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(54) Title: NEURON GENERATION, REGENERATION AND PROTECTION

(57) Abstract: The invention demonstrates that, contrary to apoptotic rabies virus G proteins, certain non-apoptotic rabies virus G proteins, such as the G protein of the CVS-NIV strain, have a neurite outgrowth promoting effect. The invention further demonstrates that this neurite outgrowth promoting effect is due to the cytoplasmic tail of said non-apoptotic rabies virus G proteins, more particularly to their PDZ-BS, which shows a single-point mutation compared to the one of apoptotic rabies virus G proteins. The invention provides means for inducing and/or stimulating neurite outgrowth, which are useful in inducing neuron differentiation, for example for the treatment of a neoplasm of the nervous system, as well as in regenerating impaired neurons, for example for the treatment of a neurodegenerative disease, disorder or condition or in the treatment of a microbial infection, or in protecting neurons from neurotoxic agents or oxidative stress.



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TITLE**Neuron generation, regeneration and protection**

5

FIELD OF THE INVENTION

The invention relates to neuron generation, regeneration and protection, more particularly to neurite outgrowth.

The invention provides means to induce and/or stimulate neuritogenic effects, which are
10 useful in the medical field, more particularly in the treatment and/or palliation and/or
prevention of disorders of the nervous system. More particularly, the invention provides
means for inducing and/or stimulating neurite outgrowth, which are useful for inducing
neuron differentiation, for example for the treatment of a neoplasm of the nervous system,
as well as in regenerating impaired neurons, for example for the treatment of a
15 neurodegenerative disease, disorder or condition or in the treatment of a microbial
infection, or in protecting neurons from neurotoxic agents or oxidative stress.

The means of the invention are based on certain non-apoptotic rabies virus G proteins,
more particularly on the cytoplasmic tail thereof.

20

BACKGROUND OF THE INVENTION

During development of the nervous system, neurons extend axons over considerable
distances in order to innervate their targets in an appropriate manner. This involves the
stimulation in the cells of specific signaling pathways which can stimulate the activity of
25 the growth cone.

While the developing nervous system, more particularly the developing central nervous
system, is highly plastic, the adult nervous system, more particularly the adult brain, has
more limited repair potential. Therefore, neurite-axon outgrowth and protection against
30 degeneration are important factors to be considered to improve the outcome of a
neurodegenerative disease, disorder or condition, such as an acute injury of the nervous
system or a chronic neurodegenerative disorder. Products, which would be capable of
inducing neurite outgrowth from such neuronal cells, would bring a very useful

therapeutic and/or preventive and/or palliative solution to such diseases, disorders or conditions.

5 At the other side of the neuron developmental process, the proliferation of neuronal progenitors, which do not differentiate into matured neuronal structures, leads to nervous system neoplasm. Products, which would be capable of inducing neurite outgrowth from such progenic cells, would bring a therapeutic and/or preventive and/or palliative solution to such neoplasms.

10 The general idea on the infection of neuronal cells by a neurotropic virus is that it has no positive impact on neuron morphology, more particularly on neurite outgrowth.

Indeed, there are numerous examples showing that neurotropic viruses cause neuronal cell death by apoptosis. This concerns both DNA viruses, such as herpes viruses, and RNA
15 viruses, either enveloped such as alphaviruses, bunyaviruses and paramyxoviruses, or unenveloped such as picornaviruses and reoviruses.

Rabies virus, more particularly attenuated rabies virus strains, has also been described as inducing neuronal apoptosis.

20

For example, WO 03/048198 relates to rabies virus G proteins and fragments thereof of at least 100 amino acids, which induce the disruption of the neuronal cell integrity and the formation of apoptotic bodies. These apoptotic bodies are capable of stimulating a humoral immune response, preferably a B-dependent humoral immune response.

25 WO 03/048198 shows that:

- attenuated rabies virus strain (such as the attenuated ERA strain) induces the apoptotic rupture of the cells it infects,
- the apoptotic bodies thereby produced stimulate a humoral immune response, more particularly a B-dependent humoral immune response;
- 30 - induction of apoptosis by a rabies virus strain is determined by the nature of its G-protein;
- a rabies virus containing the G protein from an attenuated rabies virus strain (such as the attenuated ERA strain) is able to trigger apoptosis of human cells, whereas expression of the G protein from a pathogenic rabies virus (such as a Challenge

Virus Standard -CVS- strain) is not (*cf.* more particularly example 5 and Figures 19 and 20 of WO 03/048198).

Please see also Lay *et al.* 2003 and Préhaud *et al.* 2003.

5 Thus, the G proteins of apoptotic rabies virus strains, such as the G protein of the attenuated ERA strain, are known to be useful in stimulating a humoral response, more particularly a B-dependent humoral immune response.

Since these particular G proteins induce the apoptosis of the cells they infect, they have also been proposed as candidate agents to eliminate undesirable cells by apoptotic rupture
10 of the target cells.

It has also been described that the pathogenicity of a rabies virus strain is inversely correlated with its ability to induce apoptosis (*cf.* WO 03/048198; Ugolini 1995; Sarmiento *et al.* 2005; Ugolini 2008; Jackson *et al.* 2008).

15 Therefore, the more virulent a rabies virus strain is, the less apoptotic.

The findings that virulent rabies virus strains, such as CVS strains, do not induce neuron apoptosis and thereby escape humoral detection explain why virulent rabies virus strains can propagate so extensively within the CNS before the appearance of signs and
20 symptoms of the disease.

Further studies have been conducted to analyze the changes in gene expression pattern that are induced upon infection by a neurotropic virus, such as a rabies virus or herpes simplex type 1 (HSV-1).

25 These studies have brought the demonstration that post-mitotic human neurons, in the absence of glia, have the intrinsic machinery to sense virus infection, and that neurotropic viruses, such pathogenic rabies virus or HSV-1, induce the release of cytokines from post-mitotic human neurons (Préhaud *et al.* 2005). This cytokine release is believed to further contribute to the escape of neurons from apoptosis and in the consequent spreading of
30 such neurotropic viruses.

Therefore, neuronal cell death mechanisms, as well as the capacity of neurons to raise an immune response upon viral infection, have been thoroughly investigated.

However, less is known about the processes involved in neurogeneration, neuroregeneration and neuroprotection, more particularly in neurite outgrowth from pre-mitotic neurons, such as neuronal progenitors or neoplastic neurons, or from degenerative neurons.

5

If one focuses on rabies virus, a schematic summary of the current knowledge would be that attenuated rabies virus strains are known to have medical applications due to the apoptosis they induce, and that virulent rabies virus strains are known to not induce apoptosis, but, to the contrary, to preserve the neuronal network, which favors their spreading.

10

While virulent rabies virus strains have been described as preserving the integrity of the neuronal network, they have also been reported as having a negative impact, or at the very least no positive impact, on neuronal morphology, more particularly on neurite outgrowth.

15

For example, the publication Guigoni and Coulon 2002 describes that the virulent rabies virus strain CVS-Gif-sur-Yvette do not induce neurite outgrowth from rat motoneurons (*cf.* for example, Figures 5A and 5B of this publication).

20 Negative impact on neurite outgrowth has also been reported. For example, the publication Scott *et al.*, 2008 has reported that the pathogenic CVS-11 rabies virus strain induces beading of the dendrites and axons, *i.e.*, the formation of vacuoles that are characteristic of a negative stress impact.

25 The invention provides means for the generation, regeneration and protection of neurons, which derive from certain pathogenic rabies virus strains, and which show surprising and unexpected properties.

SUMMARY OF THE INVENTION

30

The invention demonstrates that some pathogenic (and non-apoptotic) rabies virus G proteins have a neurite outgrowth promoting effect, *i.e.*, that some non-apoptotic rabies virus G proteins induce and/or stimulate neuritogenesis. This effect is not shown by

apoptotic rabies virus G proteins. Neither is it shown by all non-apoptotic rabies virus G proteins.

