

molecular weight molecules such as full-length proteins. The active transport of high molecular weight molecules through such barrier often requires specific carriers able to cross the lipid bilayer. Cell penetrating peptides (CPPs) are generally 5–30 amino acids long peptides (or motifs within a peptide) which, for their ability to cross cell membranes, are

5 widely used to deliver proteins, plasmid DNA, RNA, oligonucleotides, liposomes and anti-cancer drugs inside the cells (Borrelli et al., 2018; Bode & Löwik, 2017; Kalafatovic & Giralt, 2017; Kristensen et al., 2016).

SUMMARY

10 In the present disclosure, it has been discovered that a C-terminal fragment of the CDFN protein surprisingly protects ER stressed sympathetic and dopaminergic neurons *in vitro* and *in vivo*, and in contrast to full-length CDFN the fragment is capable of penetrating neuronal cell membrane as well as the blood-brain-barrier *in vivo*.

15 Accordingly, it is an aim of the present disclosure to provide a C-terminal CDFN fragment consisting of at least 50 consecutive amino acid residues of the sequence as set forth in SEQ ID NO:1:

MPAMKICEKL KKLDSQICEL KYEKTLDLAS VDLRKMRVAE LKQILHSWGE
ECRACAEKTD YVNLIQELAP KYAATHPKTE L

20 or a sequence which preferably has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:1.

In one aspect of the present disclosure, there is provided a use of a C-terminal CDFN fragment consisting of at least 50 consecutive amino acid residues of the sequence as set forth in SEQ ID NO:1:

25 MPAMKICEKL KKLDSQICEL KYEKTLDLAS VDLRKMRVAE LKQILHSWGE
ECRACAEKTD YVNLIQELAP KYAATHPKTE L

or a sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:1, wherein said fragment is a cell membrane penetrating peptide and has a protective effect on neuronal cells, in the manufacture of a medicament for the treatment of a

30 degenerative disease or disorder.

In another aspect, the present disclosure refers to a C-terminal CDNF fragment consisting of at least 50 consecutive amino acid residues of the sequence as set forth in SEQ ID NO:1:

MPAMKICEKL KKLDSEQICEL KYEKTLDLAS VDLRKMRVAE LKQILHSWGE
ECRACAEKTD YVNLIQELAP KYAATHPKTE L

- 5 or a sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:1, wherein said fragment is a cell membrane penetrating peptide and has a protective effect on neuronal cells, for use as a medicine.

- In another aspect, the present disclosure refers a pharmaceutical composition comprising the C-terminal CDNF fragment disclosed herein and at least one of the following: physiologically
10 acceptable carrier, buffer, excipient, preservative and stabilizer.

In another aspect of the present disclosure, there is provided a use of the pharmaceutical composition disclosed herein in the manufacture of a medicament for the treatment of a degenerative disease or disorder.

- In a further aspect, the present disclosure refers the pharmaceutical composition disclosed
15 herein for use as a medicine.

In another aspect, the present disclosure refers a use of C-terminal CDNF fragment comprising or consisting of at least 50 consecutive amino acid residues of the sequence as set forth in SEQ ID NO:1:

MPAMKICEKL KKLDSQICEL KYEKTLDLAS VDLRKMRVAE LKQILHSWGE

5 ECRACAEKTD YVNLIQELAP KYAATHPKTE L

or a sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:1, in the manufacture of a medicament for treating a degenerative disease or disorder.

The present disclosure also provides a pharmaceutical composition comprising a C-terminal
10 CDNF fragment and at least one of the following: physiologically acceptable carrier, buffer, excipient, preservative and stabilizer.

The results of the present disclosure further provides said C-terminal CDNF fragment for use in the treatment of a degenerative disease or disorder including a central nervous system (CNS) disease, diabetes or a retinal disease, wherein said CNS disease is preferably selected
15 from the group consisting of Alzheimer's disease, Parkinson's disease, Huntington's disease and other amyloid diseases, multiple system atrophy, amyotrophic lateral sclerosis, frontotemporal lobar degeneration, dementia with Lewy bodies, mild cognitive impairment, traumatic brain injury, peripheral nerve injuries, addiction and stroke.

The present disclosure also shows that in contrast to the mature MANF protein the C-terminal fragment of MANF (C-MANF) is able to penetrate the cell membrane of dopamine neurons and protects the neurons in culture.

Another aim of the present disclosure is thus to provide a C-terminal MANF fragment,

5 consisting of at least 50 consecutive amino acid residues of the sequence as set forth in SEQ ID NO:2:

ICEKLKKKDS QICELKYDKQ IDLSTVDLKK LRVKELKKIL DDWGETCKGC
AEKSDYIRKI NELMPKYAPK AASARTDL

10 or a sequence which preferably has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:2 for use in the treatment of a degenerative disease or disorder including central nervous system (CNS) diseases, wherein said fragment is administered by intravenous or peripheral administration, intraperitoneal, subcutaneous, intranasal, transdermal, intramuscular, intraocular, or intra-arterial administration.

