c retains the binding, internalization and trans-synaptic transport capabilities of undigested TeNT, but does not disrupt any neuronal processes, and is therefore nontoxic.

A need exists for a TeNT-based therapy that avoids the pre-existing anti-TeNT immune response in tetanus toxoid immunised subjects.

It is to be understood that if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art in Australia or any other country.

SUMMARY

The inventors have appreciated that exploitation of TeNT has not been fully realised because of the adaptive immune system, which upon administration of a protein therapeutic agent, produces an antibody response, thereby decreasing efficacy of the protein therapeutic agent. The adaptive immune response may be intentional, as a result of vaccination, as demonstrated by many populations that have been immunised against TeNT. Alternatively, the adaptive immune response may be unintentional, resulting from repeated exposure to the protein therapeutic agent.

The inventors have produced a family of modified TeNTs and a treatment regimen that address these problems. Specifically, the invention provides a family of PEGylated TeNTs (PEG-TeNTs) that each evade the immune system, and a tiered treatment regimen in which a different, replacement PEG-TeNT is used for treatment when efficacy of a previously administered PEG-TeNT decreases or when a patient's immunological profile precludes the use of another PEG-TeNT.

In one aspect, the present disclosure refers to a TeNT or subdomain thereof comprising, relative to SEQ ID NO: 1 R1225E and W1288A or E270A and Y374A, wherein the TeNT or subdomain thereof is inactive.

In another aspect, the present disclosure refers to a composition, optionally a therapeutic composition, comprising:

(i) a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG); and

(ii) a second TeNT, wherein the second TeNT is the TeNT as described herein.

In another aspect, the present disclosure refers to a use **of** a second TeNT comprising, relative to SEQ ID NO: 1, R1225E and W1288A or E270A and Y374A, wherein the second TeNT is inactive, in the manufacture of a medicament for treating hypotonia in a subject to be administered a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising a tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG).

In another aspect, the present disclosure refers to a use **of** a composition comprising: (i) a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising a tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG); and (ii) a second TeNT comprising, relative to SEQ ID NO: 1, R1225E and W1288A or E270A and Y374A, wherein the second TeNT is inactive, in the manufacture of a medicament for treating hypotonia in a subject.

In another aspect, the present disclosure refers to a use of : (i) a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising a tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG); and (ii) a second TeNT comprising, relative to SEQ ID NO: 1, R1225E and W1288A or E270A and Y374A, wherein the second TeNT is inactive, in the manufacture of a medicament for for treating hypotonia in a subject, wherein the first PEG-TeNT is to be administered in combination with the second TeNT.

In yet another aspect, the present disclosure refers to a kit comprising the TeNT as described herein or the composition as described herein.

A first ~~aspect~~ example provides a tetanus neurotoxin (TeNT) or fragment thereof, comprising one or more surface serine to cysteine amino acid substitutions relative to SEQ ID NO: 1.

In one example of the first ~~aspect~~example, the substituted cysteine is conjugated to polyethylene glycol (PEG).

A second ~~aspect~~ example provides a TeNT or fragment thereof comprising, relative to SEQ ID NO: 1: R1225K; R1225E; W1288A; W1288Y; W1288F; W1288L; R1225K and W1288A; R1225E and W1288A; R1225K and W1288Y; R1225E and W1288Y; R1225K and W1288F; R1225E and W1288F;

R1225K and W1288L; R1225E and W1288L; R1225del; W1288del; or R1225del and W1288del; E270A; Y374A; E270A and Y374; G270del; Y374del; or a combination thereof, wherein the TeNT or fragment is inactive.

A third ~~aspect~~ example provides a composition comprising: (i) a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG); and (ii) a second TeNT, wherein the first PEG-TeNT is not conjugated to the second TeNT.

An example provides a method for treating hypotonia, the method comprising administering to a subject: (i) a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG); and (ii) a second TeNT.

The fourth example~~aspect~~ also provides use of a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising a tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG) in the manufacture of a medicament for treating hypotonia in a subject that is to be administered a second TeNT.

The fourth ~~aspect~~ example also provides use of a second tetanus neurotoxin (TeNT) in the manufacture of a medicament for treating hypotonia in a subject that is to be administered a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising a tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG).