5 The inventors identified a sub-group of non-apoptotic (and virulent) rabies virus strains, the G protein of which has a significantly positive effect on neurite outgrowth. As described and illustrated below, a representative strain of this sub-group is the CVS-NIV strain. The G protein of this non-apoptotic (and virulent) strain differs by only 6 amino acid from the G protein of the apoptotic (and attenuated) ERA strain (CNCM I-2760).

10 The invention further demonstrates that this neurite outgrowth promoting effect is due to the cytoplasmic tail of said non-apoptotic rabies virus G proteins, more particularly to their PDZ binding site (PDZ-BS) and/or to an amino acid which, in the sequence of the G protein of the CVS-NIV strain, is at position 491, more particularly to their PDZ-BS.

15 The invention relates to polypeptides, which are or derive from certain non-apoptotic rabies virus G proteins, more particularly from their cytoplasmic tail, as well as to nucleic acids, vectors, cells and pharmaceutical compositions or drugs.

The means of the invention are notably useful as neurite outgrowth stimulating and/or inducing agents. They can notably be used for inducing neuron differentiation, for
20 example in the treatment of a neoplasm of the nervous system, as well as for regenerating impaired neurons, for example in the treatment of a neurodegenerative disease, disorder or condition or in the treatment of a microbial infection, or for protecting neurons from neurotoxic agents or oxidative stress.

25 **BRIEF DESCRIPTION OF THE FIGURES**

Some of the figures, to which the present application refers, are in color. The application as filed contains the color print-out of the figures, which can therefore be accessed by inspection of the file of the application at the patent office.

30

Figure 1: neurogeneration, neuroregeneration and neuroprotection are implicated in various neuronal disorders, conditions and diseases, including neurotoxicity, seizure, stroke, trauma, aging, neurodegenerative disease, encephalopathy.

Figure 2: schematic structure of the recombinant rabies virus (rRABV) produced by the inventors and used in the examples below.

The G protein of the CVS-NIV strain (non-apoptotic strain) differs by only 6 aa from the G protein of the ERA strain (apoptotic strain).

5 G-survival = G protein of the CVS-NIV strain

G-death = G protein of the ERA strain

G-cyto death = cytoplasmic tail of G-death in a CVS-NIV G gene background

G-cyto survival = cytoplasmic tail of G-survival in an ERA G gene background

SP = signal peptide

10 EC: extracellular domain

TM: transmembrane domain

Cyto: cytoplasmic domain

Figures 3A, 3B, 3C, 3D: the cytoplasmic tail of the G protein of the CVS-NIV strain (non-apoptotic strain) contains a molecular signature promoting neurite outgrowth.

Figure 3A: neurite outgrowth assay with rRABV G-CVS-NIV and rRABV G-ERA

Figure 3B: results of the neurite outgrowth assay with db-cAMP with rRABV G-CVS-NIV and rRABV G-ERA

Figure 3C: results of the neurite outgrowth assay without db-cAMP with rRABV G-CVS-

20 NIV, rRABV G-CVS-Cyto Death, rRABV G-ERA and rRABV G-ERA-Cyto Survival

Figure 3D: results of the neurite outgrowth assay with rRABV-G-CVS-NIV, rRABV G-CVS (LQ) and rRABV G-CVS (HE)

N.I. = non infected

rRABV = recombinant rabies virus

25 G-CVS or G-CVS-NIV = protein G of the CVS-NIV strain

G-CVS-Cyto Death = cytoplasmic tail of the G protein of the ERA strain in a CVS-NIV G gene background

G-ERA = protein G of the ERA strain

G-ERA-Cyto Survival = cytoplasmic tail of the G protein of the CVS-NIV strain in an

30 ERA G gene background

rRABV G-CVS (LQ) = recombinant rabies virus G protein of the CVS-NIV strain, wherein the amino acid H, which is at position 491 in the full length G protein of the CVS-NIV strain, has been replaced by the amino acid L

rRABV G-CVS (HE) = recombinant rabies virus G protein of the CVS-NIV strain, wherein the amino acid Q, which is at position 521 in the full length G protein of the CVS-NIV strain, has been replaced by the amino acid E

- 5 **Figures 4A, 4B:** the cytoplasmic tail of the G protein of the CVS-NIV strain (non-apoptotic strain) is an intrinsic effector promoting neuritogenesis, which works synergistically with cAMP*

Figure 4A: without db-cAMP

Figure 4B: without db-cAMP (first two histograms) and with cAMP (last two histograms)

- 10 ★ means significantly different (Student's t test with $p=0.0067$)

N.I. = non infected

rRABV = recombinant rabies virus

G-CVS or G-CVS-NIV = protein G of the CVS-NIV strain

- 15 G-CVS-Cyto Death = cytoplasmic tail of the G protein of the ERA strain in a CVS-NIV G gene background

G-ERA = protein G of the ERA strain

G-ERA-Cyto Survival = cytoplasmic tail of the G protein of the CVS-NIV strain in an ERA G gene background

- 20 **Figure 5:** the neuritogenesis effect of the cytoplasmic tail of the G protein of the CVS-NIV strain (non-apoptotic strain) is dependent on the molecular signature and not on the amount of expressed G protein.

rRABV = recombinant rabies virus

G-CVS or G-CVS-NIV = protein G of the CVS-NIV strain

- 25 G-CVS-Cyto Death = cytoplasmic tail of the G protein of the ERA strain in a CVS-NIV G gene background

G-ERA = protein G of the ERA strain

G-ERA-Cyto Survival = cytoplasmic tail of the G protein of the CVS-NIV strain in an ERA G gene background

30

Figures 6A, 6B: the cytoplasmic tail of the G protein of the CVS-NIV strain (non-apoptotic strain) confers neuroprotection against growth cone collapsing drug (LPA)

Figure 6A: with db-cAMP

Figure 6B: without db-cAMP

N.I. = non infected

rRABV = recombinant rabies virus

G-CVS or G-CVS-NIV = protein G of the CVS-NIV strain

- 5 G-CVS-Cyto Death = cytoplasmic tail of the G protein of the ERA strain in a CVS G gene background

G-ERA = protein G of the ERA strain

G-ERA-Cyto Survival = cytoplasmic tail of the G protein of the CVS-NIV strain in an ERA G gene background

10

Figure 7: the neuroprotection against the growth cone collapsing drug LPA that is induced by the cytoplasmic tail of the G protein of the CVS-NIV strain (non-apoptotic strain) is a robust one.

★ means significantly different (ANOVA test)

- 15 ▲ means not significantly different (ANOVA test)

N.I. = non infected

rRABV = recombinant rabies virus

G-CVS-NIV = protein G of the CVS-NIV strain

- 20 G-CVS-Cyto Death = cytoplasmic tail of the G protein of the ERA strain in a CVS-NIV G gene background

G-ERA = protein G of the ERA strain

G-ERA-Cyto Survival = cytoplasmic tail of the G protein of the CVS-NIV strain in an ERA G gene background

LPA = lysophosphatidic acid

25

Figure 8: the cytoplasmic tail of the G protein of the CVS-NIV strain (non-apoptotic strain) confers neuroprotection against oxidative stress (H_2O_2). These experiments were performed without db-cAMP.

★ means significantly different (Student's t test)

- 30 ▲ means not significantly different (Student's t test)

N.I. = non infected

rRABV = recombinant rabies virus

G-CVS or G-CVS-NIV = protein G of the CVS-NIV strain

G-CVS-Cyto Death = cytoplasmic tail of the G protein of the ERA strain in a CVS-NIV G gene background

G-ERA = protein G of the ERA strain

5 G-ERA-Cyto Survival = cytoplasmic tail of the G protein of the CVS-NIV strain in an ERA G gene background

Figures 9A, 9B, 9C: the cytoplasmic tail of the G protein of the CVS-NIV strain (non-apoptotic strain) confers protection against Herpes Virus Simplex type 1 (HSV-1) cytopathic effect.

