental result:

The result of OBI-858 *Clostridium botulinum* toxin Type A LC-MS/MS amino acid sequence analysis indicated that this toxin protein was composed of 71 kDa, 17 kDa, 34 kDa, 138 kDa, and 149 kDa five different size protein subunits (Table 3). The amino acid sequences SEQ ID NO. 1-5 of these subunits were achieved through protein sequencing.

Table 3: The theoretical values calculated from sequencing

Protein sequence ID	Theoretical molecular weight (Da)	Protein name	Amino acid number	Molecular formula
A5HZZ4	71391.01	HA-70	626	C ₃₂₀₀ H ₄₉₅₃ N ₈₃₇ O ₉₉₉ S ₈
A5HZZ5	17034.31	HA-17	146	C ₇₇₅ H ₁₁₆₉ N ₁₉₁ O ₂₃₀ S ₆
A5HZZ6	33872.73	HA-33	293	C ₁₅₁₅ H ₂₃₁₃ N ₄₀₉ O ₄₆₈ S ₄
A5HZZ8	138092.85	NTNH	1193	C ₆₂₄₀ H ₉₅₅₅ N ₁₅₆₉ O ₁₉₀₅ S ₃₃
A5HZZ9	149425.84	BoNT/A1	1296	C ₆₇₆₃ H ₁₀₄₅₂ N ₁₇₄₄ O ₂₀₁₁ S ₃₃

(3) Product purity measurement by using Size exclusion chromatography-High Performance Liquid Chromatography (SEC-HPLC) and Product molecular weight measurement by using multi-angle light scattering (MALS).

Experimental materials/method:

SEC-MALS analysis is combined size-exclusion high performance liquid chromatography and static light scattering analysis, which was performed using an Agilent 1260 HPLC system and connected in-line to a WYATT_DAWN HELEOS static light scattering detector with a SRT SEC-300 (5 μ m, 7.8 $\times$ 300mm) column. The Agilent 1260 HPLC system was controlled using Chemstation software (Agilent Technologies). The light scattering detector was controlled and analyzed using the ASTRA V software (Wyatt Technology). Isocratic elution was performed at a flow rate of 0.5 mL/min using a mobile phase of 150mM PB/0.1M NaCl and 200 ppm NaN₃; pH6.0. The concentration of the OBI-858 complex during the light scattering experiments was monitored by UV absorbance at 280 nm. The molecular mass for the OBI-858 complex was calculated from the light scattering data using a specific refractive index increment (dn/dc) value of 0.185 mL/g and UV Extinction Coefficient 1.49 mL/(mg*cm).

Experimental result:

Figure 1 indicated product purity measurement by using Size exclusion chromatography-High Performance Liquid Chromatography (SEC-HPLC) and the purity was 99.36%. **Figure 2** indicated product molecular weight measurement by using multi-angle light scattering (MALS) and the molecular weight was closed to 760 kDa protein.

(4) Electron microscopy measurement

Experimental materials/method:

The negatively stained sample preparation was followed the standard procedure. Briefly, a 4 μ L drop of sample solution was adsorbed to a glow-discharged carbon-coated copper grid, washed with one drops of deionized water, stained with 2% uranyl acetate, and air dry. Images of sample were recorded using a JEM-2100F Transmission Electron Microscope (TEM) equipped with a field emission electron source and operated at an acceleration voltage of 200 kV. Images were recorded on a DE12 camera.

Experimental result:

Figure 3 indicated product total protein molecular weight measurement by using electron microscopy. Fig. 3a indicated OBI-858, the arrow indicated the toxin particles; Fig. 3b indicated reference studies of 760 kDa L-PTC/A, the arrow indicated the four different orientations. Therefore, this product differs to 900 kDa *Clostridium botulinum* toxin Type A on the market.

Example 3: OBI-858 Efficacy Study

The purpose of this study was to evaluate the single-dose acute intramuscular (IM) efficacy of the test article, OBI-858, in female ICR mice. The efficacy of the test article was determined by Digit Abduction Scoring assay (DAS assay) for calculation of IM median effective dose (ED₅₀). Safety margin was determined as a ratio of 72 h IM LD₅₀ to 48 h IM ED₅₀.

Experimental materials/method:

Test Article, Reference Article, Vehicle, and Control

Test Article

Name: OBI-858

Supplier: OBI Pharma, Inc.

Batch number: 14004-25, 14004-30, 14004-35, 14004-45

Storage Condition: -15 – -25 °C

Other Characteristics: One vial of sterilized powder containing 1,350,440 units

Reference Article

Name: BOTOX®

Supplier: Allergan Inc.

Batch number: C3461 C2

Storage Condition: -15 – -25 °C

Other Characteristics: 50 Units of *Clostridium botulinum* toxin Type A [per vial]

Vehicle and Control

Dilution buffer (30 mM phosphate buffered saline with 0.2% w/v gelatin, pH 6.8) was used as vehicle for formulation preparation and negative control at dosing.

Preparation and disposition of dose formulations

All formulations were freshly prepared for same-day use on the scheduled dosing days.

Test article reconstitution and dose formulation

All dose formulation preparation procedures were performed in a Class II biosafety cabinet

located in the Testing Facility. Dose formulation preparation was performed on the dosing day prior to the dosing procedures.

Test article was packaged as lyophilized material in a sterile sealed vial. To reconstitute the lyophilized test article, 1 mL of diluent buffer was injected into the vial containing the test article. The vial was shaken vigorously to dissolve the test article until no particulate matters in the reconstituted stock were visible by naked eye. If particulate matters are visible after vigorous shaking lasting for at least 3 minutes, OBI will be contacted for replacement of the test article vial. The reconstituted test article had a stock concentration of 1,350,440 LD₅₀ units (U) per mL. Serial dilutions were then performed in the following Table 4 with the reconstituted test article:

Table 4: Dilution Scheme for Test Article Starting Stock for Dose Formulation Preparation

Stock ID	0 (Reconstituted TA)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Group	N/A	N/A	N/A	N/A	N/A	12	11	10	9	8	7	6	5	4	3	2
Diluent Buffer Volume (μL)	1000	700	600	600	2000	2445	402	396	406	394	508	488	509	781	778	800
Stock ID	N/A	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Stock Volume to ADD (μL)	N/A	100	100	100	500	1000	2000	2000	2000	2000	1000	1000	1000	1000	1000	1000
Total Volume (μL)	1000	800	700	700	2500	3445	2402	2396	2406	2394	1508	1488	1509	1781	1778	1800
Volume for Dosing (μL)	N/A	N/A	N/A	N/A	N/A	1445	402	396	406	1394	508	488	509	781	778	1800
Dilution Factor	1	8	56	392	1960	6752.2	8109.4	9715.1	11687.2	13989.6	21096.3	31391.3	47369.5	84365	150001	270001.8
Fold Dilution	1	8	7	7	5	3.445	1.201	1.198	1.203	1.197	1.508	1.488	1.509	1.781	1.778	1.8
Dose concentration (U/mL)	1350440	168805	24115	3445	689	200	166.528	139.005	115.549	96.532	64.013	43.020	28.509	16.007	9.003	5.002

To prepare each serial dilution, the volume of diluent buffer required (see Serial Dilution Schedule, above) was transferred by micropipettes to a fresh glass vial with cap. The required volume of stock to be diluted was then transferred by micropipettes to the vial containing the diluent buffer. Mixing was performed by repeated gentle pipetting for several times followed by repeated gentle inversion, with cap screwed tight on the vial, for several times. Used micropipette tips were discarded at completion of each transfer step. The resultant Stock 5 through Stock 15 at completion of the dose formulation preparation were used as the test article

dose formulations.