15 Also a pharmaceutical composition comprising said C-terminal MANF fragment, and at least one of the following: physiologically acceptable carrier, buffer, excipient and stabilizer, is provided for use in the treatment of a degenerative disease or disorder including central nervous system (CNS) diseases, wherein said fragment is administered by intravenous or peripheral administration, intraperitoneal, subcutaneous, intranasal, transdermal, intramuscular, intraocular, or intra-arterial administration.

20 Further aim of the present disclosure is to provide a C-terminal MANF fragment consisting of at least 50 consecutive amino acid residues of the sequence as set forth in SEQ ID NO:2:

ICEKLKKKDS QICELKYDKQ IDLSTVDLKK LRVKELKKIL DDWGETCKGC
AEKSDYIRKI NELMPKYAPK AASARTDL

25 or a sequence which preferably has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:2 for use in the treatment of type 1 or type 2 diabetes or a retinal disease.

The present disclosure also provides a pharmaceutical composition comprising the C-terminal MANF fragment and at least one of the following: physiologically acceptable carrier, buffer, excipient, preservative and stabilizer for use in the treatment of type 1 or type 2 diabetes or a
30 retinal disease.

The aforementioned and other advantages and benefits of the present disclosure are achieved in the manner described as characteristics in the accompanying claims.

BRIEF DESCRIPTION OF THE DRAWINGS

5 **Figure 1.** (A) CDNF has two domains: The N-terminal domain and the C-terminal domain. N-terminal domain can bind oxidized phospholipids (and at least MANF N-terminal domain also lipid sulfatide, also known as 3-O-sulfogalactosylceramide, see Bai et al., 2018) and is a saposin-like domain. The C-terminal domain has the C-X-X-C (i.e. C-R-A-C) sequence and C-terminal ER retention signal KTEL and is a SAPLIP-like domain. CDNF can be
10 proteolytically cleaved *in vitro* yielding these two domains. (B) Schematic view on the structures of MANF and CDNF. Black vertical bars show the location of 8 conserved cysteine residues.

Figure 2. CDNF and C-terminal fragment of CDNF expressed from plasmids rescue ER stressed superior cervical ganglia (SCG) sympathetic neurons. In the experiment, SCG
15 neurons from 7 days old rats/mice were microinjected with indicated plasmids expressing CDNF, the C-terminal fragment of CDNF (C-CDNF), the control plasmid PCR3.1, and positive control when nerve growth factor (NGF at 10 ng/ml) was added to the culture medium. Next day tunicamycin (TM) at 2 μ M was added to trigger ER stress-induced cell death, then after three days living and fluorescent neurons were counted and results are
20 disclosed as percentage of initial neurons.

Figure 3. CDNF and CDNF fragment proteins rescue ER stressed SCG neurons when microinjected into the cytoplasm. In the experiment, SCG neurons were prepared from postnatal day 1 old mice, cultured for 7 days, and then injected with the recombinant human CDNF or C-CDNF protein, respectively. Next day tunicamycin (2 μ M) was added, and after 3
25 days the living fluorescent neurons were counted. The results are disclosed as a percentage of initial neurons.

Figure 4. C-terminal fragment of MANF (C-MANF) protects dopaminergic neurons in culture. Dissociated cultures of embryonic day 13 (E13) NMRI mouse midbrain floors were grown on a 96-well plate for 5 days with C-MANF, GDNF added to the culture medium
30 (positive control) or without growth factors as control. The cultures were thereafter stained for tyrosine hydroxylase (TH). Images were scanned by CellInsight™ and immunopositive

CLAIMS

1. Use of a C-terminal CDFN fragment consisting of at least 50 consecutive amino acid residues of the sequence as set forth in SEQ ID NO:1:

MPAMKICEKL KKLDSQICEL KYEKTLDLAS VDLRKMRVAE LKQILHSWGE
ECRACAEKTD YVNLIQELAP KYAATHPKTE L

or a sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:1, wherein said fragment is a cell membrane penetrating peptide and has a protective effect on neuronal cells, in the manufacture of a medicament for the treatment of a degenerative disease or disorder.

2. A C-terminal CDFN fragment consisting of at least 50 consecutive amino acid residues of the sequence as set forth in SEQ ID NO:1:

MPAMKICEKL KKLDSQICEL KYEKTLDLAS VDLRKMRVAE LKQILHSWGE
ECRACAEKTD YVNLIQELAP KYAATHPKTE L

or a sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:1, wherein said fragment is a cell membrane penetrating peptide and has a protective effect on neuronal cells, for use as a medicine.

3. The fragment for use according to claim 2 in the treatment of a degenerative disease or disorder.

4. The use or fragment for use according to claim 1 or 3, wherein said degenerative disease is a neurodegenerative disease.

