VARIANTS OF HUMAN GDNF

The present invention is in the field of medicine, particularly in the field of therapeutic proteins. Specifically, the present invention relates to novel variants of human glial cell-derived neurotrophic factor (GDNF). The novel variants of GDNF may be useful for the treatment of Parkinson's disease.

GDNF is a well known neurotrophic factor that is reported to provide trophic support to dopaminergic neurons *in vitro* and *in vivo*. Further, it has been reported that GDNF provides functional improvements and has neuroprotective actions in rodent and primate models of Parkinson's disease. Wild type GDNF protein from *E. coli* has been administered centrally to patients suffering from Parkinson's disease with mixed results. In two small open labeled studies, the wild type GDNF was reported to produce long-lasting improvement in motor function. However, in a randomized, placebo-controlled, Phase IIa trial of 34 patients, intra-putamenal delivery of GDNF showed no symptomatic improvement at 6 months. An increase in biomarker signal was only evident in the immediate tissue surrounding the infusion site. One recent report states that GDNF may be a promising molecule to rescue dying nerves; however, delivering the molecule to the correct area of the brain remains a daunting challenge. ***Nature***, Vol 466:19 August 2010.

Truncated GDNF proteins are reported in WO97/11964 (PCT/US96/14915); however, there continues to be a need for new GDNF variants with desired pharmacological properties, stability and bio-distribution properties. There is a need for a variant form of GDNF that is stable in the delivery device, and facilitates desired brain bio-distribution, while demonstrating desired potency and acceptable immunogenicity properties. GDNF variants offering one or more of these desirable properties may be a new pharmaceutically useful medicinal therapy, particularly for use in the treatment of Parkinson's disease.

The present disclosure~~invention~~ provides a novel truncated GDNF variant of mature human GDNF domain lacking the first 31 amino acids at the N-terminus ("Δ31-N-terminus truncated GDNF"), with certain amino acid substitutions introduced to provide stable, suitably potent, GDNF variants offering desirable bio-distribution properties and a

pharmaceutically acceptable immunogenicity profile. The present disclosure~~invention~~ provides certain variants of human GDNF that impart one or more advantages over mature human wild-type GDNF including variants that have improved pharmaceutical stability, as well as improved bio-distribution, reduced heparin binding, reduced deamidation, reduced susceptibility to succinimide formation, and reduced immunogenicity potential compared to human wild-type GDNF. Certain new GDNF variants may be a useful new treatment option for Parkinson's disease patients.

The present disclosure~~invention~~ provides human GDNF variant comprising SEQ ID NO: 23 RGQRGKQRGCVLTAIHLNVTDLGLGYETKEELIFRYCSGSCDAAETTYDKILXaa$_{84}$NLSXaa$_{88}$NXaa$_{90}$RLVSEKVGQACCRPIAFDDDLSFLDDNLVYHILRXaa$_{125}$HSAKXaa$_{130}$CGCI (SEQ ID NO: 23), wherein:

i) Xaa$_{84}$ is K or A;

ii) Xaa$_{88}$ is R or K;

iii) Xaa$_{90}$ is R or K;

iv) Xaa$_{125}$ is K or E; and

v) Xaa$_{130}$ is R or E.

In a first aspect of the present disclosure, there is provided a human GDNF variant comprising the amino acid sequence of SEQ ID NO:23:
RGQRGKQRGCVLTAIHLNVTDLGLGYETKEELIFRYCSGSCDAAETTYD
KILXaa$_{84}$NLSXaa$_{88}$NXaa$_{90}$RLVSEKVGQACCRPIAFDDDLSFLDDNLVYHI
LRXaa$_{125}$HSAKXaa$_{130}$CGCI
wherein:

i) Xaa$_{84}$ is K or A;

ii) Xaa$_{88}$ is R or K;

iii) Xaa$_{90}$ is R or K;

iv) Xaa$_{125}$ is K or E; and

v) Xaa$_{130}$ is R or E.

The present disclosure~~invention~~ further provides a human GDNF variant wherein said variant is selected from the group consisting of

RGQRGKQRGCVLTAIHLNVTDLGLGYETKEELIFRYCSGSCDAAETTYDKILKNL SRNRRLVSEKVGQACCRPIAFDDDLSFLDDNLVYHILRKHSAKRCGCI (SEQ ID NO:9),

RGQRGKQRGCVLTAIHLNVTDLGLGYETKEELIFRYCSGSCDAAETTYDKILANL SKNKRLVSEKVGQACCRPIAFDDDLSFLDDNLVYHILRKHSAKRCGCI (SEQ ID NO:12), and

RGQRGKQRGCVLTAIHLNVTDLGLGYETKEELIFRYCSGSCDAAETTYDKILANL SKNKRLVSEKVGQACCRPIAFDDDLSFLDDNLVYHILREHSAKECGCI (SEQ ID NO:15).

~~In an aspect, t~~The present disclosure also~~invention~~ provides a human GDNF variant comprising an amino acid sequence as shown in SEQ ID NO: 9:

RGQRGKQRGCVLTAIHLNVTDLGLGYETKEELIFRYCSGSCDAAETTYDKILKNL SRNRRLVSEKVGQACCRPIAFDDDLSFLDDNLVYHILRKHSAKRCGCI.