10 N.I. = non infected

rRABV = recombinant rabies virus

G-CVS or G-CVS-NIV = protein G of the CVS-NIV strain

G-CVS-Cyto Death = cytoplasmic tail of the G protein of the ERA strain in a CVS-NIV G gene background

15 G-ERA = protein G of the ERA strain

G-ERA-Cyto Survival = cytoplasmic tail of the G protein of the CVS-NIV strain in an ERA G gene background

Figures 10A, 10B: cell proliferation of human neuroblastoma cells treated with the all-trans-retinoic acid (ATRA) pro-differentiative drug (Figure 10A: flow cytometry; Figure 10B: MTT assay).

20 N.I. = non infected

Figures 11A, 11B: the cytoplasmic tail of the G protein of the CVS-NIV strain (non-apoptotic strain) confers anti-proliferative properties: cell proliferation (Figure 11A) and neurite length (Figure 11B) of human neuroblastoma cells treated with rRABV G-CVS-NIV or rRABV G-ERA

25 N.I. = non infected

rRABV = recombinant rabies virus

30 G-CVS-NIV = protein G of the CVS-NIV strain

G-ERA = protein G of the ERA strain

Figures 12A, 12B: the cytoplasmic tail of the G protein of the CVS-NIV strain (non-apoptotic strain) confers anti-proliferative properties: cell proliferation of human

neuroblastoma cells treated with rRABV G-CVS, rRABV G-ERA, rRABV G-CVS-Cyto death or rRABV G-ERA-Cyto survival (Figure 12A: flow cytometry; Figure 12B: MTT assay) (N.I. = non infected)

N.I. = non infected

5 rRABV = recombinant rabies virus

G-CVS or G-CVS-NIV = protein G of the CVS-NIV strain

G-CVS-Cyto Death = cytoplasmic tail of the G protein of the ERA strain in a CVS-NIV G gene background

G-ERA = protein G of the ERA strain

10 G-ERA-Cyto Survival = cytoplasmic tail of the G protein of the CVS-NIV strain in an ERA G gene background

Figures 13A, 13B: nucleic acid and protein sequences of the G protein of the CVS-NIV strain (Figure 13A) and of the ERA strain (Figure 13B). In Figure 13A, the PDZ-BS motif of the G protein of the CVS-NIV strain is underlined (QTRL).

aa = amino acid

Figure 14: sequence alignment of the G proteins of the CVS-NIV and ERA strains; the G proteins differ by only 6 amino acids (shown in bold in Figure 14):

20

Table 1:

Position of the amino acid in the sequence of the full length G protein	G protein of the CVS-NIV strain (non-apoptotic strain)	G protein of the ERA strain (apoptotic strain)
48	V	I
139	H	R
179	P	S
219	A	V
491	H	L
521	Q	E

The alignment shown in Figure 14 has been performed using the following parameters:

Comparison matrix: BLOSUM62

Number of alignments computed: 20

Gap open penalty: 12

Gap extension penalty: 4

5 The result of this alignment is:

98.9% identity in 524 residues overlap;

Score: 2787.0;

Gap frequency: 0.0%.

10 **Figure 15:** nucleic acid and protein sequences of the cytoplasmic domain of the G protein of the CVS-NIV strain and of the ERA strain. In Figure 15, the PDZ-BS motif of the G protein of the CVS-NIV strain is underlined (QTRL).

aa = amino acid

15 **Figure 16:** nucleic acid and protein sequences of the PDZ-BS of the G protein of the CVS-NIV strain and of the ERA strain.

aa = amino acid

20 **Figure 17:** alignment of the G protein of the CVS-NIV strain (SEQ ID NO: 2) and of the G protein of the CVS-Gif-sur-Yvette strain (SEQ ID NO: 15).

Figure 18: alignment of the G protein of the CVS-NIV strain (SEQ ID NO: 2) and of the G proteins of three CVS-11 strains (SEQ ID NO: 16; SEQ ID NO: 17; SEQ ID NO: 18).

25 **Figure 19:** sequence of the cytoplasmic fragment of the G protein of the CVS-NIV strain (SEQ ID NO: 6) and of two conservative variants deriving therefrom (variant sequence A of SEQ ID NO: 19; variant sequence B of SEQ ID NO: 20).

30 **Figure 20:** the deletion of the PDZ-BS affects the neurite outgrowth phenotype (average neurite length in μm at 8h post-infection, in the presence of db-cAMP).

N.I. = non infected;

G-CVS-NIV = protein G of the CVS-NIV strain;

G-CVS-NIV-DeltaPDZ-BS = protein G of the CVS-NIV strain from which the PDZ-BS has been deleted.

Figure 21: schematic structure of single-point mutants of the invention (variants B and A). Mutant viruses CVS HE (I-4143) and CVS LQ (I-4142) of the invention differ from CVS-NIV (I-4140) by their G proteins, in which a single-point mutation has been introduced.

5 CVS HE (variant B) = single-point mutation of the first PDZ-BS amino acid of the G protein CVS-NIV strain, which is E in CVS HE (position 521) instead of Q.

CVS LQ (variant A) = single-point mutation of the amino acid at position 491, which is E instead of Q.

SP: signal peptide

10 EC: extracellular domain

TM: transmembrane domain

Cyto: cytoplasmic domain

Figure 22: amino acid sequences of domains of the G protein of a non-apoptotic virus strain and of construct inserts

- Sequences of G-CVS domains:

- **G full length** (SEQ ID NO: 2): full-length G protein of an apoptotic rabies virus strain (CVS-NIV);

20 - **Signal peptide (SP; SEQ ID NO: 21)**: signal peptide of an apoptotic rabies virus strain (CVS-NIV);

- **Ectodomain (EC; SEQ ID NO: 22)**: ectodomain of an apoptotic rabies virus strain (CVS-NIV);

- **Transmembrane domain (TM. SEQ ID NO: 23)**: transmembrane domain of an apoptotic rabies virus strain (CVS-NIV);

25 - **Cytoplasmic domain** (Cyto; SEQ ID NO: 6): cytoplasmic domain of an apoptotic rabies virus strain (CVS-NIV).

- Amino acid sequences coded by construct inserts:

- Amino acid M + SEQ ID NO: 6 = **SEQ ID NO: 24 (construct G-Cyto)**;

30 - Transmembrane domain of SEQ ID NO: 23 + cytoplasmic domain of SEQ ID NO: 6 = **SEQ ID NO: 25**;

- Two amino acids from the C-terminal end of the ectodomain of G-CVS-NIV (amino acids GK) + transmembrane domain of SEQ ID NO: 23 + cytoplasmic domain of SEQ ID NO: 6 = **SEQ ID NO: 26**;

- Signal peptide of SEQ ID NO: 21 + two amino acids from the C-terminal end of the ectodomain of G-CVS-NIV (amino acids GK) + transmembrane domain of SEQ ID NO: 23 + cytoplasmic domain of SEQ ID NO: 6 = **SEQ ID NO: 27** (**construct G-(SP-[2a]-TM-Cyto**).

5

Figure 23: alignment of the amino acid sequences coded by the inserts of three constructs (SEQ ID NO: 2 coded by the G-full construct; SEQ ID NO: 27 coded by the G-(SP)-[2a]-TM-Cyto (=GSP2aaTMCyto) construct; SEQ ID NO: 24 coded by the G-Cyto construct).

10 **Figure 24:** schematic representation of three RABV G gene constructs (G-full of SEQ ID NO: 2 (= G survival); G-Cyto of SEQ ID NO: 24 (= G survival-Cyto); G-(SP)-[2a]-TM-Cyto of SEQ ID NO: 27 (= G Survival-ΔEC).

15 **Figure 25:** RABV G expression (arbitrary units) for control plasmid, G-Full construct (SEQ ID NO: 2), G-(SP-[2a]-TM-Cyto) construct (SEQ ID NO: 27), and G-Cyto construct (SEQ ID NO: 24) in the SH-SY5Y cell line (human neuroblastoma cell line).

Figures 26 and 27: effect on neurite outgrowth of the expression:

- of the full length G protein of a non-apoptotic rabies virus strain (SEQ ID NO: 2; G-Full construct),
 - of the transmembrane and cytoplasmic domains of a non-apoptotic rabies virus strain (SEQ ID NO: 27; G-(SP-[2a]-TM-Cyto) construct), or
 - of the cytoplasmic domain of a non-apoptotic rabies virus strain (SEQ ID NO: 24; G-Cyto construct),
- 25 in human neuroblastoma cells (SH-SY5Y cell line in the presence of db c-AMP), compared to control (no DNA) and to the control plasmid.