Reference article reconstitution and dose formulation

Reference article was packaged as lyophilized material in a sterile sealed vial. To reconstitute the lyophilized reference article, 0.2 mL of diluent buffer was injected into the vial containing the test article. The vial was then shaken vigorously to dissolve the test article until no particulate matter in the reconstituted stock was visible by naked eye. The reconstituted test article would have the stock concentration of 250 LD₅₀ units (U) per mL. Serial dilutions were then prepared from the reconstituted reference article in the following Table 5:

Table 5: Dilution Scheme for Reference Article Dose Formulation Preparation

Stock ID	0 (Reconstituted TA)	1	2	3	4	5	6	7	8	9	10	11
Group	N/A	12	11	10	9	8	7	6	5	4	3	2
Diluent Buffer Volume (μL)	1600	360	281	253	229	187	366	327	305	391	373	360
Stock ID	N/A	0	1	2	3	4	5	6	7	8	9	10
Stock Volume to ADD (μL)	0	1440	1400	1280	1130	950	720	670	600	500	480	450
Total Volume (μL)	1600	1800	1681	1533	1359	1137	1086	997	905	891	853	810
Volume for Dosing (μL)	0	400	401	403	409	417	416	397	405	411	403	810
Dilution Factor	1	1.25	1.501	1.798	2.162	2.587	3.903	5.807	8.759	15.609	27.739	49.930
Fold Dilution	1	1.25	1.201	1.198	1.203	1.197	1.508	1.488	1.508	1.782	1.777	1.8
Dose concentration (U/mL)	250	200	166.568	139.078	115.642	96.623	64.059	43.049	28.541	16.016	9.013	5.007

To prepare each serial dilution, the volume of diluent buffer required (see Serial Dilution Schedule, above) was transferred by micropipette to a fresh glass vial with cap. The required volume of stock to be diluted was then transferred by micropipette to the vial containing the diluent buffer. Mixing was performed by repeated gentle pipetting for several times followed by repeated gentle inversion, with cap screwed tight on the vial, for several times. Used micropipette tips were discarded at completion of each transfer step. The resultant Stock 1 through Stock 11 at completion of the dose formulation preparation were used as the reference article dose formulations.

Study animals

Study animals are described in following:

Strain and species: ICR mice

Source: BioLASCO Taiwan Co., Ltd (Taipei, Taiwan)

Number of animals for group assignment: 236 females per batch; 4 batches

Number of spare animals: 14 females per batch; 4 batches

5 Weights at dosing day: 18-22 g (Body weight range will be within $\pm 10\%$ of the mean weight)

Identification: Ear-notch, body or tail marking with dye, and cage card

Justification for selection: The established assay procedures for efficacy and toxicity of the drug class of test article and reference article in this study are designed with the mouse as the test system. Female animals were used in the development of the procedures and were also shown in previous study to be more susceptible to the pharmacological effect and toxicity of the drug class of the test and reference articles. On the basis of the established procedures and previous study results, the female mouse is selected as the test system in this study.

Environmental conditions

Housing condition of study animals are described in following:

15 Animals were individually housed in polycarbonate cages with bedding in Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) certified facility. Temperature 20.7 – 23.1 °C, Relative humidity 51.2 – 67.1%, Light cycle 12-hour light/12-hour dark cycle, Diet Autoclaved Rodent Diet 5010 (PMI® Nutrition International, Inc., MO, USA) was supplied ad libitum. Water ad libitum. Food and water are routinely analyzed. The results of food and water analyses are maintained in the archive of Testing Facility. No contaminants are expected to be present in food and water that would interfere with the outcome of this study.

Experimental Design

Animal acclimation and selection

25 Animals were quarantined at the Testing Facility for 2 days and acclimated for between 3 and 4 days before dosing. At least one day before dosing, animals were randomly assigned to the study groups (Groups 1–12, each with two Subsets, A and B) in the proposed study design. A four-digit serial number was given to each assigned animal. There was no statistically significant ($p < 0.05$) difference among the mean body weights of the study groups or study group subsets, as determined by one- way analysis of variance (ANOVA) or Kruskal-Wallis one- way ANOVA on ranks. Unassigned animals were designated as spare animals. Assigned animals would be replaced with spare animals if health issues [including body weight

exceeding acceptable limit (24 g)] and/or suspected dosing errors arise on or before Day 0 of the respective batch. Collection of spare animal data was not performed after completion of the acclimation period. Spare animals were excluded from the study after Day 1.

Study Design

Dose groups, nominal dose concentrations, dose volumes, nominal dose levels, and the number of animals per dose group are listed in the following Table 6:

Table 6: Study Design

Group	Nominal Dose Concentration (U/mL) ^a	Dose Volume (mL/animal)	Nominal Dose Level (U/animal) ^b	Total Animals (F: Female)	
				OBI-858	BOTOX [®]
1	0 ^c	0.02	0	10F	
				Subset A	Subset B
2	5	0.02	0.1	10F	10F
3	9	0.02	0.18	10F	10F
4	16	0.02	0.32	10F	10F
5	28.5	0.02	0.57	10F	10F
6	43	0.02	0.86	10F	10F
7	64	0.02	1.28	10F	10F
8	96.5	0.02	1.93	10F	10F
9	115.5	0.02	2.31	10F	10F
10	139	0.02	2.78	10F	10F
11	166.5	0.02	3.33	10F	10F
12	200	0.02	4	10F	10F
13	844,025	0.1	84,402.5	3F+ 3F ^d	0

a: See Sec. 4.3.4 for information on the calculated dose concentration.

b: Except for Group 13, the actual dose level is multiplied by dose volume.

c: Dilution buffer was used for dosing of Group 1 (control) animals.

d: Group 13 was for verification of test article integrity. Group 13 animals were selected from spare animals on dosing day.

Verification of Test Article Integrity

On the dosing day (Day 0) of each batch, 3 animals were randomly selected from the pool

of spare animals. Separately, a single vial of test article was reconstituted (Stock 0), and a portion of Stock 0 was diluted 1.6-fold. The selected test animals were then intravenously injected via the tail vein with 0.1 mL of the reconstituted and diluted test article. Subsequent dosing of the main study animals would proceed when the test article integrity was confirmed by the deaths of at least two IV-dosed test animals within 1 hour post dose. If at least 2 animals failed to die within 1 hour, the particular vial of test article Stock 0 would have been discarded. In that event, fresh vial of Stock 0 was to be prepared from a second test article vial, with the verification test repeated with the stock of the second vial. If the second vial also failed the verification test, the dosing of the particular batch would have been stopped due to unacceptable test article potency. Since all test article vials passed the test, no follow-up procedures as described above took place after the initial tests.

Administration of Test Article: Intramuscular injection of the right gastrocnemius muscle.

Rationales for selection of dose levels and dosing route

The nominal dose levels were selected by OBI in order to determine the intramuscular median effective dose (IM ED₅₀) and intramuscular median lethal dose (IM LD₅₀), which were expected to differ by one order.

Frequency of administrations: Single dose.

Administration of Negative Control

As negative control, 20 µL of diluent buffer was intramuscularly injected in the right gastrocnemius muscle of Group 1 dose animal. The procedures for control injection were identical to those of test article injection.

Observations and Examinations

Animals were observed for mortality once at least 6 hours post dose on Day 0. Thereafter animals were observed for mortality daily throughout the study period concurrent with conducting of DAS assay (up to Day 3). Mortality was individually scored and tallied on a per group basis for calculation of LD₅₀.

