

Claims

1. A modified botulinum neurotoxin A (BoNT/A) L-chain protease that cleaves human SNAP-23 (hSNAP-23), and having a modified amino acid sequence relative to the wild-type BoNT/A L-chain (SEQ ID NO: 1), that comprises:
- a) at least one amino acid residue change located within a first BoNT/A L-chain protease binding pocket for binding to the P182 /D178 binding site of hSNAP-23;
 - b) wherein said first BoNT/A L-chain protease binding pocket is defined by amino acid residues E148, T307, A308 and Y312 of wild-type BoNT/A L-chain (SEQ ID NO: 1);
 - c) and wherein said at least one amino acid residue change comprises:
 - i. an amino acid residue selected from the group consisting of asparagine and tyrosine at the position on the modified L-chain protease amino acid sequence that corresponds to amino acid residue E148 of wild-type BoNT/A L-chain (SEQ ID NO: 1); and/ or
 - ii. an amino acid residue selected from the group consisting of phenylalanine, isoleucine and leucine at the position on the modified L-chain protease amino acid sequence that corresponds to amino acid residue T307 of wild-type BoNT/A L-chain (SEQ ID NO: 1); and/ or
 - iii. an amino acid residue selected from the group consisting of proline, asparagine, threonine and isoleucine at the position on the modified L-chain protease amino acid sequence that corresponds to amino acid residue A308 of wild-type BoNT/A L-chain (SEQ ID NO: 1); and/ or
 - iv. an amino acid residue selected from the group consisting of lysine, valine, methionine and leucine at the position on the modified L-chain protease amino acid sequence that corresponds to amino acid residue Y312 of wild-type BoNT/A L-chain (SEQ ID NO: 1).

2. The modified BoNT/ A L-chain protease according to claim 1, further comprising:
- 5 a) an amino acid residue change located within a second BoNT/A L-chain protease binding pocket for binding to the D189/D192 binding site of hSNAP-23;
 - b) wherein said second BoNT/A L-chain protease binding pocket is defined by amino acid residue Q29 of wild-type BoNT/A L-chain (SEQ ID NO: 1);
 - 10 c) and wherein said amino acid residue change comprises:
 - i. an amino acid residue selected from the group consisting of alanine at the position on the modified L-chain protease amino acid sequence that corresponds to amino acid residue Q29 of wild-type BoNT/A L-chain (SEQ ID NO: 1).
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3. The modified BoNT/A L-chain protease according to claim 1 or claim 2, further comprising:
- 20 a) an amino acid residue change located within a third BoNT/A L-chain protease binding pocket for binding to the I198 binding site of hSNAP-23;
 - b) wherein said third BoNT/A L-chain protease binding pocket is defined by amino acid residue K166 of wild-type BoNT/A L-chain (SEQ ID NO: 1);
 - 25 c) and wherein said amino acid residue change comprises:
 - i. an amino acid residue selected from the group consisting of valine at the position on the modified L-chain protease amino acid sequence that corresponds to amino acid residue K166 of wild-type BoNT/A L-chain (SEQ ID NO: 1).
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4. The modified BoNT/A L-chain protease according to any one of the preceding claims, further comprising:

- 5 a) at least one amino acid residue change located within a fourth BoNT/A L-chain protease binding pocket for binding to the K185 binding site of hSNAP-23;
- b) wherein said fourth BoNT/A L-chain protease binding pocket is defined by amino acid residue G305 of wild-type BoNT/A L-chain (SEQ ID NO: 1);
- c) and wherein said at least one amino acid residue change comprises:
- 10 i. an amino acid residue selected from the group consisting of glutamate and aspartate at the position on the modified L-chain protease amino acid sequence that corresponds to amino acid residue G305 of wild-type BoNT/A L-chain (SEQ ID NO: 1).
- 15 5. The modified BoNT/A L-chain protease according to any one of the preceding claims, further comprising:
- a) an amino acid residue change located within a fifth BoNT/A L-chain protease binding pocket for binding to the R186 binding site of hSNAP-23;
- 20 b) wherein said fifth BoNT/A L-chain protease binding pocket is defined by amino acid residue S143 of wild-type BoNT/A L-chain (SEQ ID NO: 1);
- c) and wherein said amino acid residue change comprises:
- 25 i. an amino acid residue selected from the group consisting of glutamine, glutamate and aspartate at the position on the modified L-chain protease amino acid sequence that corresponds to amino acid residue S143 of wild-type BoNT/A L-chain (SEQ ID NO: 1).
- 30 6. The modified BoNT/ A L-chain protease according to any one of the preceding claims, further comprising:
- a) at least one amino acid residue change located within a sixth BoNT/A L-chain protease binding pocket for binding to the K206 binding site of hSNAP-23;

- b) wherein said sixth BoNT/A L-chain protease binding pocket is defined by amino acid residue Y251 of wild-type BoNT/A L-chain (SEQ ID NO: 1);
- c) and wherein said at least one amino acid residue change comprises:
- 5 i. an amino acid residue selected from the group consisting of glutamate and aspartate at the position on the modified L-chain protease amino acid sequence that corresponds to amino acid residue Y251 of wild-type BoNT/A L-chain
- 10 (SEQ ID NO: 1).
7. A nucleic acid construct comprising or consisting of a nucleic acid sequence encoding the modified BoNT/A L-chain protease as defined in any preceding Claim.
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8. A delivery vehicle, comprising:
- a) the modified BoNT/A L chain protease as defined in any Claims 1 to 6, or the nucleic acid construct of Claim 7; and
- b) means for delivering said modified BoNT/A L chain protease, or
- 20 said nucleic acid construct, into a target cell, preferably into a non-neuronal target cell.
9. The delivery vehicle according to Claim 8, wherein the means b) for delivering said modified BoNT/A L chain protease to a target cell comprises:
- 25 i) a targeting moiety that binds the delivery vehicle to the target cell; and
- ii) a translocation peptide that translocates the modified BoNT/A L-chain protease or the nucleic acid construct into the target cell,
- 30 preferably into a non-neuronal target cell.
10. A method of cleaving hSNAP-23, comprising contacting hSNAP-23 with a (BoNT/A) L-chain protease according to any of Claims 1 to 6, or with

a nucleic acid construct according to Claim 7, or with a delivery vehicle according to Claim 8 or 9.

5 11.A (BoNT/A) L-chain protease according to any of Claims 1 to 6, or a nucleic acid construct according to Claim 7, or a delivery vehicle according to Claim 8 or 9, for use in a method according to Claim 10.

10 12.A (BoNT/A) L-chain protease according to any of Claims 1 to 6, or a nucleic acid construct according to Claim 7, or a delivery vehicle according to Claim 8 or 9 for use as a medicament.