Figure 26: transient expression.

Figure 27: stable expression.

30 **Figure 28:** the expression of the transmembrane and cytoplasmic domains of a non-apoptotic rabies virus strain (SEQ ID NO: 27; construct G-(SP-[2a]-TM-Cyto) induces neurite outgrowth from human neuroblastoma cells (SH-SY5Y cell line in the presence of

db c-AMP), and confers protection against the growth cone collapsing drug LPA to the neurites that have grown.

Figures 29 and 30: the (stable) expression of the cytoplasmic domain of a non-apoptotic rabies virus strain induces and/or stimulates the differentiation of an embryonic carcinoma cell line (NTERA 2cl.-D1; ATCC CRL-1973) into mature post-mitotic human neurons (5 days post differentiation procedure: effect of the G-Full construct -insert of SEQ ID NO: 2-, of the G-(SP-[2a]-TM-Cyto) construct -insert of SEQ ID NO: 27-, or of the G-Cyto construct -insert of SEQ ID NO: 24-, compared to control plasmid).

Figure 29: colorized images.

Figure 30: neurite tips.

Figure 31: the (stable) expression of the cytoplasmic domain of a non-apoptotic rabies virus strain induces and/or stimulates the differentiation of an embryonic carcinoma cell line (NTERA 2cl.-D1; ATCC CRL-1973) into mature post-mitotic human neurons, and induces and/or stimulates the organisation of a neuronal network with long axons (colorized images of live neurons, 50 days post differentiation procedure: effect of the G-Full construct -insert of SEQ ID NO: 2-, of the G-(SP-[2a]-TM-Cyto) construct -insert of SEQ ID NO: 27-, or of the G-Cyto construct -insert of SEQ ID NO: 24-, compared to control plasmid).

Figures 32 and 33: expression of the transmembrane and cytoplasmic domains of a non-apoptotic rabies virus strain induces and/or stimulates the regeneration of wounded mature post-mitotic human neurons (neurons seeded on PDL-laminin plastic ware, 3 days post scratching with injection needle (26GX1/2", 12-4.5); effect of the polypeptide of SEQ ID NO: 27 (construct G-(SP-[2a]-TM-Cyto) -neurite regeneration-, compared to control plasmid -neurite degeneration-).

Figure 32: colorized images

Figure 33: percentage of neurite regeneration

DETAILED DESCRIPTION OF THE INVENTION

The present application relates to the subject-matter as defined in the claims as filed and as herein described and illustrated.

Pathogenicity of rabies virus, a neurotropic virus causing fatal encephalitis in most mammalian species, correlates with the ability of the infected neurons to survive. Attenuation of laboratory strains obtained in search of candidate live vaccine is always
5 linked to the ability of those vaccine strains to trigger cell death.
Subversion of an infected cell by a given virus implies the perturbation of specific signaling pathways.

Rabies virus is an enveloped, bullet-shaped virus belonging to the *Rhabdoviridae* family
10 and the *Lyssavirus* genus. The viral particle consists of a membrane composed of host lipids and two viral proteins, the G and M proteins, surrounding a helical nucleocapsid (NC). The NC is composed of a viral negative-strand RNA molecule protected by the N protein, the P protein, and the RNA-dependent RNA polymerase, the L protein. The rabies virus proteins are not synthesized in equal amounts in infected cells and are not present at
15 the same ratios in viral particles. Indeed, the N, G and M proteins are, in this order, the most prominent species in virions.

The strategic decision between preservation of the neuronal network integrity (favoring rabies virus spreading) and neuronal cell death (favoring immunogenicity) necessarily
20 involves a choice between at least two signaling networks.

The invention brings the demonstration that the cytoplasmic tail of the G protein of rabies virus strain has a crucial role in the command of the pro-survival (*i.e.*, preservation of the neuronal network integrity, which favors rabies virus spreading) *versus* pro-apoptotic (*i.e.*,
25 cell death, which favors immunogenicity) decision process.

The invention further uncovers unexpected effects of the pro-survival strategy.
Indeed, the invention shows that the G proteins of some non-apoptotic (and virulent) rabies virus strains, more particularly of the CVS-NIV strain, have a neurite outgrowth
30 promoting effect, *i.e.*, that these non-apoptotic rabies virus G proteins induce and/or stimulate neuritogenesis.

This effect is not shown by apoptotic rabies virus G proteins, such as the G proteins of the ERA and other attenuated rabies virus strains.

Furthermore, this effect is not shown by all apoptotic and/or virulent rabies virus G proteins. More particularly, it is not shown by the G protein of the CVS-Gif-sur-Yvette strain (Préhaud *et al.* 1988), although this G protein has a high identity score with the G protein of the CVS-NIV strain (*cf.* Figure 17). Said effect is not shown by the G proteins
5 of the CVS-11 strains (e.g., ACA57830, AAC34683, ABV24348), although these G proteins also have a high identity score with the G protein of the CVS-NIV strain (*cf.* Figure 18). Neither is it shown by the G proteins of the N2C or CVS-24 strains.

Therefore, not all virulent rabies virus strains have a G protein, which shows a significantly positive effect on neurite outgrowth.

10 Hence, the inventors identified a sub-group of non-apoptotic (and virulent) rabies virus strains, the G protein of which has a significantly positive effect on neurite outgrowth. A representative strain of this sub-group is the CVS-NIV strain. These G proteins may herein be referred to as G proteins of the CVS-NIV type.

The CVS-NIV strain has been deposited at the CNCM on the 1st of April 2009 under
15 deposit number I-4140.

A plasmid expressing the G protein of the CVS-NIV strain has been deposited at the CNCM on the 30th of November, 2001 under deposit number I-2578.

The G protein of the CVS-NIV strain has been described in Préhaud *et al.* 2003.

The sequence of the G protein of the CVS-NIV strain is available under accession number
20 AF 406694.

A sequence of the G protein of the CVS-NIV strain is the sequence of SEQ ID NO: 2 shown in Figure 13A.

A rabies virus G protein is a type I transmembrane glycoprotein that forms the trimeric
25 spikes of the viral envelope and that is found at the membrane of infected cells. The rabies virus G protein is a 524 amino acid long protein, which consists of an ectodomain, a transmembrane segment and a 44 amino acid long cytoplasmic domain.

The very last 4 amino acids of this C-terminal domain form a PDZ binding site (PDZ-BS).

30 PDZ (PSD-95, Discs Large, ZO-1) domains form globular structures of 80-100 aa organized into six beta-strands and two alpha helices creating a socket where the C terminal sequence of a partner protein could be inserted.

A PDZ binding site (PDZ-BS) is a 4 amino acid sequence; the PDZ-BS sequence is the sequence of SEQ ID NO: 13:

$x_1 - x_2 - x_3 - x_4$, wherein:

x_1 is any amino acid, and

x_2 is T or S or I, and

x_3 is any amino acid, and

5 x_4 is L or V.

The invention notably demonstrates that it is the cytoplasmic tail of said non-apoptotic rabies virus G proteins of the CVS-NIV type, which is responsible for this neurite outgrowth effect.

10

The invention further demonstrates that this is notably due to the PDZ-BS that is contained in the cytoplasmic tail of said non-apoptotic rabies virus G proteins of the CVS-NIV type.

15 The PDZ-BS motif of said non-apoptotic rabies virus G proteins of the CVS-NIV type shows a single-point mutation compared to the one of apoptotic rabies virus G proteins. This single-point mutation concerns the first PDZ-BS amino acid, which is not an E in non-apoptotic rabies virus G proteins.

20 The invention further demonstrates that the amino acid, which, in the full length non-apoptotic rabies virus G protein sequence of the CVS-NIV strain, is at position 491, also contributes to this neurite outgrowth effect. This amino acid is H in the G protein of the CVS-NIV strain (*cf.* SEQ ID NO: 2). Position 491 in the full length G protein corresponds to position 11 in the cytoplasmic fragment of this protein (SEQ ID NO: 6 in Figure 15).