Digit Abduction Scoring (DAS) Assay

For each batch, study animals were observed for muscle weakness 24-26, 48-50, and 72-74 hours after the dosing of the first animal on Day 0 of respective batch. Muscle weakness was scored by DAS assay. For each animal, two individual personnel independently recorded the score of its muscle weakness. The two individually observed scores were averaged for analysis. The DAS assay was conducted as follows (excerpt from U.S. Patent Application Publication

No. US 2010/0168023A1): The muscle paralysis was measured using digit abduction score (DAS) assay as reported by Aoki, K. R. in “A comparison of the safety margins of botulinum neurotoxin serotypes A, B, and F in mice”, Toxicon 2001; 39(12):1815-1820. In the DAS assay, a mouse is briefly suspended by its tail to cause a characteristic startle response in which the mouse extends its hind limbs and abducts its hind digits. The extent to which the mouse is able to exhibit this startle response is scored on a five-point scale (from 0-4), with zero representing a normal startle response and four representing maximal reduction in digit abduction and leg extension. Scoring of digit abduction was based on the following Table 7:

Table 7: Digit abduction score (DAS) assay scoring

Digit Abduction Score	4	Curved foot, 5 digits touching.
		Death
	3	Flat foot, 5 digits touching.
		Curved foot, 4 digits touching.
	2	Flat foot, slight space open at tips of all digits.
		Flat foot, 3 digits touching.
	1	Flat foot, a difference in the width of digits abduction compared to the noninjected leg.
		Flat foot, 2 digits touching and the rest spread completely.
	0	Normal (no change)

Animals that were dead at the time of DAS session were assigned the score “4” for the purpose of DAS assay.

Body Weight

Body weights were measured at least once for grouping and on Day 0 prior to dosing.

Statistical Analysis

Probit analysis was used for fitting probit and logit sigmoid toxin unit/DAS responses curves and for calculating IM ED₅₀ as well as IM LD₅₀ values. Analysis was performed using Minitab®. Safety margins of respective batches were calculated based on IM ED₅₀ (DAS) at 48 hours post dose and IM LD₅₀ at 72 hours post dose. The acceptance criteria for the regression analysis is $R^2 > 0.9$.

Computer System

Online data collection system, Pristima® (Version 6.3.2; Xybion Medical Systems

Corporation) was used for the capture and analysis of body weight data in the Testing Facility.

Results and discussion

Tests of OBI-858 RDS Batch Integrity

5 Summary of the OBI-858 integrity tests is presented in the following Table 8:

Table 8: OBI-858 integrity tests

Batch	Batch-Vial No.	Mean Survival (minute post-dose)
1	14004-25	34.0 ± 2.6
2	14004-30	33.3 ± 1.5
3	14004-35	34.7 ± 3.1
4	14004-45	31.7 ± 2.3

All vials of the test article passed the integrity test with mean post-dose survival less than 1 hour.

10

Body weight

For each batch, the body weight ranges of individual dose groups were within ±10% of the respective mean weights.

DAS Assay and ED₅₀ Calculations

15 OBI-858

Animals exhibited signs of digit abduction as early as 24 hours post dose. The severity of digit abduction correlated to increasing dosage of OBI-858 RDS before plateauing at maximum severity (including death) at higher doses. For 48 h IM ED₅₀, all except Batch 1 passed the acceptance criteria ($R^2 > 0.9$) of the regression analysis. For Batches 2, 3, and 4, the ED₅₀ at 48 hours post dose are 0.33, 0.32, and 0.36 U/animal, respectively.

20

Table 9: Test Article OBI-858 ED₅₀ at 48 hours (U/animal)

Batch	ED ₅₀	Low 95%	High 95%	R ² value
1	0.21	0.13	0.35	0.89
2	0.33	0.27	0.41	0.97
3	0.32	0.23	0.46	0.92

4	0.36	0.27	0.47	0.95
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BOTOX®

Animals exhibited signs of digit abduction as early as 24 hours post dose. The severity of digit abduction correlated to increasing dosage of BOTOX®, but the plateau of maximum severity (including death) was attained by BOTOX® at smaller doses than OBI-858 RDS. For 48 h IM ED₅₀, all except Batch 1 passed the acceptance criteria ($R^2 > 0.9$) of the regression analysis. For Batches 2, 3, and 4, the ED₅₀ at 48 hours post dose are 0.22, 0.24, and 0.23 U/animal, respectively. Overall, the 48 h IM ED₅₀ of BOTOX® are lower than that of OBI-858.

Table 10: BOTOX® ED₅₀ at 48 hours (U/animal)

Batch	ED ₅₀	Low 95%	High 95%	R ² value
1	0.36	0.21	0.60	0.82
2	0.22	0.19	0.25	0.99
3	0.24	0.22	0.26	1.00
4	0.23	0.19	0.29	0.98

Mortalities and LD₅₀ Calculations

OBI-858

The majority of deaths took place at 72 hours post dose, although deaths were observed as early as 48 hours post dose among Groups 11 and 12 for some batches. At 72 hours post dose, LD₅₀ of all four batches were 3.00, 3.42, 3.33, and 3.35 U/animal, respectively. However, at 72 hours post dose only Batches 2, 3, and 4 had sufficient dose groups within the linear range of dose correlation for regression analysis, with the percentage dead between 0% and 100%. (At least three dose groups per analysis were required.) Among these three batches, only Batches 2 and 3 passed the acceptance criteria for the regression analysis.

Table 11: Test Article OBI-858 LD₅₀ at 72 hours (U/animal)

Batch	LD ₅₀	Low 95%	High 95%	R ² value
1	3.00			
2	3.42	2.70	4.32	0.99
3	3.33	3.31	3.36	1.00

4	3.35	1.66	6.75	0.86
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BOTOX®

Deaths took place among BOTOX®-dosed animals sooner (as early as 24 hours post dose) and at doses lower than those seen in OBI-858 RDS-dosed animals (Group 6 through Group 12 at 72 hours post dose). At 72 hours post dose, the calculated LD₅₀ of all four batches were 2.21, 1.63, 2.14, and 1.57 U/animal, respectively. However, none of the four batches of death rate outcomes passed the acceptance criteria for the regression analysis. For Batches 2, 3, and 4, there were no dose groups within the linear range of dose correlation at 72 hours post dose. Consequently, no regression analysis could be performed for the calculated LD₅₀ of these batches. For Batch 1, the R² value was 0.74, below the acceptance criteria.

Table 12: BOTOX® LD₅₀ at 72 hours (U/animal)

Batch	LD ₅₀	Low 95%	High 95%	R ² value
1	2.21	0.80	6.11	0.74
2	1.63			
3	2.14			
4	1.57			

Assessment of Impact of Body Weight Deviations on ED₅₀ Outcomes

For both OBI-858 RDS and BOTOX®, there was no consistent trend of differences in the calculated ED₅₀ between animals within the required body weight range (18-22 g) and animals overweight (greater than 22 g) at dosing. Exclusion of overweight animals from ED₅₀ calculations led to no consistent changes in ED₅₀ values, when compared to overall ED₅₀ values, as shown in the following Table 13:

Table 13: Comparison of IM 48 h ED₅₀ by Body Weight Range (U/animal)

Batch	OBI-858			BOTOX®		
	48 h IM ED ₅₀	48 h IM ED ₅₀ (Body Weight: 18-22 g)	48 h IM ED ₅₀ (Body Weight > 22 g)	48 h IM ED ₅₀	48 h IM ED ₅₀ (Body Weight: 18-22 g)	48 h IM ED ₅₀ (Body Weight > 22 g)
1	0.21	0.19	0.23	0.36	0.36	0.41

2	0.33	0.33	0.40	0.22	0.21	0.22
3	0.32	0.37	0.32	0.24	0.25	0.25
4	0.36	0.38	0.36	0.23	0.24	0.25

In addition, one animal was found to be underweight at 17.7 g at dosing (below the 18 g threshold). The underweight animal [ID 0193; Group 9, Subset B (Botox[®]); Batch 2] received similar Digital Abduction scores as the other animals in the same group in all scoring sessions.