The fourth ~~aspect~~ example also provides use of: (i) a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising a tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG); and (ii) a second TeNT in the manufacture of a medicament for treating hypotonia.

~~In another aspect, the present disclosure refers to a use of a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising a tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG) in the manufacture of a medicament for use in combinatorial therapy for treating hypotonia, wherein said medicament is to be administered in combination with a second TeNT.~~

The fourth example also provides a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising a tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG) for use in a method for treating hypotonia in a subject administered a second TeNT.

The fourth example also provides a second tetanus neurotoxin (TeNT) for use in a method for treating hypotonia in a subject administered a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising a tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG).

The fourth example also provides: (i) a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising a tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG); and (ii) a second TeNT for use in a method for treating hypotonia.

In examples of the fourth ~~aspect~~example, a composition may comprise the first PEG-TeNT and the second TeNT.

The fourth example also provides a composition comprising: (i) a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG); and (ii) a second TeNT for use in a method for treating hypotonia.

In one example, the first PEG-TeNT or the second TeNT comprises a PEGylated TeNT light chain (LC), a PEGylated TeNT heavy chain (HC), a PEGylated TeNT heavy chain (HC) and PEGylated TeNT light chain (LC), or a PEGylated TeNT fragment c (c). In one example, the first PEG-TeNT or the second TeNT comprises PEG-TeNT-LC-HC.

In another example, the first PEG-TeNT or the second TeNT is PEG-TeNT-HC comprising a PEGylated HC. In this example, LC is not PEGylated. In another example, the first PEG-TeNT or the second TeNT is PEG-TeNT-LC-c comprising a PEGylated LC and a PEGylated c. In this example, HN is not PEGylated.

In a further example, the first PEG-TeNT is PEG-TeNT-HC, and the second TeNT is PEG-TeNT-LC-c.

In one example, the second TeNT comprises an inactivated TeNT. Relative to SEQ ID NO: 1, the inactivated TeNT may comprise: R1225K; R1225E; W1288A; W1288Y; W1288F; W1288L; R1225K and W1288A; R1225E and W1288A; R1225K and W1288Y; R1225E and W1288Y; R1225K and W1288F; R1225E and W1288F; R1225K and W1288L; R1225E and W1288L; R1225del; W1288del; R1225del or W1288del; E270A; Y374A; E270A and Y374A; G270del; Y374del; or a combination thereof. In one example, the second TeNT comprises an inactivated TeNT comprising R1225E and W1288A.

In one example, the subject is administered: a PEG-TeNT comprising PEGylated c (PEG-TeNT-c) until efficacy decreases; then a composition comprising PEG-TeNT-HC and PEG-TeNT-LC-c until efficacy decreases; then a PEG-TeNT comprising a PEGylated LC and a PEGylated HC (PEG-TeNT-LC-HC).

In one example, treating comprises adminstering to the subject: a PEG-TeNT comprising PEGylated c (PEG-TeNT-c); then a composition comprising PEG-TeNT-HC and PEG-TeNT-LC-c; then a PEG-TeNT comprising a PEGylated LC and a PEGylated HC (PEG-TeNT-LC-HC), to determine the immunological profile of anti-TeNT antibodies of the subject and determine the effective composition of PEG-TeNTs based on that profile.

In one example, the hypotonia is obstructive sleep apnoea.

In another example, the composition is a therapeutic composition. In another example, the composition is a cosmetic composition.

Also disclosed is a method for preparing a protein consisting of TeNT LC and TeNT HN (LC-HN), the method comprising expressing in a host cell a nucleic acid molecule encoding an amino acid comprising SEQ ID NO: 6.

A fifth example ~~aspect provides a PEGylated tetanus neurotoxin (PEG-TeNT), comprising tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG), wherein PEG is conjugated to a cysteine residue, wherein the cysteine residue is a surface serine to cysteine substitution relative to SEQ ID NO: 1. In one example, the disclosure~~ provides a PEGylated tetanus neurotoxin (PEG-TeNT), comprising tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG).