25 The inventors demonstrate that the amino acids, which, in the full length non-apoptotic rabies virus G protein sequence of the CVS-NIV strain (SEQ ID NO: 2), are at positions 491 and 521, are both important for the neurite outgrowth effect of the invention. More particularly, they show that H (but not L) at position 491 and Q (but not E) at position 521, both favors the neurite outgrowth effect of the invention.

30 Mutant virus, which differ from the CVS-NIV strain in that their G proteins are H491L single-point mutated or Q521E single point mutated, have been constructed and produced by the inventors (CNCM I-4142 and I-4143).

The H491L mutant G protein still has Q at position 521.

The Q521E mutant G protein still has H at position 491.

Both mutant proteins induce a significantly positive neurite outgrowth effect, although at a lesser extent than the CVS-NIV G protein, which has both H at position 491 and Q at position 521.

The cytoplasmic fragments of these mutant G proteins are shown in Figure 19 (SEQ ID NO: 19 and SEQ ID NO: 20).

Definition of a polypeptide of the invention:

The invention relates to a **polypeptide, which is:**

10

- a **polypeptide, which comprises:**

15

- the **parent sequence** selected from the sequence of SEQ ID NO: 6, the cytoplasmic fragment of the G protein of the strain I-4140, the cytoplasmic fragment of the G protein produced by the plasmid I-2578, the cytoplasmic fragment of the G protein of sequence AF406694, the cytoplasmic fragment of the G protein of SEQ ID NO: 2,

or

20

- a **conservative variant sequence** of said parent sequence,

or

25

- a **conservative fragment** of said parent sequence or of said conservative variant sequence.

30

Said parent sequences, conservative variant sequences and conservative fragments sequences advantageously have the function of inducing and/or stimulating neurite outgrowth, for example as below illustrated, more particularly neurite outgrowth from human pre-mitotic neurons, *e.g.*, from the human neuroblastoma cell line SH-SY5Y (ATCC CRL-2266; please see example 1 below for illustrative experimental conditions and materials).

The invention more particularly relates to said polypeptide, for use in stimulating and/or inducing neurite outgrowth, more particularly for use in the treatment and/or palliation and/or prevention of a disease, disorder or condition involving an insufficient or impaired neurite outgrowth, more particularly an insufficient or impaired neuritogenesis.

5

Preferably, the amino acid length of said polypeptide is of less than 100 amino acids, more preferably of less than 90 amino acids.

10 The sequence of the above-mentioned variant proteins can be a sequence, which does not correspond to any known or identifiable rabies virus G protein.

Indeed, one of average skill in the art will recognize that starting from the sequence of a given rabies virus G protein, one or several amino acid substitution(s) and/or addition(s) and/or deletion(s) can be made, while still retaining the capacity of inducing and/or stimulating neurite outgrowth. Such conservative amino acid substitution(s) and/or
15 addition(s) and/or deletion(s) are herein encompassed.

Without being bound by theory, each of the following eight groups are usually considered as containing amino acids that are conservative substitutions for one another:

- 1) Alanine (A), Glycine (G);
- 2) Aspartic acid (D), Glutamic acid (E);
- 20 3) Asparagine (N), Glutamine (Q);
- 4) Arginine (R), Lysine (K);
- 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);
- 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);
- 7) Serine (S), Threonine (T); and
- 25 8) Cysteine (C), Methionine (M).

Therefore, the above-mentioned variant proteins notably encompass proteins, which have been engineered by man, and which differ from said rabies virus G protein by one or several amino acid substitution(s) and/or addition(s) and/or deletion(s), provided that the
30 resulting variant protein still has the capacity of inducing and/or stimulating neurite outgrowth, more particularly neurite outgrowth from human pre-mitotic neurons, *e.g.*, from the human neuroblastoma cell line SH-SY5Y (ATCC CRL-2266; please see example 1 below for illustrative experimental conditions and materials).

Preferably, said conservative variant sequence is:

- a variant sequence of said parent sequence, which:

- is of 34 to 54 amino acids,

5 - is at least 94% identical to said parent sequence over the entire length of the shortest of the parent and variant sequences,

- comprises a PDZ-BS sequence (preferably the last four C-terminal amino acids), wherein said PDZ-BS sequence is:

x_1 is any amino acid except E (preferably Q), and

10 x_2 is T or S or I (preferably not I, more preferably T), and

x_3 is any amino acid (preferably R), and

x_4 is L or V (preferably L)

(SEQ ID NO: 14),

said variant sequence being referred to as **variant sequence A**,

15

or

- a variant sequence of said parent sequence, which:

- is of 44 amino acids,

20 - is at least 94% identical to said parent sequence,

- comprises a PDZ-BS sequence (preferably the last four C-terminal amino acids), wherein said PDZ-BS sequence is:

x_1 is E, and

x_2 is T or S or I (preferably not I, more preferably T), and

25 x_3 is any amino acid (preferably R), and

x_4 is L or V (preferably L)

(SEQ ID NO: 13 with $x_1 = E$),

and

- does not comprise the amino acid L at position 11 (preferably
30 comprises the amino acid H at this position),

said variant sequence being referred to as **variant sequence B**.

Preferably, said sequence identity is of at least 95% (preferably at least 96%, more preferably at least 97%, still more preferably at least 97.5%, even still more preferably at least 98%, most preferably at least 98.5%, still most preferably at least 99%).

- 5 More preferably, said conservative variant sequence is a variant sequence A.

Preferably, said conservative fragment is a fragment of at least 34 amino acids, which has retained the PDZ-BS sequence of said parent sequence or of said variant sequence A or of said variant sequence B, respectively

10

For the sake of conciseness, the term “polypeptide” is herein intended as encompassing proteins, and conversely.

- This term also encompasses polypeptides (or proteins), which have been modified by post-transcriptional modification and/or by synthetic chemistry, e.g., by adjunction of a non-peptidic chemical group and/or by modification of the tertiary structure of the polypeptide, e.g., by acetylation, acylation, hydroxylation, cyclisation, racemisation, phosphorylation, etc., as long as the resulting modified polypeptide has retained the capacity of inducing and/or stimulating neurite outgrowth, more particularly neurite outgrowth from human pre-mitotic neurons, e.g., from the human neuroblastoma cell line SH-SY5Y (ATCC
- 15
- 20 CRL-2266; please see example 1 below for illustrative experimental conditions and materials).

The polypeptide of the invention advantageously is, on and of its own, neurotropic.

- If required or desired, the polypeptide of the invention can nevertheless be coupled to, or fused with an agent that improves its neurotropicity, more particularly its tropicity for brain neurons, such as RVG-9R as described by Kumar *et al.* 2007.
- 25

- Amino acid sequences and nucleotide sequences are herein given in accordance with the standard orientation, *i.e.*, from N-terminal end to C-terminal end for amino acid sequences and from 5'-terminal end to 3'-terminal end for nucleotide sequences.
- 30

According to an embodiment of the invention, the PDZ-BS sequence of said variant sequence A is QTRL (SEQ ID NO: 10).

According to an embodiment of the invention, said variant sequence A is of 44 amino acids and has the amino acid H or L, preferably H, at position 11.

An illustrative variant sequence A, which is of 44 amino acids and which has the amino acid L at position 11 is the sequence of SEQ ID NO: 19 (*cf.* Figure 19).

5

According to an embodiment of the invention, the PDZ-BS sequence of said variant sequence B is ETRL (SEQ ID NO: 12).

According to an embodiment of the invention, said variant sequence B has the amino acid H at position 11.

10 An illustrative variant sequence B, which is of 44 amino acids and which has the amino acid H at position 11 is the sequence of SEQ ID NO: 20 (*cf.* Figure 19).

According to an embodiment of the invention, said fragment is the fragment 11-44 from the sequence of SEQ ID NO: 6.