5 The event therefore had no impact on the study outcome.

Estimate of Margins of Safety (MOSs)

Safety margins are calculated as the ratio of 72 h IM LD₅₀ to 48 h IM ED₅₀.

10 For the four batches of tests, OBI-858 has the MOS values of 14.07, 10.32, 10.33, and 9.29, respectively. For the four batches of tests, BOTOX[®] has the MOS values of 6.20, 7.58, 8.98, and 6.73, respectively. The MOS of OBI-858 RDS is consistently higher than that of BOTOX[®] (Table 16).

Conclusion

15 All vials of OBI-858 tested in this study passed the integrity test. Elevated body weight (between 22 to 24 g) of some of the study animals at dosing had no significant impact on the outcomes of ED₅₀ calculations. For OBI-858, the respective 72 h IM LD₅₀ and 48 h IM ED₅₀ values for the acceptable batches are 3.42 and 0.33 U/animal (Batch 2) and 3.33 and 0.32 U/animal (Batch 3). The corresponding MOS values are 10.32 and 10.33 for Batch 2 and Batch
20 3, respectively. For BOTOX[®], the respective 72 h IM LD₅₀ and 48 h IM ED₅₀ values are 1.63 and 0.22 U/animal (Batch 2) and 2.14 and 0.24 U/animal (Batch 3). The corresponding MOS values are 7.58 and 8.98 for Batch 2 and Batch 3, respectively. In short, OBI-858 has a potency comparable to that of BOTOX[®]. It has a higher 72 h IM LD₅₀ and safety margin value than BOTOX[®].

Table 14: Test Article OBI-858 Mouse DAS Score at 48 hr by Body Weight Range

Group (Dose Level (U/animal))	Batch 1		Batch 2		Batch 3		Batch 4	
	18 ≤ BW ≤ 22 g (n)	> 22 g (n)	18 ≤ BW ≤ 22 g (n)	> 22 g (n)	18 ≤ BW ≤ 22 g (n)	> 22 g (n)	18 ≤ BW ≤ 22 g (n)	> 22 g (n)
1 (0.00)	0.00 ± 0.00 (6)	0.00 ± 0.00 (4)	0.00 ± 0.00 (7)	0.00 ± 0.00 (3)	0.00 ± 0.00 (9)	0.00 ± 0.00 (1)	0.21 ± 0.43 (7)	0.00 ± 0.00 (3)
2 (0.10)	1.39 ± 0.50 (9)	1.00 ± 0.00 (1)	0.90 ± 0.32 (5)	0.40 ± 0.52 (5)	0.33 ± 0.52 (3)	1.00 ± 0.68 (7)	0.80 ± 0.42 (5)	0.90 ± 0.99 (5)
3 (0.18)	2.33 ± 0.78 (6)	2.00 ± 0.76 (4)	0.94 ± 0.44 (8)	1.50 ± 0.58 (2)	1.00 ± 0.69 (9)	1.00 ± 0.00 (1)	1.00 ± 0.67 (5)	1.70 ± 1.06 (5)
4 (0.32)	2.44 ± 1.10 (9)	2.00 ± 0.00 (1)	2.17 ± 0.83 (6)	1.88 ± 1.25 (4)	2.92 ± 0.67 (6)	2.13 ± 1.13 (4)	1.71 ± 0.83 (7)	1.83 ± 0.98 (3)
5 (0.57)	2.88 ± 1.31* (8)	3.50 ± 0.71 (1)	2.50 ± 0.85 (5)	2.90 ± 0.57 (5)	2.80 ± 0.79 (5)	2.90 ± 1.10 (5)	2.40 ± 0.52 (5)	2.80 ± 0.42 (5)
6 (0.86)	3.44 ± 0.51 (8)	3.25 ± 0.50 (2)	3.43 ± 0.65 (7)	3.17 ± 0.41 (3)	3.42 ± 0.51 (6)	3.63 ± 0.52 (4)	3.08 ± 0.79 (6)	2.63 ± 0.92 (4)
7 (1.28)	4.00 ± 0.00 (6)	3.88 ± 0.35 (4)	3.17 ± 0.75 (3)	3.29 ± 0.47 (7)	3.25 ± 0.46 (4)	3.25 ± 0.45 (6)	3.14 ± 0.53 (7)	3.17 ± 0.75 (3)
8 (1.93)	4.00 ± 0.00 (8)	3.75 ± 0.50 (2)	3.75 ± 0.45 (6)	4.00 ± 0.00 (4)	3.58 ± 0.51 (6)	3.38 ± 0.52 (4)	3.57 ± 0.51 (7)	2.83 ± 0.75 (3)
9 (2.31)	3.78 ± 0.43 (9)	4.00 ± 0.00 (1)	4.00 ± 0.00 (9)	3.00 ± 0.00 (1)	3.50 ± 0.52 (6)	3.75 ± 0.46 (4)	3.75 ± 0.46 (4)	3.50 ± 0.52 (6)
10 (2.78)	3.92 ± 0.29 (6)	4.00 ± 0.00 (4)	4.00 ± 0.00 (8)	4.00 ± 0.00 (2)	3.90 ± 0.32 (5)	3.80 ± 0.42 (5)	4.00 ± 0.00 (6)	3.75 ± 0.46 (4)
11 (3.33)	4.00 ± 0.00 (6)	4.00 ± 0.00 (4)	4.00 ± 0.00 (8)	4.00 ± 0.00 (2)	3.90 ± 0.32 (5)	4.00 ± 0.00 (5)	4.00 ± 0.00 (8)	4.00 ± 0.00 (2)
12 (4.00)	4.00 ± 0.00 (8)	4.00 ± 0.00 (2)	4.00 ± 0.00 (7)	4.00 ± 0.00 (3)	4.00 ± 0.00 (8)	4.00 ± 0.00 (2)	4.00 ± 0.00 (7)	4.00 ± 0.00 (3)

*: One animal was dead during procedure; the incident was unrelated to test article effect. The animal was excluded from DAS assay at 48 hours.