In one example, the TeNT light chain (LC) is PEGylated, the TeNT heavy chain (HC) is PEGylated, or the TeNT fragment c (c) is PEGylated. In one example, LC is PEGylated and HC is PEGylated (PEG-TeNT-LC-HC), or LC is PEGylated and c is PEGylated (PEG-TeNT-LC-c).

In another example, PEG is conjugated to a lysine residue of TeNT. In another example, PEG is conjugated to a cysteine residue of TeNT. In one example, PEG is conjugated to a cysteine residue of TeNT, wherein the cysteine residue is either native or a substitution for a serine residue relative to SEQ ID NO: 1.

In one example, the PEG has a molecular weight of about 2 kDa, about 5 kDa, about 10 kDa, or about 20 kDa, or about 30 kDa.

A sixth example provides a method for treating hypotonia, the method comprising administering to a subject the PEG-TeNT of the fifth ~~aspect~~example, wherein the PEG-TeNT is not conjugated to a second TeNT.

The sixth ~~aspect~~ example also provides use of the PEG-TeNT of the fifth ~~aspect~~ example in the manufacture of a medicament for treating hypotonia, wherein the PEG-TeNT is not conjugated to a second TeNT.

Alternatively, the sixth example provides the PEG-TeNT of the fifth ~~aspect~~ example for use in a method for treating hypotonia, the method comprising administering to a subject the PEG-TeNT, wherein the PEG-TeNT is not conjugated to a second TeNT.

In one example, the subject is administered a first PEG-TeNT comprising a PEGylated HC (PEG-TeNT-HC) and a second PEG-TeNT comprising a PEGylated LC and a PEGylated c (PEG-TeNT-LC-c).

In another example, the subject is administered: a PEG-TeNT comprising a PEGylated c (PEG-TeNT-c); and/or a first PEG-TeNT comprising a PEGylated HC (PEG-TeNT-HC) and a second PEG-TeNT comprising a PEGylated LC-c (PEG-TeNT-LC-c); and/or a PEG-TeNT comprising a PEGylated HC and a PEGylated LC (PEG-TeNT-LC-HC).

In one example, treating comprises administering to the subject: a PEG-TeNT comprising PEGylated c (PEG-TeNT-c) until efficacy decreases. Thereafter, treating may comprise administering to the subject: a PEG-TeNT-HC and a PEG-TeNT-LC-c until efficacy decreases. Thereafter, treating may comprise administering to the subject: a PEG-TeNT comprising a PEGylated LC and a PEGylated HC (PEG-TeNT-LC-HC).

In one example, treating comprises administering to the subject: a PEG-TeNT comprising PEGylated c (PEG-TeNT-c) until efficacy decreases; then a PEG-TeNT-HC and a PEG-TeNT-LC-c until efficacy decreases; then a PEG-TeNT comprising a PEGylated LC and a PEGylated HC (PEG-TeNT-LC-HC).

In one example, treating comprises administering to the subject: a PEG-TeNT comprising PEGylated c (PEG-TeNT-c) comprising PEG having a molecular weight of about 5 kDa until efficacy decreases; then a PEG-TeNT comprising PEGylated c (PEG-TeNT-c) comprising PEG having a molecular weight of about 10 kDa until efficacy decreases; then a PEG-TeNT comprising PEGylated c (PEG-TeNT-c) comprising PEG having a molecular weight of about 20 kDa until efficacy decreases. Thereafter, treating may comprise administering to the subject: a PEG-TeNT-HC and a PEG-TeNT-LC-c, either or both of which comprise PEG having a molecular weight of 5 kDa, until efficacy decreases; then a PEG-TeNT-HC and a PEG-TeNT-LC-c, either or both of which comprise PEG having a molecular weight of 10 kDa, until efficacy decreases; then a PEG-TeNT-HC and a PEG-

TeNT-LC-c, either or both of which comprise PEG having a molecular weight of 20 kDa, until efficacy decreases. Thereafter, treating may comprise administering to the subject: a PEG-TeNT comprising a PEGylated LC and a PEGylated HC (PEG-TeNT-LC-HC), either or both of which comprise PEG having a molecular weight of 5 kDa, until efficacy decreases; then a PEG-TeNT comprising a PEGylated LC and a PEGylated HC (PEG-TeNT-LC-HC), either or both of which comprise PEG having a molecular weight of 10 kDa, until efficacy decreases; then a PEG-TeNT comprising a PEGylated LC and a PEGylated HC (PEG-TeNT-LC-HC), either or both of which comprise PEG having a molecular weight of 20 kDa.