The present disclosure~~invention~~ further provides an intermediate, useful for preparing a Δ31-N-terminus truncated variant of mature human GDNF. The intermediate comprises the amino acid sequence of SEQ ID NO: 23

RGQRGKQRGCVLTAIHLNVTDLGLGYETKEELIFRYCSGSCDAAETTYDKILXaa$_{84}$ NLSXaa$_{88}$NXaa$_{90}$RLVSEKVGQACCRPIAFDDDLSFLDDNLVYHILRXaa$_{125}$HSAKXaa $_{130}$CGCI (SEQ ID NO: 23), which is extended at the N-terminus with a signal secretion peptide. Numbers of signal secretion peptide sequences can be used herein. Exemplary signal secretion peptide sequences include murine kappa leader signal secretion peptide having a sequence of METDTLLLWVLLLWVPGSTG (SEQ ID NO: 25), and human growth hormone signal secretion peptide having a sequence of MATGSRTSLLLAFGLLCLPWLQEGSA (SEQ ID NO: 32).

Intermediates having these signal secretion peptides can produce the claimed human GDNF variants with increased yield over truncated GDNF constructs having other leader sequences. The disclosed intermediates including signal secretion peptide can thus have an amino acid sequence of METDTLLLWVLLLWVPGSTGRGQRGKQRGCVLTAIHLNVTDLGLGYETKEELIF RYCSGSCDAAETTYDKILKNLSRNRRLVSEKVGQACCRPIAFDDDLSFLDDNLVY HILRKHSAKRCGCI (SEQ ID NO: 28); or MATGSRTSLLLAFGLLCLPWLQEGSARGQRGKQRGCVLTAIHLNVTDLGLGYET KEELIFRYCSGSCDAAETTYDKILKNLSRNRRLVSEKVGQACCRPIAFDDDLSFLD DNLVYHILRKHSAKRCGCI (SEQ ID NO: 35).

In a second aspect of the present disclosure, there is provided an intermediate for preparing a Δ31-N-terminus truncated GDNF variant comprising an amino acid sequence selected from the group consisting of METDTLLLWVLLLWVPGSTGRGQRGKQRGCVLTAIHLNVTDLGLGYET KEELIFRYCSGSCDAAETTYDKILKNLSRNRRLVSEKVGQACCRPIAFDD

DLSFLDDNLVYHILRKHSAKRCGCI (SEQ ID NO: 28); and

MATGSRTSLLLAFGLLCLPWLQEGSARGQRGKQRGCVLTAIHLNVTDL GLGYETKEELIFRYCSGSCDAAETTYDKILKNLSRNRRLVSEKVGQACC RPIAFDDDLSFLDDNLVYHILRKHSAKRCGCI (SEQ ID NO: 35).

The present disclosure~~invention~~ provides a pharmaceutical composition, comprising a variant of human GDNF as claimed by the present disclosure~~invention~~ and one or more pharmaceutically acceptable diluents, carriers or excipients. The present disclosure~~invention~~ provides a variant of human GDNF for use as a medicament. The present disclosure~~invention~~ further provides a variant of human

GDNF for use in the treatment of Parkinson's disease. The present disclosure~~invention~~ provides a variant of GDNF for use as a therapy.

In a third aspect of the present disclosure, there is provided a pharmaceutical composition comprising a human GDNF variant as defined herein and one or more pharmaceutically acceptable diluents, carriers or excipients.

In a fourth aspect of the present disclosure, there is provided a human GDNF variant as defined herein for use as a medicament.

In a fifth aspect of the present disclosure, there is provided a human GDNF variant as defined herein for use in the treatment of Parkinson's disease.

In a sixth aspect of the present disclosure, there is provided a human GDNF variant as defined herein for use as a therapy.

A variant wherein Xaa_{84} is A, Xaa_{88} is K, and Xaa_{90} is K is preferred.

A variant wherein Xaa_{84} is K, Xaa_{88} is R, and Xaa_{90} is R is preferred.

A variant wherein Xaa_{125} is K and Xaa_{130} is R is preferred.

A variant wherein Xaa_{84} is A, Xaa_{88} is K, and Xaa_{90} is K, Xaa_{125} is E and Xaa_{130} is E is preferred.

DETAILED DESCRIPTION

It has been reported that wild type GDNF binds to heparin and extracellular matrix, likely through the positive charges located in the N-terminal 1-31 amino acid residues, therefore limiting the distribution of GDNF upon delivery in the brain (Lin et al., *J Neurochem* 63, 758-768, 1994; Rickard et al., *Glycobiology* 13, 419-426, 2003; Piltonen et al., *Experimental Neurology* 219, 499-506, 2009) . It has also been reported that GDNF provides functional improvements and has neuroprotective actions in rodent and primate models of Parkinson's disease (Tomac et al, 1995; Gash et al., 1996). Wild type GDNF protein from *E. coli* has been administered centrally to patients suffering from Parkinson's disease with mixed results. In two small open labelled studies, GDNF produced long-lasting improvement in motor function (Gill et al., 2003, Slevin et al., 2005). In addition, increased dopaminergic neuron sprouting was evident in one patient who died of unrelated causes – myocardial infarction (Love et al 2005). However, in a randomized, placebo-controlled, Phase IIa trial of 34 patients conducted by Amgen, intra-

putamen delivery of GDNF (Liatermin) showed no symptomatic improvement at 6 months (Lang et al., 2006). The claimed GDNF variants exhibit improved properties compared to the previously tested wild type GDNF protein from *E. coli*.

Wild type GDNF full length construct sequence (211aa) containing the signal peptide (the first 19 amino acids, SEQ ID NO: 4), pro-domain (italics, SEQ ID NO: 5), and mature peptide (underlined, SEQ ID NO: 3) is indicated as SEQ ID NO: 1:

MKLWDVVAVCLVLLHTASA*FPLPAGKRPPEAPAEDRSLGRRRAPFALSSDSNMPE DYPDQFDDVMDFIQATIKRLKR*SPDKQMAVLPRRERNRQAAAANPENSRGKGRR GQRGKNRGCVLTAIHLNVTDLGLGYETKEELIFRYCSGSCDAAETTYDKILKNLS