Table 15: Botox[®] Mouse DAS Score at 48 hr by Body Weight Range

Group (Dose Level (U/animal))	Batch 1		Batch 2		Batch 3		Batch 4	
	18 $\leq$ BW $\leq$ 22 g (n)	> 22 g (n)	18 $\leq$ BW $\leq$ 22 g (n)	> 22 g (n)	18 $\leq$ BW $\leq$ 22 g (n)	> 22 g (n)	18 $\leq$ BW $\leq$ 22 g (n)	> 22 g (n)
1 (0.00)	0.00 $\pm$ 0.00 (6)	0.00 $\pm$ 0.00 (4)	0.00 $\pm$ 0.00 (7)	0.00 $\pm$ 0.00 (3)	0.00 $\pm$ 0.00 (9)	0.00 $\pm$ 0.00 (1)	0.21 $\pm$ 0.43 (7)	0.00 $\pm$ 0.00 (3)
2 (0.10)	1.39 $\pm$ 0.50 (9)	1.00 $\pm$ 0.00 (1)	0.90 $\pm$ 0.32 (5)	0.40 $\pm$ 0.52 (5)	0.33 $\pm$ 0.52 (3)	1.00 $\pm$ 0.68 (7)	0.80 $\pm$ 0.42 (5)	0.90 $\pm$ 0.99 (5)
3 (0.18)	2.33 $\pm$ 0.78 (6)	2.00 $\pm$ 0.76 (4)	0.94 $\pm$ 0.44 (8)	1.50 $\pm$ 0.58 (2)	1.00 $\pm$ 0.69 (9)	1.00 $\pm$ 0.00 (1)	1.00 $\pm$ 0.67 (5)	1.70 $\pm$ 1.06 (5)
4 (0.32)	2.44 $\pm$ 1.10 (9)	2.00 $\pm$ 0.00 (1)	2.17 $\pm$ 0.83 (6)	1.88 $\pm$ 1.25 (4)	2.92 $\pm$ 0.67 (6)	2.13 $\pm$ 1.13 (4)	1.71 $\pm$ 0.83 (7)	1.83 $\pm$ 0.98 (3)
5 (0.57)	2.88 $\pm$ 1.31* (8)	3.50 $\pm$ 0.71 (1)	2.50 $\pm$ 0.85 (5)	2.90 $\pm$ 0.57 (5)	2.80 $\pm$ 0.79 (5)	2.90 $\pm$ 1.10 (5)	2.40 $\pm$ 0.52 (5)	2.80 $\pm$ 0.42 (5)
6 (0.86)	3.44 $\pm$ 0.51 (8)	3.25 $\pm$ 0.50 (2)	3.43 $\pm$ 0.65 (7)	3.17 $\pm$ 0.41 (3)	3.42 $\pm$ 0.51 (6)	3.63 $\pm$ 0.52 (4)	3.08 $\pm$ 0.79 (6)	2.63 $\pm$ 0.92 (4)
7 (1.28)	4.00 $\pm$ 0.00 (6)	3.88 $\pm$ 0.35 (4)	3.17 $\pm$ 0.75 (3)	3.29 $\pm$ 0.47 (7)	3.25 $\pm$ 0.46 (4)	3.25 $\pm$ 0.45 (6)	3.14 $\pm$ 0.53 (7)	3.17 $\pm$ 0.75 (3)
8 (1.93)	4.00 $\pm$ 0.00 (8)	3.75 $\pm$ 0.50 (2)	3.75 $\pm$ 0.45 (6)	4.00 $\pm$ 0.00 (4)	3.58 $\pm$ 0.51 (6)	3.38 $\pm$ 0.52 (4)	3.57 $\pm$ 0.51 (7)	2.83 $\pm$ 0.75 (3)
9 (2.31)	3.69 $\pm$ 0.48 (8)	3.75 $\pm$ 0.50 (2)	4.00 $\pm$ 0.00 (4)	4.00 $\pm$ 0.00 (6)	4.00 $\pm$ 0.00 (4)	4.00 $\pm$ 0.00 (6)	4.00 $\pm$ 0.00 (8)	4.00 $\pm$ 0.00 (2)
10 (2.78)	3.61 $\pm$ 0.50 (9)	3.00 $\pm$ 0.00 (1)	4.00 $\pm$ 0.00 (5)	4.00 $\pm$ 0.00 (5)	4.00 $\pm$ 0.00 (5)	4.00 $\pm$ 0.00 (5)	4.00 $\pm$ 0.00 (5)	4.00 $\pm$ 0.00 (5)
11 (3.33)	4.00 $\pm$ 0.00 (8)	4.00 $\pm$ 0.00 (2)	4.00 $\pm$ 0.00 (7)	4.00 $\pm$ 0.00 (3)	4.00 $\pm$ 0.00 (7)	4.00 $\pm$ 0.00 (3)	4.00 $\pm$ 0.00 (8)	4.00 $\pm$ 0.00 (2)
12 (4.00)	4.00 $\pm$ 0.00 (6)	4.00 $\pm$ 0.00 (4)	4.00 $\pm$ 0.00 (7)	4.00 $\pm$ 0.00 (3)	4.00 $\pm$ 0.00 (5)	4.00 $\pm$ 0.00 (5)	4.00 $\pm$ 0.00 (9)	4.00 $\pm$ 0.00 (1)

Table 16: The safety margin value of OBI-858 and BOTOX®

Batch	OBI-858	BOTOX®
1	14.07	6.20
2	10.32	7.58
3	10.33	8.98
4	9.29	6.73

Example 4: The stability test of OBI-858 formulation

Experimental material/method:

5 (1) LF08 formulation:

0.5 mg/mL Human Serum Albumin (HSA), 11.25 mg/mL sodium chloride, and 0.188 mg/mL Tween 80 dissolved in 50 mM phosphate buffer (pH 6.3)。

(2) LF09 formulation:

10 0.5 mg/mL Human Serum Albumin (HSA), 11.25 mg/mL sodium chloride, and 0.188 mg/mL Tween 80 dissolved in 50 mM Citric acid (Sodium Citrate) buffer (pH 5.6)。

(3) LF10 formulation:

15 0.5 mg/mL Methionine, 11.25 mg/mL sodium chloride, 0.188 mg/mL Tween 80 and 150 mM Arginine dissolved in 50 mM Citric acid (Sodium Citrate) buffer (pH 6.0)。

(4) LF11 formulation:

11.25 mg/mL sodium chloride and 0.188 mg/mL Tween dissolved in 50 mM Citric acid (Sodium Citrate) buffer (pH 6.3)。

20 (5) Experimental animal:

Eight female ICR mice (Body weight range: 18-22 g). The selected test animals were then intravenously injected via the tail vein with 0.1 mL of OBI-858 to test lethal dose (LD₅₀/mL).

25 **Experimental result:**

(1) Stability test between different formulations:

Lyophilized OBI-858 DS powder was reconstituted within four formulations. After stored in 45 °C for fourteen days, these test articles were transferred to 4 °C for twenty-four hours. The selected test animals were then intravenously injected via the tail after warming up in room temperature for 1 hour. Table 17 showed the lethal dose (LD₅₀/mL) of these four formulations. It indicated that the best stability existed in LF09 formulation.

Table 17: Stability test between different formulations

Formulation	LF08	LF09	LF10	LF11
lethal dose before test (LD ₅₀ /mL)	6.15E+05	8.12E+05	6.75E+05	1.63E+05
Test temperature	45 °C			
Test time	14 Days			
lethal dose after test (LD ₅₀ /mL)	0	2.58E+05	0	0

(2) 4-60°C accelerated stability test of LF09 formulation:

Lyophilized OBI-858 DS powder was reconstituted within LF09 formulation. After stored in four temperatures (4 °C, 25 °C, 45 °C, and 60 °C) for seven days, these test articles were transferred to 4 °C for twenty-four hours. These test articles were then intravenously injected via the tail after warming up in room temperature for 1 hour. Table 18 showed the lethal dose (LD₅₀/mL) of LF09 formulation. It indicated that the stability still existed in storage within 45 °C.

Table 18: accelerated stability test of LF09 formulation

Test temperature	4 °C	25 °C	45 °C	60 °C
lethal dose before	5.07E+05	5.07E+05	5.07E+05	5.07E+05

test (LD ₅₀ /mL)				
Test time	7 Days			
lethal dose after test (LD ₅₀ /mL)	5.07E+05	5.07E+05	4.06E+05	0

It should be understood that the exemplary embodiments described herein should be considered in a descriptive sense only and not for the purpose of limitation. It should generally be considered that the description of features or aspects within each exemplary embodiment can be used for other similar features or aspects in other exemplary embodiments. Although one or more exemplary embodiments have been described with reference to the accompanying drawings, those of ordinary skill in the art should understand that they can be carried out without departing from the spirit and scope of the inventive concept as defined by the appended claims various changes in form and detail.