In one example, treating comprises adminstering to the subject: a PEG-TeNT comprising PEGylated c (PEG-TeNT-c); then a composition comprising PEG-TeNT-HC and PEG-TeNT-LC-c; then a PEG-TeNT comprising a PEGylated LC and a PEGylated HC (PEG-TeNT-LC-HC), to determine the immunological profile of anti-TeNT antibodies of the subject and determine the effective composition of PEG-TeNTs based on that profile.

In another example, treating with a TeNT further comprises administering an inactivated TeNT. Relative to SEQ ID NO: 1, the inactivated TeNT may comprise: R1225K; R1225E; W1228A; W1288Y; W1288F; W1288L; R1225K and W1288A; R1225E and W1288A; R1225K and W1288Y; R1225E and W1288Y; R1225K and W1288F; R1225E and W1288F; R1225K and W1288L; R1225E and W1288L; R1225del; W1288del; R1225del or W1288del. In one example, the second TeNT comprises an inactivated TeNT comprising R1225E and W1288A; E270A; Y374A; E270A and Y374A; G270del; Y374del; or a combination thereof.

In one example, the hypotonia is obstructive sleep apnoea.

A seventh ~~aspect~~ example provides a kit comprising the TeNT of the first ~~aspect~~example, the composition of the second ~~aspect~~ example or the PEG-TeNT of the fifth ~~aspect~~example.

In one example, the composition or PEG-TeNT is used according to the method of the third or sixth ~~aspect~~example, respectively.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a schematic representation of example PEG-TeNTs of the invention: A PEG-TeNT-c; B PEG-TeNT-HC, C PEG-TeNT-LC-c,

CLAIMS

~~1. A tetanus neurotoxin (TeNT) or fragment thereof comprising one or more surface serine to cysteine amino acid substitutions relative to SEQ ID NO: 1.~~

~~2. The TeNT or fragment thereof of claim 1, wherein the substituted serine comprises one or more of S81C, S120C, S144C, S248C, S335C, S428C, S600C, S963C, S1041C, S1155C, and S1187C relative to SEQ ID NO: 1.~~

~~3. The TeNT or fragment thereof of claim 1 or claim 2, wherein the substituted cysteine is conjugated to polyethylene glycol (PEG).~~

~~4. The TeNT or fragment thereof of claim 3, wherein the PEG is about 2 kDa, about 5 kDa, about 10 kDa, or about 20 kDa.~~

~~5. The TeNT or fragment thereof of claim 3 or claim 4, wherein the PEG is linear or branched.~~

1~~6~~. A TeNT or subdomain~~fragment~~ thereof comprising, relative to SEQ ID NO: 1~~: R1225K; R1225E; W1288A; W1288Y; W1288F; W1288L; R1225K and W1288A;~~ R1225E and W1288A~~; R1225K and W1288Y; R1225E and W1288Y; R1225K and W1288F; R1225E and W1288F; R1225K and W1288L; R1225E and W1288L; R1225del; W1288del; R1225del and W1288del;~~ or E270A and Y374A~~; E270del; or a combination thereof~~, wherein the TeNT or subdomain thereof~~fragment~~ is inactive.

2. The TeNT of claim 1, further comprising, relative to SEQ ID NO: 1, E270A or E270del.

3~~7~~. A composition, optionally a therapeutic composition, comprising:

(i) a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG); and

(ii) a second TeNT, wherein the second TeNT is the TeNT of claim 1 or claim 2.

4. The composition of claim 3, wherein the first PEG-TeNT comprises one or more surface serine to cysteine amino acid substitutions relative to SEQ ID NO: 1, and wherein the substituted cysteine is conjugated to polyethylene glycol (PEG).