5. The use or fragment for use according to claim 4, wherein said neurodegenerative disease is a central nervous system (CNS) disease selected from the group consisting of: Alzheimer's disease, Parkinson's disease, multiple system atrophy, amyotrophic lateral sclerosis, frontotemporal lobar degeneration, dementia with Lewy bodies, mild cognitive impairment, Huntington's disease, traumatic brain injury, drug addiction and stroke.

6. The use or fragment for use according to claim 5, wherein said CNS disease is Parkinson's disease.

7. The use or fragment for use according to any one of claims 1 to 3 in the treatment of type I or type II diabetes.
8. The use or fragment for use according to claim 4 in the treatment of amyotrophic lateral sclerosis.
9. The use or fragment for use according to any one of claims 1 to 3 in the treatment of retinal disorders such as retinitis pigmentosa.
10. The use or fragment for use according to any of claims 1-9, wherein said fragment is to be administered by intravenous administration, intraperitoneal, subcutaneous, intrathecal, intracerebroventricular, intranasal, transdermal, intracerebral, intramuscular, intraocular, or intraarterial administration or said fragment is to be administered via a viral expression vector.
11. The use or fragment for use according to claim 10, wherein said intravenous administration is peripheral administration.
12. The use or fragment for use according to any of the preceding claims, wherein said sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:1 consists of at least 50 consecutive amino acid residues of the sequence of SEQ ID NO:3:
MPAMKICEKL KKLDSEQICEL KYEKTLDLAS VDLRKMRVAE LKQILHSWGE
ECXXCAEKTD YVNLIQELAP KYAATHPKTE L
wherein X is any amino acid.
13. The use or fragment for use according to claim 12, wherein said sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:1 comprises sequence CKGC in positions 52-55 of SEQ ID NO:1.
14. The use or fragment for use according to any one of claims 1-11, wherein said fragment has the sequence of SEQ ID NO:4:
KYEKTLDLAS VDLRKMRVAE LKQILHSWGE ECRACAEKTD YVNLIQELAP
KYAATHPKTE L

or a sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:4.

15. The use or fragment for use according to any of claims 12-14, wherein said fragment comprises the C-terminal amino acid L in position 81 of SEQ ID NO:1.

16. The use or fragment for use according to any of claims 1-14, wherein said fragment lacks the ER retention signal KTEL corresponding to positions 78-81 of SEQ ID NO:1.

17. The use or fragment for use according to any of the preceding claims, wherein the fragment further comprises modifications protecting the fragment from enzymatic degradation preferably selected from the group consisting of amidation of the C-terminus and acetylation of the N-terminus.

18. The use or fragment for use according to any of the preceding claims further conjugated to a detectable chemical or biochemical moiety.

19. The use or fragment for use according to any of the preceding claims, wherein said fragment is a cell-penetrating peptide and is capable of penetrating human blood-brain barrier.

20. A pharmaceutical composition comprising the C-terminal CDNF fragment as defined in any of claims 1-19 and at least one of the following: physiologically acceptable carrier, buffer, excipient, preservative and stabilizer.

21. The pharmaceutical composition according to claim 20 for intravenous administration preferably peripheral administration, intraperitoneal, subcutaneous, intrathecal, intracerebroventricular, intranasal, transdermal, intracerebral, intramuscular, intraocular, or intraarterial administration.

22. The pharmaceutical composition according to claim 20 or 21, wherein the C-terminal CDNF fragment comprises modifications protecting the fragment from enzymatic degradation preferably selected from the group consisting of amidation of the C-terminus and acetylation of the N-terminus.
23. The pharmaceutical composition according to claim 20 or 21, wherein the C-terminal CDNF fragment is conjugated to a detectable chemical or biochemical moiety.
24. Use of the pharmaceutical composition according to any one of claims 20-23 in the manufacture of a medicament for the treatment of a degenerative disease or disorder.
25. The pharmaceutical composition according to any one of claims 20-23 for use as a medicine.
26. The pharmaceutical composition for use according to claim 25 in the treatment of a degenerative disease or disorder.
27. The use or pharmaceutical composition for use according to claim 24 or 26, wherein said degenerative disease or disorder is a central nervous system (CNS) disease selected from the group consisting of: Alzheimer's disease, Parkinson's disease, multiple system atrophy, amyotrophic lateral sclerosis, frontotemporal lobar degeneration, dementia with Lewy bodies, mild cognitive impairment, Huntington's disease, traumatic brain injury, drug addiction and stroke.
28. The use or pharmaceutical composition for use according to any one of claims 24 to 26 in the treatment of type I or type II diabetes.
29. The use or pharmaceutical composition for use according to any one of claims 24 to 26 in the treatment of retinal disorders such as retinitis pigmentosa.

30. Use of C-terminal CDFN fragment comprising or consisting of at least 50 consecutive amino acid residues of the sequence as set forth in SEQ ID NO:1:

MPAMKICEKL KKLD SQICEL KYEKTLDLAS VDLRKMRVAE LKQILHSWGE
ECRACAEKTD YVNLIQELAP KYAATHPKTE L

or a sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:1, in the manufacture of a medicament for treating a degenerative disease or disorder.