CLAIMS

1. A pharmaceutical composition comprising a *Clostridium botulinum* toxin Type A complex and a pharmaceutically or dermatologically acceptable carrier, wherein the *Clostridium botulinum* toxin Type A complex comprises a HA70 component, a HA17 component, a HA33 component, a NTNH component and a BoNT/A1 component; wherein

(1) the HA70 component comprises the amino acid sequence of SEQ ID NO. 1 with retaining the function of the HA70 component;

(2) the HA17 component comprises the amino acid sequence of SEQ ID NO. 2 with retaining the function of the HA17 component;

(3) the HA33 component comprises the amino acid sequence of SEQ ID NO. 3 with retaining the function of the HA33 component;

(4) the NTNH component comprises the amino acid sequence of SEQ ID NO. 4 with retaining the function of the NTNH component;

(5) wherein the BoNT/A1 component comprises the amino acid sequence of SEQ ID NO. 5 with retaining the function of the BoNT/A1 component; and

wherein the *Clostridium botulinum* toxin Type A complex has a molecular weight of 740-790 kDa.

2. A liquid formulation comprising a *Clostridium botulinum* toxin Type A complex, wherein the *Clostridium botulinum* toxin Type A complex comprises a HA70 component, a HA17 component, a HA33 component, a NTNH component and a BoNT/A1 component; wherein

(1) the HA70 component comprises the amino acid sequence of SEQ ID NO. 1 with retaining the function of the HA70 component;

(2) the HA17 component comprises the amino acid sequence of SEQ ID NO. 2 with retaining the function of the HA17 component;

(3) the HA33 component comprises the amino acid sequence of SEQ ID NO. 3 with retaining the function of the HA33 component;

(4) the NTNH component comprises the amino acid sequence of SEQ ID NO. 4 with retaining the function of the NTNH component;

(5) wherein the BoNT/A1 component comprises the amino acid sequence of SEQ ID NO. 5 with retaining the function of the BoNT/A1 component; and

wherein the *Clostridium botulinum* toxin Type A complex has a molecular weight of 740-790 kDa.

3. The liquid formulation of claim 2, comprising human serum albumin, methionine, arginine, sodium chloride, Tween 80, citrate buffer, and/or phosphate buffer.

4. The pharmaceutical composition of claim 1, or the liquid formulation of claim 2 or 3, for use in treating or preventing a disease.

5. An aesthetic method comprising administering an effective amount of the liquid formulation of claim 2 or 3 to a subject in need thereof.

6. The aesthetic method of claim 5, comprising reducing the appearance of fine lines and/or wrinkles, enlarging eyes, lifting the corners of mouth, smoothing lines that appear on the upper lip, or relieving muscle tone.

7. Use of the pharmaceutical composition of claim 1 or the liquid formulation of claim 2 or 3 in the preparation of a medicament for treating or preventing a disease caused by synaptic transmission or release of acetylcholine,

wherein the disease is selected from the group consisting of neuromuscular disorders characterized by skeletal muscle hyperactivity, neuropathic pain, migraine, overactive bladder, rhinitis, sinusitis, acne, dystonia, dystonic contraction, hyperhidrosis, hypersecretion of one or more glands controlled by the cholinergic nervous system, hemifacial spasm, adult-onset spastic torticollis, anal fissure, blepharospasm, cerebral

palsy, cervical dystonia, strabismus, temporomandibular joint disorders and various types of muscle convulsions and spasms.

8. The liquid formulation of claim 2 or 3, for use in reducing the appearance of fine lines and/or wrinkles, enlarging eyes, lifting the corners of mouth, smoothing lines that appear on the upper lip, or relieving muscle tone.

9. The pharmaceutical composition of claim 1, wherein the composition is in a lyophilized form.

10. The aesthetic method of claim 6, wherein the appearance of fine lines and/or wrinkles is on the face.

11. The use of claim 8, wherein the appearance of fine lines and/or wrinkles is on the face.

Sequence Listing

<110> OBI PHARMA, INC.

<120> Clostridium botulinum toxin Type A composition, formulation and use thereof

<130> F20W6191

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<170> PatentIn version 3.5

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Arg	Arg	Gly	Asp	Gly	Trp	Ile	Leu	Ser	Arg	Gln	Asn	Gln	Ile	Leu	Gly
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Gly	Ser	Val	Ile	Ser	Asn	Gly	Ser	Thr	Gly	Ile	Val	Gly	Asp	Leu	Arg
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Val	Asn	Asp	Asn	Ala	Ile	Pro	Tyr	Tyr	Tyr	Pro	Thr	Pro	Ser	Phe	Asn
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Glu	Glu	Tyr	Ile	Lys	Asn	Asn	Ile	Gln	Thr	Val	Phe	Thr	Asn	Phe	Thr
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Glu	Ala	Asn	Gln	Ile	Pro	Ile	Gly	Phe	Glu	Phe	Ser	Lys	Thr	Ala	Pro
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Ser	Asn	Lys	Asn	Leu	Tyr	Met	Tyr	Leu	Gln	Tyr	Thr	Tyr	Ile	Arg	Tyr
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Ser Thr Ser Leu Phe Lys Phe Val Glu Glu Ala Pro Ser Asp Lys Asn
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Tyr Gln Asn Gly Ser Ile Val Asn Lys Ala Glu Tyr Tyr Leu Pro Ser

325

330

335

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Val Lys Met Gln Val Ala Glu Asp Gly Phe Ile Gln Asn Gly Pro Glu
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Glu Glu Ile Val Val Gly Val Ile Asp Pro Ser Glu Asn Ile Gln Glu
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Asp Asp Asp Ile Lys Ala Ile Asn Tyr Ile Thr Gly Phe Asp Ser Pro
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Tyr Ile Arg Val Pro Gln Thr Ser Ser Asn Ile Glu Asn Gln Ile Gln
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Phe Lys Arg Glu Glu Gly Asp Leu Arg Asn Leu Met Asn Ser Ser Val
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Tyr Asn Gln Gly Ile Gly Thr Leu Phe Arg Val Thr Glu Thr Ile Asp
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Gly Tyr Asn Leu Ile Asn Ile Gln Gln Asn Leu His Leu Leu Asn Asn
580 585 590

Thr Asn Ser Ile Arg Leu Leu Asn Gly Ala Ile Tyr Ile Leu Lys Val
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Asp Ile His Asn Thr Asn Leu Val Leu Thr Trp Asn Ala Pro Thr His