5. The composition of claim 4, wherein the one or more substituted serine in the first PEG-TeNT comprises one or more of S81C, S120C, S144C, S248C, S335C, S428C, S600C, S963C, S1041C, S1155C, and S1187C relative to SEQ ID NO: 1.

6. The composition of claim 4 or claim 5, wherein the PEG is about 2 kDa, about 5 kDa, about 10 kDa, or about 20 kDa, optionally wherein the PEG is linear or branched.

~~8. The composition of claim 7, wherein the first PEG-TeNT comprises the TeNT of any one of claims 3 to 5.~~

~~9. The composition of claim 7 or claim 8, wherein the second TeNT is the TeNT of any one of claims 1 to 6.~~

7. The composition of any one of claims 3 to 6, wherein the first PEG-TeNT comprises a PEGylated TeNT light chain (LC), a PEGylated TeNT heavy chain (HC), or a PEGylated TeNT fragment c (c).

8. The composition of any one of claims 3 to 7, wherein the first PEG-TeNT is PEG-TeNT-HC comprising a PEGylated HC, or is PEG-TeNT-LC-c comprising a PEGylated LC and a PEGylated c.

9. The composition of any one of claims 3 to 8, wherein the first PEG-TeNT is PEG-TeNT-HC, and further comprises PEG-TeNT-LC-c.

10. Use of a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising a tetanus neurotoxin (TeNT) conjugated to polyethylene

glycol (PEG) in the manufacture of a medicament for treating hypotonia in a subject ~~that is~~ to be administered a second TeNT comprising, relative to SEQ ID NO: 1, R1225E and W1288A or E270A and Y374A, wherein the second TeNT is inactive.

11. Use of a second TeNT comprising, relative to SEQ ID NO: 1, R1225E and W1288A or E270A and Y374A, wherein the second TeNT is inactive, in the manufacture of a medicament for treating hypotonia in a subject ~~that is~~ to be administered a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising a tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG).

12. Use of a composition comprising: (i) a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising a tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG); and (ii) a second TeNT comprising, relative to SEQ ID NO: 1, R1225E and W1288A or E270A and Y374A, wherein the second TeNT is inactive, in the manufacture of a medicament for treating hypotonia in a subject.

13. Use of: (i) a first PEGylated tetanus neurotoxin (PEG-TeNT) comprising a tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG); and (ii) a second TeNT comprising, relative to SEQ ID NO: 1, R1225E and W1288A or E270A and Y374A, wherein the second TeNT is inactive, in the manufacture of a medicament for ~~use in combinatorial therapy~~ for treating hypotonia in a subject, wherein ~~said~~ the ~~medicament~~ first PEG-TeNT is to be administered in combination with the~~a~~ second TeNT.

~~14. The composition of any one of claims 7 to 9, or use of any one of claims 10 to 13, wherein the first PEG-TeNT or the second TeNT comprises a PEGylated TeNT light chain (LC), a PEGylated TeNT heavy chain (HC), or a PEGylated TeNT fragment c (c).~~

~~15. The composition or use of any one of claims 7 to 14, wherein the first PEG-TeNT or the second TeNT is PEG-TeNT-HC comprising a~~

~~PEGylated HC, or is PEG-TeNT-LC-c comprising a PEGylated LC and a PEGylated c.~~

~~16. The composition or use of any one of claims 7 to 15, wherein the first PEG-TeNT is PEG-TeNT-HC, and the second TeNT isPEG-TeNT-LC-c.~~

~~17. The composition or use of any one of claims 7 to 16, wherein the second TeNT comprises an inactivated TeNT.~~

~~18. The composition or use of claim 17, wherein, relative to SEQ ID NO: 1, the inactivated TeNT comprises: R1225K; R1225E; W1228A; W1288Y; W1288F; W1288L; R1225K and W1288A; R1225E and W1288A; R1225K and W1288Y; R1225E and W1288Y; R1225K and W1288F; R1225E and W1288F; R1225K and W1288L; R1225E and W1288L; R1225del; W1288del; R1225del and W1288del; E270A; Y374A; E270A and Y374A; E270del; Y374del; or a combination thereof.~~

~~19. The use of any one of claims 9 to 18, wherein the subject is to be administered:~~