15

A polypeptide of the invention may comprise, or consist of said pro-neurite outgrowth sequence and of the transmembranar part of a G rabies virus protein or any other anchoring means that the person of average skill in the art may find appropriate to anchor the polypeptide on or in the membrane of a cell, such as a cell as below-defined.

20

A polypeptide of the invention can be inserted into the structure of an engineered antibody, for example a single chain antibody.

25 According to an embodiment of the invention, said polypeptide is the G protein of a rabies virus strain, or a cytoplasmic fragment thereof (*e.g.*, SEQ ID NO: 6), or a sub-fragment of such a cytoplasmic fragment (*e.g.*, fragment 11-54 of SEQ ID NO: 6). Said rabies virus G protein more preferably is a non-apoptotic rabies virus G protein. For example, said rabies virus strain is the strain deposited at the CNCM under I-4140.

30 According to an embodiment of the invention, said polypeptide comprises, or consists of, a variant sequence A or B (as above-defined) of such a cytoplasmic fragment or sub-fragment. For example, said polypeptide comprises, or consist of, the cytoplasmic fragment of the G protein of the rabies virus strain deposited at the CNCM under I-4142 or I-4143 (*cf.* Figure 21), whose G proteins are single-point mutant proteins of the G

protein of the I-4140 strain (cytoplasmic fragments of SEQ ID NO: 19 and 20, respectively; cf. Figure 19).

CNCM is the Collection Nationale de Cultures de Microorganismes ; Institut Pasteur ; 28,
5 rue du Docteur Roux ; F-75724 Paris Cedex 15 ; France. Said deposits have been made under the terms of the Budapest Treaty.

Variant sequence A:

10 A variant sequence A of the invention comprises the sequence of SEQ ID NO: 14, i.e., the sequence $x_1 - x_2 - x_3 - x_4$, wherein:

x_1 is any amino acid except E, and

x_2 is T or S or I, and

x_3 is any amino acid, and

15 x_4 is L or V.

This sequence is a particular PDZ-BS motif. It usually is located at the C-terminal end of the polypeptide, most usually this sequence is the sequence of the very last four C-terminal amino acids of the polypeptide.

20

Preferably, said amino acid x_1 is Q.

Preferably, said amino acid x_2 is not I. Preferably it is T.

25 Preferably, said amino acid x_3 is R.

Preferably, said amino acid x_3 is L.

Preferably, the sequence of said PDZ-BS motif is the sequence of SEQ ID NO: 14,
30 wherein:

x_1 is Q, and

x_2 is T or S or I, and

x_3 is R, and

x_4 is L or V.

Most preferably, the sequence of said PDZ-BS motif is the sequence of SEQ ID NO: 14, wherein:

5 x_1 is Q, and
 x_2 is T, and
 x_3 is R, and
 x_4 is L or V.

10 Most preferably, the sequence of said PDZ-BS motif is the sequence of SEQ ID NO: 14, wherein:

x_1 is Q, and
 x_2 is T or S or I, and
 x_3 is R, and
 x_4 is L.

15

More preferably, the sequence of said PDZ-BS motif is the sequence of SEQ ID NO: 14, wherein:

x_1 is Q, and
 x_2 is T, and
20 x_3 is R, and
 x_4 is L,

i.e., it is the sequence of SEQ ID NO: 10 (*cf.* Figure 16).

This QTRL sequence is the sequence of the PDZ-BS motif of the G protein of the CVS-NIV strain, said strain being available from the CNCM under deposit number I-4140, and/or of the protein coded by the plasmid available from the CNCM under deposit
25 number I-2758 (recombinant *E. coli* containing said plasmid), and/or of the protein of SEQ ID NO: 2 (*cf.* Figure 13A).

Appropriate conditions for the cultivation of the recombinant *E. coli* strain containing the
30 plasmid CNCM I-2758 coding for the G protein of CVS-NIV comprise the incubation of said recombinant *E. coli* strain at 37°C on a standard LB-TYM growth medium (in the presence of ampicillin); *cf.* WO 03/048198.

Appropriate conditions for the propagation of the virus I-4140 (recombinant rabies virus) comprise the incubation of said virus at 37°C under 5% CO₂ with BHK-21 cells (sub-

clone BSR) on a DMEM growth medium containing glucose (*e.g.*, 4.5 g/L), sodium pyruvate and glutamax (Invitrogen 31966047) and 5% FBS; *cf.* example 1 below.

Preferably, a variant sequence A of the invention is of 44 amino acids and has the amino acid H or L, preferably H, at position 11 of its 44aa-sequence.

Illustrative variant A polypeptides notably comprise the G protein of the recombinant rabies virus strain deposited on the 1st of April, 2009 at the CNCM under deposit number I-4142 (whose G protein is a single-point mutant protein of the G protein of I-4140; *cf.* Figure 21), the cytoplasmic fragment thereof and the conservative cytoplasmic sub-fragments thereof.

The I-4142 strain differs from the CVS-NIV strain (CNCM I-4140) in that its G protein has amino acid L at position 491 (position computed with respect to the full length G protein), instead of H (*cf.* table 2 below and figure 3D).

Appropriate conditions for the propagation of the virus I-4142 (recombinant rabies virus) comprise the incubation of said virus at 37°C under 5% CO₂ with BHK-21 cells (sub-clone BSR) on a DMEM growth medium containing glucose (*e.g.*, 4.5 g/L), sodium pyruvate and glutamax (Invitrogen 31966047) and 5% FBS; *cf.* example 1 below.

Illustrative variant A polypeptides notably comprise polypeptides comprising the cytoplasmic fragment of SEQ ID NO: 19 or at least one conservative sub-fragment thereof. The sequence of SEQ ID NO: 19 is an illustrative conservative variant of the sequence of SEQ ID NO: 6 (*cf.* Figure 19). It stills has a positive neurite outgrowth effect, although at a lesser level than the sequence of SEQ ID NO: 6 (*cf.* figure 3D and associated comments in the examples section).

Variant sequence B:

A variant sequence B of the invention is of 44 amino acids.

It comprises the sequence of SEQ ID NO: 13 with $x_1 = E$, i.e., the sequence $x_1 - x_2 - x_3 - x_4$, wherein:

x_1 is E, and

x_2 is T or S or I (preferably not I, more preferably T), and

x₃ is any amino acid (preferably R), and
x₄ is L or V (preferably L).

This sequence is a particular PDZ-BS motif. It usually is located at the C-terminal end of
5 the polypeptide, most usually this sequence is the sequence of the very last four C-terminal amino acids of the polypeptide.

Preferably, said sequence of SEQ ID NO: 13 is ETRL (SEQ ID NO: 12).

10 In a variant sequence B of the invention, the amino acid, which is at position 11, is not L. It is most preferably H.

Illustrative variant B polypeptides notably comprise the G protein of the recombinant rabies virus strain deposited on the 1st of April, 2009 at the CNCM under deposit number
15 I-4143 (whose G protein is a single-point mutant protein of the G protein of I-4140; *cf.* Figure 21), the cytoplasmic fragment thereof and the conservative cytoplasmic sub-fragments thereof.

The I-4143 strain differs from the CVS-NIV strain (CNCM I-4140) in that its G protein has amino acid E at position 521 (position computed with respect to the full length G
20 protein), instead of Q (*cf.* table 2 below and figure 3D).

Appropriate conditions for the propagation of the virus I-4143 (recombinant rabies virus) comprise the incubation of said virus at 37°C under 5% CO₂ with BHK-21 cells (sub-clone BSR) on a DMEM growth medium containing glucose (*e.g.*, 4.5 g/L), sodium
25 pyruvate and glutamax (Invitrogen 31966047) and 5% FBS; *cf.* example 1 below.

Illustrative variant B polypeptides notably comprise polypeptides comprising the cytoplasmic fragment of SEQ ID NO: 20 or at least one conservative sub-fragment thereof. The sequence of SEQ ID NO: 20 is an illustrative conservative variant of the sequence of
30 SEQ ID NO: 6 (*cf.* Figure 19). It stills has a positive neurite outgrowth effect, although at a lesser level than the sequence of SEQ ID NO: 6 (*cf.* figure 3D and associated comments in the examples section).