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Leu	Leu	Lys	Asp	Ile	Gly	Asn	Asn	Ser	Phe	Ile	Ile	Ala	Ser	Tyr	Lys
			100					105					110		
Asn	Pro	Asn	Leu	Val	Leu	Tyr	Ala	Asp	Thr	Val	Ala	Arg	Asn	Leu	Lys
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Leu	Ser	Thr	Leu	Asn	Asn	Ser	Asn	Tyr	Ile	Lys	Phe	Ile	Ile	Glu	Asp
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Tyr	Ile	Ile	Ser	Asp	Leu	Asn	Asn	Phe	Thr	Cys	Lys	Ile	Ser	Pro	Ile
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Leu	Asp	Leu	Asn	Lys	Val	Val	Gln	Gln	Val	Asp	Val	Thr	Asn	Leu	Asn
				165					170					175	
Val	Asn	Leu	Tyr	Thr	Trp	Asp	Tyr	Gly	Arg	Asn	Gln	Lys	Trp	Thr	Ile
			180					185					190		
Arg	Tyr	Asn	Glu	Glu	Lys	Ala	Ala	Tyr	Gln	Phe	Phe	Asn	Thr	Ile	Leu
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Ser	Asn	Gly	Val	Leu	Thr	Trp	Ile	Phe	Ser	Asn	Gly	Asn	Thr	Val	Arg
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Val	Ser	Ser	Ser	Asn	Asp	Gln	Asn	Asn	Asp	Ala	Gln	Tyr	Trp	Leu	Ile
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Asn	Pro	Val	Ser	Asp	Thr	Asp	Glu	Thr	Tyr	Thr	Ile	Thr	Asn	Leu	Arg
				245					250					255	
Asp	Thr	Thr	Lys	Ala	Leu	Asp	Leu	Tyr	Gly	Gly	Gln	Thr	Ala	Asn	Gly
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Asp Ser Asn Phe Leu Ser Gln Asp Ser Glu Lys Asp Lys Phe Leu Gln
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Ala Ile Ile Thr Leu Leu Lys Arg Ile Asn Ser Thr Asn Ala Gly Glu
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Lys Leu Leu Ser Leu Ile Ser Thr Ala Ile Pro Phe Pro Tyr Gly Tyr
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Ile Gly Gly Gly Tyr Tyr Ala Pro Asn Met Ile Thr Phe Gly Ser Ala
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Pro Lys Ser Asn Lys Lys Leu Asn Ser Leu Ile Ser Ser Thr Ile Pro

130

135

140

Phe Pro Tyr Ala Gly Tyr Arg Glu Thr Asn Tyr Leu Ser Ser Glu Asp
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Asn Lys Ser Phe Tyr Ala Ser Asn Ile Val Ile Phe Gly Pro Gly Ala
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Asn Ile Val Glu Asn Asn Thr Val Phe Tyr Lys Lys Glu Asp Ala Glu
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Asn Gly Met Gly Thr Met Thr Glu Ile Trp Phe Gln Pro Phe Leu Thr
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Tyr Lys Tyr Asp Glu Phe Tyr Ile Asp Pro Ala Ile Glu Leu Ile Lys
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Cys Leu Ile Lys Ser Leu Tyr Phe Leu Tyr Gly Ile Lys Pro Ser Asp
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Asp Leu Val Ile Pro Tyr Arg Leu Arg Ser Glu Leu Glu Asn Ile Glu
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Tyr Ser Gln Leu Asn Ile Val Asp Leu Leu Val Ser Gly Gly Ile Asp
 260 265 270

Pro Lys Phe Ile Asn Thr Asp Pro Tyr Trp Phe Thr Asp Asn Tyr Phe
 275 280 285

Ser Asn Ala Lys Lys Val Phe Glu Asp His Arg Asn Ile Tyr Glu Thr
 290 295 300

Glu Ile Glu Gly Asn Asn Ala Ile Gly Asn Asp Ile Lys Leu Arg Leu
 305 310 315 320

Lys Gln Lys Phe Arg Ile Asn Ile Asn Asp Ile Trp Glu Leu Asn Leu
 325 330 335

Asn Tyr Phe Ser Lys Glu Phe Ser Ile Met Met Pro Asp Arg Phe Asn
340 345 350

Asn Ala Leu Lys His Phe Tyr Arg Lys Gln Tyr Tyr Lys Ile Asp Tyr
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Pro Glu Asn Tyr Ser Ile Asn Gly Phe Val Asn Gly Gln Ile Asn Ala
370 375 380

Gln Leu Ser Leu Ser Asp Arg Asn Gln Asp Ile Ile Asn Lys Pro Glu
385 390 395 400

Glu Ile Ile Asn Leu Leu Asn Gly Asn Asn Val Ser Leu Met Arg Ser
405 410 415

Asn Ile Tyr Gly Asp Gly Leu Lys Ser Thr Val Asp Asp Phe Tyr Ser
420 425 430

Asn Tyr Lys Ile Pro Tyr Asn Arg Ala Tyr Glu Tyr His Phe Asn Asn
435 440 445

Ser Asn Asp Ser Ser Leu Asp Asn Val Asn Ile Gly Val Ile Asp Asn
450 455 460

Ile Pro Glu Ile Ile Asp Val Asn Pro Tyr Lys Glu Asn Cys Asp Lys
465 470 475 480

Phe Ser Pro Val Gln Lys Ile Thr Ser Thr Arg Glu Ile Asn Thr Asn
485 490 495

Ile Pro Trp Pro Ile Asn Tyr Leu Gln Ala Gln Asn Thr Asn Asn Glu
500 505 510

Lys Phe Ser Leu Ser Ser Asp Phe Val Glu Val Val Ser Ser Lys Asp
515 520 525

Lys Ser Leu Val Tyr Ser Phe Leu Ser Asn Val Met Phe Tyr Leu Asp

530

535

540

Ser Ile Lys Asp Asn Ser Pro Ile Asp Thr Asp Lys Lys Tyr Tyr Leu
 545 550 555 560

Trp Leu Arg Glu Ile Phe Arg Asn Tyr Ser Phe Asp Ile Thr Ala Thr
 565 570 575

Gln Glu Ile Asn Thr Asn Cys Gly Ile Asn Lys Val Val Thr Trp Phe
 580 585 590

Gly Lys Ala Leu Asn Ile Leu Asn Thr Ser Asp Ser Phe Val Glu Glu
 595 600 605

Phe Gln Asn Leu Gly Ala Ile Ser Leu Ile Asn Lys Lys Glu Asn Leu
 610 615 620

Ser Met Pro Ile Ile Glu Ser Tyr Glu Ile Pro Asn Asp Met Leu Gly
 625 630 635 640

Leu Pro Leu Asn Asp Leu Asn Glu Lys Leu Phe Asn Ile Tyr Ser Lys
 645 650 655

Asn Thr Ala Tyr Phe Lys Lys Ile Tyr Tyr Asn Phe Leu Asp Gln Trp
 660 665 670

Trp Thr Gln Tyr Tyr Ser Gln Tyr Phe Asp Leu Ile Cys Met Ala Lys
 675 680 685

Arg Ser Val Leu Ala Gln Glu Thr Leu Ile Lys Arg Ile Ile Gln Lys
 690 695 700

Lys Leu Ser Tyr Leu Ile Gly Asn Ser Asn Ile Ser Ser Asp Asn Leu
 705 710 715 720

Ala Leu Met Asn Leu Thr Thr Thr Asn Thr Leu Arg Asp Ile Ser Asn
 725 730 735

Glu Ser Gln Ile Ala Met Asn Asn Val Asp Ser Phe Leu Asn Asn Ala
740 745 750

Ala Ile Cys Val Phe Glu Ser Asn Ile Tyr Pro Lys Phe Ile Ser Phe
755 760 765

Met Glu Gln Cys Ile Asn Asn Ile Asn Ile Lys Thr Lys Glu Phe Ile
770 775 780

Gln Lys Cys Thr Asn Ile Asn Glu Asp Glu Lys Leu Gln Leu Ile Asn
785 790 795 800

Gln Asn Val Phe Asn Ser Leu Asp Phe Glu Phe Leu Asn Ile Gln Asn
805 810 815

Met Lys Ser Leu Phe Ser Ser Glu Thr Ala Leu Leu Ile Lys Glu Glu
820 825 830

Thr Trp Pro Tyr Glu Leu Val Leu Tyr Ala Phe Lys Glu Pro Gly Asn
835 840 845

Asn Val Ile Gly Asp Ala Ser Gly Lys Asn Thr Ser Ile Glu Tyr Ser
850 855 860

Lys Asp Ile Gly Leu Val Tyr Gly Ile Asn Ser Asp Ala Leu Tyr Leu
865 870 875 880

Asn Gly Ser Asn Gln Ser Ile Ser Phe Ser Asn Asp Phe Phe Glu Asn
885 890 895

Gly Leu Thr Asn Ser Phe Ser Ile Tyr Phe Trp Leu Arg Asn Leu Gly
900 905 910

Lys Asp Thr Ile Lys Ser Lys Leu Ile Gly Ser Lys Glu Asp Asn Cys
915 920 925

Gly Trp Glu Ile Tyr Phe Gln Asp Thr Gly Leu Val Phe Asn Met Ile

930

935

940

Asp Ser Asn Gly Asn Glu Lys Asn Ile Tyr Leu Ser Asp Val Ser Asn
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Asn Ser Trp His Tyr Ile Thr Ile Ser Val Asp Arg Leu Lys Glu Gln
 965 970 975