~~a PEG-TeNT comprising PEGylated c (PEG-TeNT-c) until efficacy decreases; then~~

~~a composition comprising PEG-TeNT-HC and PEG-TeNT-LC-c until efficacy decreases; then~~

~~a PEG-TeNT comprising a PEGylated LC and a PEGylated HC (PEG-TeNT-LC-HC).~~

~~20. The use of any one of claims 10 to 19, wherein the hypotonia is obstructive sleep apnoea.~~

~~21. The composition of any one of claims 7 or 14 to 18, wherein the composition is a therapeutic composition.~~

~~22. A PEGylated tetanus neurotoxin (PEG-TeNT), comprising tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG), wherein PEG is conjugated to a cysteine residue, wherein the cysteine~~

~~residue is a surface serine to cysteine substitution relative to SEQ ID NO: 1.~~

~~23. The PEG-TeNT of claim 22, wherein the TeNT light chain (LC) is PEGylated, the TeNT heavy chain (HC) is PEGylated, or the TeNT fragment c (c) is PEGylated.~~

~~24. The PEG-TeNT of claim 22 or claim 23, wherein LC is PEGylated and HC is PEGylated (PEG-TeNT-LC-HC), or LC is PEGylated and c is PEGylated (PEG-TeNT-LC-c).~~

~~25. The PEG-TeNT of any one of claims 22 to 24, wherein the PEG has a molecular weight of about 5 kDa, about 10 kDa or about 20 kDa.~~

~~26. Use of a PEGylated tetanus neurotoxin (PEG-TeNT), comprising tetanus neurotoxin (TeNT) conjugated to polyethylene glycol (PEG), optionally the PEG-TeNT of any one of claims 22 to 25, in the manufacture of a medicament for treating hypotonia.~~

~~27. The use of claim 26, wherein the subject is to be administered a first PEG-TeNT comprising a PEGylated HC (PEG-TeNT-HC) and a second PEG-TeNT comprising a PEGylated LC and a PEGylated c (PEG-TeNT-LC-c).~~

14~~28~~. The use of any one of claims 10 to 13~~claim 26 or claim 27~~, wherein the subject is to be administered:

a PEG-TeNT comprising a PEGylated c (PEG-TeNT-c); and/or

a first PEG-TeNT comprising a PEGylated HC (PEG-TeNT-HC) and a second PEG-TeNT comprising a PEGylated LC-c (PEG-TeNT-LC-c); and/or

a PEG-TeNT comprising a PEGylated HC and a PEGylated LC (PEG-TeNT-LC-HC).

15~~29~~. The use ~~of any one~~ of claim~~s~~ 14~~26 to 28~~, wherein the subject is to be administered:

a PEG-TeNT comprising PEGylated c (PEG-TeNT-c) until efficacy decreases; then

a PEG-TeNT-HC and a PEG-TeNT-LC-c until efficacy decreases; then

a PEG-TeNT comprising a PEGylated LC and a PEGylated HC (PEG-TeNT-LC-HC).

~~30. The use of any one of claims 26 to 29, wherein the subject is to be further administered an inactivated TeNT.~~

16~~31~~. The use of claim 14~~30~~ or claim 15, wherein the inactive TeNT, relative to SEQ ID NO: 1, ~~the inactivated TeNT~~further comprises~~:~~ ~~R1225K; R1225E; W1288A; W1288Y; W1288F; W1288L; R1225K and W1288A; R1225E and W1288A; R1225K and W1288Y; R1225E and W1288Y; R1225K and W1288F; R1225E and W1288F; R1225K and W1288L; R1225E and W1288L; R1225del; W1288del; R1225del and W1288del; E270A; Y374A;~~ E270A and Y374A~~; E270del; Y374del; or a combination thereof~~.

17~~32~~. The use of any one of claims 10~~26~~ to 16~~31~~, wherein the hypotonia is obstructive sleep apnoea.

18~~33~~. A kit comprising the TeNT ~~of any one~~ of claim~~s~~ 1 or claim 2~~to 6~~, or the composition of any one of claims 3 to 9~~7, 14 to 18 or 21, or the PEG-TeNT of any one of claims 22 to 25~~.