The G proteins of the I-4142 and the I-4143 strains induce a significantly positive neurite outgrowth effect, although this effect is significantly lower than the one of the G protein of the CVS-NIV strain (I-4140); *cf.* example 1 and Figure 3D.

Therefore, each of the two amino acid positions contributes to the neurite outgrowth effect,

5 *i.e.*:

- the amino acid position, which, in the G protein of the CVS-NIV strain, is position 491 (*i.e.*, H in the G protein of the CVS-NIV strain), and
 - the amino acid position, which, in the G protein of the CVS-NIV strain, is the first amino acid of the PDZ-BS of the G protein, *i.e.*, position 521 in the G protein of
- 10 the CVS-NIV strain (*i.e.*, amino acid Q in the G protein of the CVS-NIV strain).

Alternative or complementary definition of a polypeptide of the invention:

A polypeptide of the invention is a pro-neurite outgrowth polypeptide.

- 15 A polypeptide of the invention comprises a parent sequence and/or a conservative variant sequence (variant sequence A or B) and/or a conservative fragment sequence as above-defined.

- Alternatively or complementarily, a polypeptide of the invention can be defined as being
- 20 the G protein of a rabies virus strain, or a variant sequence thereof which derives therefrom by one or several amino acid substitution(s) and/or addition(s) and/or deletion(s), or a fragment sequence of such a G protein or variant G protein, more particularly a cytoplasmic fragment or sub-fragment thereof, provided that said polypeptide has the function of inducing and/or stimulating neurite outgrowth, for
- 25 example as below illustrated, more particularly neurite outgrowth from human pre-mitotic neurons, *e.g.*, from the human neuroblastoma cell line SH-SY5Y (ATCC CRL-2266; please see example 1 below for illustrative experimental conditions and materials).

- More particularly, a polypeptide of the invention can alternatively or complementarily be
- 30 defined as being the G protein of a non-apoptotic rabies virus strain, or a variant sequence thereof which derives therefrom by one or several amino acid substitution(s) and/or addition(s) and/or deletion(s), or a fragment sequence of such a G protein or variant protein, more particularly a cytoplasmic fragment or sub-fragment thereof, provided that said variant sequence or said fragment has retained the function of inducing and/or

stimulating neurite outgrowth, for example as below illustrated, more particularly neurite outgrowth from human pre-mitotic neurons, *e.g.*, from the human neuroblastoma cell line SH-SY5Y (ATCC CRL-2266; please see example 1 below for illustrative experimental conditions and materials).

5

Illustrative pro-neurite outgrowth proteins, which are not the G protein of the CVS-NIV strain and/or which are other than the protein coded by the plasmid available from the CNCM under deposit number I-2758, and/or which are other than the protein of SEQ ID NO: 2, but which still are suitable pro-neurite outgrowth polypeptides notably comprise the G proteins of the recombinant rabies virus strains deposited on the 1st of April, 2009 at the CNCM under deposit numbers I-4142 and I-4143.

10

The I-4142 strain differs from the CVS-NIV strain (CNCM I-4140) in that its G protein has amino acid L at position 491 (position computed with respect to the full length G protein), instead of H (*cf.* table 2 below and figure 3D).

15

The I-4143 strain differs from the CVS-NIV strain (CNCM I-4140) in that its G protein has amino acid E at position 521 (position computed with respect to the full length G protein), instead of Q (*cf.* table 2 below and figure 3D).

The G proteins of the I-4142 and the I-4143 strains induce a significantly positive neurite outgrowth effect, although this effect is significantly lower than the one of the G protein of the CVS-NIV strain (I-4140).

20

Therefore, each of the two amino acid positions contributes to the neurite outgrowth effect, *i.e.*:

- the amino acid position, which, in the G protein of the CVS-NIV strain, is position 491 (*i.e.*, H in the G protein of the CVS-NIV strain), and
- the amino acid position, which, in the G protein of the CVS-NIV strain, is the first amino acid of the PDZ-BS of the G protein, *i.e.*, position 521 in the G protein of the CVS-NIV strain (*i.e.*, amino acid Q in the G protein of the CVS-NIV strain).

25

Hence, illustrative pro-neurite outgrowth polypeptides comprise the G protein of the I-4142 strain and the G protein of the I-4143 strain, as well as the fragments of these proteins, more particularly the cytoplasmic fragments thereof (SEQ ID NO: 19 and SEQ ID NO: 20 in Figure 19) and the cytoplasmic sub-fragments thereof, provided that these sub-fragments have retained the function of inducing and/or stimulating neurite outgrowth, for example as below illustrated, more particularly neurite outgrowth from human pre-

30

mitotic neurons, *e.g.*, from the human neuroblastoma cell line SH-SY5Y (ATCC CRL-2266; please see example 1 below for illustrative experimental conditions and materials).

5 The invention more particularly relates to said polypeptide, for use in stimulating and/or inducing neurite outgrowth, more particularly for use in the treatment and/or palliation and/or prevention of a disease, disorder or condition involving an insufficient or impaired neurite outgrowth, more particularly an insufficient or impaired neuritogenesis.

10 Preferably, the amino acid length of said polypeptide is of less than 100 amino acids, more preferably of less than 90 amino acids.

The expression "non-apoptotic rabies virus G protein" is herein intended according to its ordinary meaning in the field.

15 The PDZ-BS of a non-apoptotic rabies virus G protein is of SEQ ID NO: 14.

A non-apoptotic rabies virus G protein can be additionally or alternatively characterized by the fact that its sequence is the sequence of a G protein of a rabies virus strain, and that it does not trigger the apoptosis of human neurons.

20 Illustrative means to check that a candidate rabies virus G protein is a non-apoptogenic one are known to the person of ordinary skill in the art.

25 One of the means comprises checking that the rabies virus strain, which comprises this G protein, is a non-apoptotic strain when it infects neurons, more particularly human neurons, such as SK-N-SH neuroblastoma cell line (ATCC HTB11) or the SH-SY5Y neuroblastoma cell line (ATCC CRL-2266), preferably the SK-N-SH neuroblastoma cell line (ATCC HTB11). Such means are notably useful when a naturally-occurring rabies virus strain comprising the candidate G protein is available for analysis.

30 Other means comprises genetically engineering cells to make them express the candidate G protein, infecting neuron cells with said expressed candidate G protein, and determining that apoptosis is not induced by said infection.

An illustration of such means is described in Préhaud *et al.* 2003.

Illustrative genetically engineering cells comprise the transgenic Jurkat cell line that is described in Préhaud *et al.* 2003, more particularly at page 10538 (*cf.* § “inducible transgenic cell lines”).

5 Illustrative neuron cells comprise the SK-N-SH neuroblastoma cell line (ATCC HTB11) or the SH-SY5Y neuroblastoma cell line (ATCC CRL-2266), preferably the SK-N-SH neuroblastoma cell line (ATCC HTB11).

Illustrative experimental conditions to make the genetically engineering cells infect the neuron cells comprise those described in Préhaud *et al.* 2003, more particularly at page 10538 (*cf.* § “inducible transgenic cell lines”).

10

Detection that the rabies virus strain or the genetically engineered cell does not induce the apoptosis of the neurons cells is within the ambit of the person of average skill in the art.

Illustrative means comprise those described in Préhaud *et al.* 2003 and Préhaud *et al.* 2005.

15 Illustrative means comprise detecting that no significant DNA fragmentation is induced, *e.g.*, by Hoechst staining (*cf.* Préhaud *et al.* 2003, more particularly at page 10538, § “Detection of nuclear fragmentation by Hoechst staining”), and/or by the TUNEL method (*cf.* Préhaud *et al.* 2003, more particularly at page 10538 (*cf.* § “Detection of nuclear fragmentation by the TUNEL method”), and/or by DNA electrophoresis (*cf.* Préhaud *et al.* 2005).

20 Additional or alternate illustrative means to detect that no significant DNA fragmentation is induced comprise the detection that caspase 8 is not activated, *e.g.*, following the procedure described in Préhaud *et al.* 2003, more particularly at page 10538, § “Detection of caspase activation”), with the proviso that that the neuron cells used should then contain caspase 8, which is the case of the SK-N-SH cell line.