Leu Leu Ile Phe Ile Asp Asp Asn Leu Val Ala Asn Glu Ser Ile Lys
 980 985 990

Glu Ile Leu Asn Ile Tyr Ser Ser Asn Ile Ile Ser Leu Leu Ser Glu
 995 1000 1005

Asn Asn Pro Ser Tyr Ile Glu Gly Leu Thr Ile Leu Asn Lys Pro
 1010 1015 1020

Thr Thr Ser Gln Glu Val Leu Ser Asn Tyr Phe Glu Val Leu Asn
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Asn Ser Tyr Ile Arg Asp Ser Asn Glu Glu Arg Leu Glu Tyr Asn
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Lys Thr Tyr Gln Leu Tyr Asn Tyr Val Phe Ser Asp Lys Pro Ile
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Cys Glu Val Lys Gln Asn Asn Asn Ile Tyr Leu Thr Ile Asn Asn
 1070 1075 1080

Thr Asn Asn Leu Asn Leu Gln Ala Ser Lys Phe Lys Leu Leu Ser
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Ile Asn Pro Asn Lys Gln Tyr Val Gln Lys Leu Asp Glu Val Ile
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Ile Ser Val Leu Asp Asn Met Glu Lys Tyr Ile Asp Ile Ser Glu
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Asp Asn Arg Leu Gln Leu Ile Asp Asn Lys Asn Asn Ala Lys Lys
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Met Ile Ile Ser Asn Asp Ile Phe Ile Ser Asn Cys Leu Thr Leu
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<400> 5

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Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
 35 40 45

Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu
 50 55 60

Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
 65 70 75 80

Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu

85

90

95

Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val
 100 105 110

Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys
 115 120 125

Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr
 130 135 140

Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile
 145 150 155 160

Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr
 165 170 175

Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe
 180 185 190

Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu
 195 200 205

Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu
 210 215 220

Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn
 225 230 235 240

Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu
 245 250 255

Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys
 260 265 270

Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn
 275 280 285

Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val
290 295 300

Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys
305 310 315 320

Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu
325 330 335

Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp
340 345 350

Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn
355 360 365

Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr
370 375 380

Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn
385 390 395 400

Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu
405 410 415

Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg
420 425 430

Gly Ile Ile Thr Ser Lys Thr Lys Ser Leu Asp Lys Gly Tyr Asn Lys
435 440 445

Ala Leu Asn Asp Leu Cys Ile Lys Val Asn Asn Trp Asp Leu Phe Phe
450 455 460

Ser Pro Ser Glu Asp Asn Phe Thr Asn Asp Leu Asn Lys Gly Glu Glu
465 470 475 480

Ile Thr Ser Asp Thr Asn Ile Glu Ala Ala Glu Glu Asn Ile Ser Leu

485

490

495

Asp Leu Ile Gln Gln Tyr Tyr Leu Thr Phe Asn Phe Asp Asn Glu Pro
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Glu Asn Ile Ser Ile Glu Asn Leu Ser Ser Asp Ile Ile Gly Gln Leu
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Glu Leu Met Pro Asn Ile Glu Arg Phe Pro Asn Gly Lys Lys Tyr Glu
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Leu Asp Lys Tyr Thr Met Phe His Tyr Leu Arg Ala Gln Glu Phe Glu
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His Gly Lys Ser Arg Ile Ala Leu Thr Asn Ser Val Asn Glu Ala Leu
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Leu Asn Pro Ser Arg Val Tyr Thr Phe Phe Ser Ser Asp Tyr Val Lys
 580 585 590

Lys Val Asn Lys Ala Thr Glu Ala Ala Met Phe Leu Gly Trp Val Glu
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Gln Leu Val Tyr Asp Phe Thr Asp Glu Thr Ser Glu Val Ser Thr Thr
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Asp Lys Ile Ala Asp Ile Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala
 625 630 635 640

Leu Asn Ile Gly Asn Met Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu
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Ile Phe Ser Gly Ala Val Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala
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Ile Pro Val Leu Gly Thr Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys
 675 680 685

Val Leu Thr Val Gln Thr Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu
690 695 700

Lys Trp Asp Glu Val Tyr Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys
705 710 715 720

Val Asn Thr Gln Ile Asp Leu Ile Arg Lys Lys Met Lys Glu Ala Leu
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Glu Asn Gln Ala Glu Ala Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn
740 745 750

Gln Tyr Thr Glu Glu Glu Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp
755 760 765

Leu Ser Ser Lys Leu Asn Glu Ser Ile Asn Lys Ala Met Ile Asn Ile
770 775 780

Asn Lys Phe Leu Asn Gln Cys Ser Val Ser Tyr Leu Met Asn Ser Met
785 790 795 800

Ile Pro Tyr Gly Val Lys Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys
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820 825 830

Gln Val Asp Arg Leu Lys Asp Lys Val Asn Asn Thr Leu Ser Thr Asp
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Ile Pro Phe Gln Leu Ser Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser
850 855 860

Thr Phe Thr Glu Tyr Ile Lys Asn Ile Ile Asn Thr Ser Ile Leu Asn
865 870 875 880

Leu Arg Tyr Glu Ser Asn His Leu Ile Asp Leu Ser Arg Tyr Ala Ser

885

890

895

Lys Ile Asn Ile Gly Ser Lys Val Asn Phe Asp Pro Ile Asp Lys Asn
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Gln Ile Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile Glu Val Ile Leu
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Lys Asn Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn Phe Ser Thr Ser
 930 935 940

Phe Trp Ile Arg Ile Pro Lys Tyr Phe Asn Ser Ile Ser Leu Asn Asn
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Glu Tyr Thr Ile Ile Asn Cys Met Glu Asn Asn Ser Gly Trp Lys Val
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Ser Leu Asn Tyr Gly Glu Ile Ile Trp Thr Leu Gln Asp Thr Gln Glu
 980 985 990

Ile Lys Gln Arg Val Val Phe Lys Tyr Ser Gln Met Ile Asn Ile Ser
 995 1000 1005

Asp Tyr Ile Asn Arg Trp Ile Phe Val Thr Ile Thr Asn Asn Arg
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Leu Asn Asn Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile Asp Gln
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Lys Pro Ile Ser Asn Leu Gly Asn Ile His Ala Ser Asn Asn Ile
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Met Phe Lys Leu Asp Gly Cys Arg Asp Thr His Arg Tyr Ile Trp
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Ile Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys
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Asp Phe Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro Tyr Tyr Met
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1115 1120 1125

Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser Val
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Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly Asn Lys Asp Asn Ile
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Val Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val Lys Asn
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Lys Glu Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val Glu
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Lys Ile Leu Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser
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Gln Val Val Val Met Lys Ser Lys Asn Asp Gln Gly Ile Thr Asn
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Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly
1235 1240 1245

Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys Leu Val Ala
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Ser Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg Thr Leu

1265

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