25

Illustrative values of non significant apoptosis are shown in Figure 5D of Préhaud *et al.* 2003: *cf.* penultimate column with the heading “JrtTA-G-CVS”:

- only 19% of apoptotic cells as measured by Hoechst staining;
 - only 4.7% of apoptotic cells as measured by assessment of the activation of
- 30 caspase 8.

The rabies virus strains which are attenuated strains (*i.e.*, non pathogenic, *e.g.*, non pathogenic when injected intramuscularly in immunocompetent mice), such as the

attenuated ERA strain, the RV194-2 strain, the AVO-1 strain, the SN10 strain, the SN-10-SAD strain, the SAG2 strain, are apoptogenic strains.

Those rabies virus strains, which are pathogenic, *i.e.*, *in vivo* neurovirulent (such as the
5 CVS-NIV strain) are non-apoptotic.

Therefore, a non-apoptogenic rabies virus strain is a pathogenic (neurovirulent) strain (*e.g.*, pathogenic when injected intramuscularly in immunocompetent mice).

The current knowledge is that:

- 10 - when a non-apoptogenic rabies virus strain infect a human neuron cell, the G protein it encodes accumulates in the cytoplasm of said neuron cell under the form of perinuclear globular structures, and is not diffusively distributed in the cytoplasm of said neuron cell;
- and that, to the contrary, when an apoptogenic rabies virus infect a human neuron
15 cell, the G protein it encodes does not accumulate in the cytoplasm of said neuron cell under the form of perinuclear globular structures, but is diffusively distributed in the cytoplasm of said neuron cell.

As above-mentioned and below illustrated, a polypeptide of the invention can be the G
20 protein of a rabies virus strain.

Preferably, said rabies virus G protein is:

i. the G protein of the CVS-NIV strain, said strain being available from the CNCM under deposit number I-4140 (the deposit date being the 1st of April, 2009), and/or the
25 protein coded by the plasmid available from the CNCM under deposit number I-2758, (the deposit date being the 30th of November, 2001) and/or the protein of SEQ ID NO: 2; or

ii. a variant protein of said rabies virus G protein of i., wherein said variant protein still is a rabies virus G protein, and wherein the sequence of the PDZ-BS motif of said variant rabies virus G protein still is the sequence of SEQ ID NO: 14.

30

Preferably, said rabies virus G protein is:

i. the G protein of the CVS-NIV strain which is available from the CNCM under deposit number I-4140, and/or the protein coded by the plasmid available from the CNCM under deposit number I-2758, and/or the protein of SEQ ID NO: 2; or

ii. a variant protein of said rabies virus G protein of i., wherein:

- said variant protein consists of a sequence which is at least 95% identical (preferably at least 96%, more preferably at least 97%, still more preferably at least 97.5%, even still more preferably at least 98%, most preferably at least 98.5%, still most preferably at least 99%) to the sequence of said rabies virus G protein of i. over the entire length of the sequence of said rabies virus G protein of i., and
- the sequence of the PDZ-BS motif of said variant rabies virus G protein is the sequence of SEQ ID NO: 14.

Preferably, said rabies virus G protein is:

i. the G protein of the CVS-NIV strain which is available from the CNCM under deposit number I-4140, and/or the protein coded by the plasmid available from the CNCM under deposit number I-2758, and/or the protein of SEQ ID NO: 2; or

ii. a variant protein of said rabies virus G protein of i., wherein said variant protein still is a rabies virus G protein, and wherein:

- said variant protein consists of a sequence which is at least 95% identical (preferably at least 96%, more preferably at least 97%, still more preferably at least 97.5%, even still more preferably at least 98%, most preferably at least 98.5%, still most preferably at least 99%) to the sequence of said rabies virus G protein of i. over the entire length of the sequence of said rabies virus G protein of i., and
- the sequence of the PDZ-BS motif of said variant rabies virus G protein is the sequence of SEQ ID NO: 14.

Preferably, said rabies virus G protein is:

i. the G protein of the CVS-NIV strain which is available from the CNCM under deposit number I-4140, and/or the protein coded by the plasmid available from the CNCM under deposit number I-2758, and/or the protein of SEQ ID NO: 2; or

ii. a variant protein of said rabies virus G protein of i., wherein said variant protein still is a rabies virus G protein, and wherein said variant protein consists of a sequence which:

- is at least 95% identical (preferably at least 96%, more preferably at least 97%, still more preferably at least 97.5%, even still more preferably at least 98%,

most preferably at least 98.5%, still most preferably at least 99%) to the sequence of said rabies virus G protein of i. over the entire length of the sequence of said rabies virus G protein of i., and

- 5 - has retained the PDZ-BS motif of SEQ ID NO: 14 of said rabies virus G protein of i. and/or contains a PDZ-BS motif, the sequence of which is QTRL (SEQ ID NO: 14 with $x_1=Q$; $x_2=T$; $x_3=R$; $x_4=L$).

Preferably, the amino acid length of said variant rabies virus G protein of ii. does not exceed the length of said rabies virus G protein of i. of more than 50 amino acids.

- 10 Preferably, the amino acid length of said variant rabies virus G protein of ii. is no more than 50 amino acid lower than the length of said rabies virus G protein of i., for example of the same length as the sequence of said rabies virus G protein of i.

- More preferably, the amino acid length of said variant rabies virus G protein of ii. is comprised is at least the length of said rabies virus G protein of i. minus 50 amino acids, and of at most the length of said rabies virus G protein of i. plus 50 amino acids, for example of the same length as the sequence of said rabies virus G protein of i.
- 15

- Illustrative pro-neurite outgrowth rabies virus G proteins, which are not the G protein of the CVS-NIV strain (I-4140) and/or which are other than the protein coded by the plasmid available from the CNCM under deposit number I-2758, and/or which are other the protein of SEQ ID NO: 2, but which still are suitable pro-neurite outgrowth rabies virus G proteins notably comprise the G proteins of the recombinant rabies virus strain deposited on the 1st of April, 2009 at the CNCM under deposit number I-4142.
- 20

- The I-4142 strain differs from the CVS-NIV strain (CNCM I-4140) in that its G protein has amino acid L at position 491 (position computed with respect to the full length G protein), instead of H (*cf.* table 2 below and figure 3D).
- 25

An illustrative fragment of the sequence of SEQ ID NO: 19 (*cf.* figure 19).

Other sequences that may additionally be present:

30

As above-mentioned and below illustrated, a polypeptide of the invention comprises an amino acid sequence, which has an effect of pro-neurite outgrowth (and/or of neurite spouting and/or of axon growth and/or of dendritic tree extension).

As above-mentioned and below illustrated, said pro-neurite outgrowth sequence can be defined as a parent sequence and/or a conservative variant sequence (variant sequence A or B) and/or a conservative fragment sequence as above-defined.

As above-mentioned and below illustrated, said pro-neurite outgrowth sequence can,
5 alternatively or complementarily, be defined as being the sequence of the G protein of a (non-apoptotic) rabies virus strain (*e.g.*, the G protein of CNCM I-4140, I-4142 or I-4143), or a variant sequence thereof, which derives therefrom by one or several amino acid substitution(s) and/or addition(s) and/or deletion(s), or a fragment sequence of such a G protein or variant G protein, more particularly a cytoplasmic fragment or sub-fragment
10 thereof.

Illustrative examples of such fragments of G protein or variant G protein notably comprise those fragments, which have retained the cytoplasmic domain and the transmembrane domain of said G protein or variant G protein.

Such a transmembrane domain notably has the advantage of anchoring the cytoplasmic
15 fragment in intracellular compartment(s) of the cells, more particularly in the endoplasmic reticulum and/or the Golgi membrane of the cells, whereby said cytoplasmic fragment exerts more efficiently its effects of stimulation and/or induction of neurogeneration, neuroregeneration and neuroprotection. Please see examples 4 and 5 below.

20 Hence, in addition to said pro-neurite outgrowth sequence, a polypeptide of the invention may further comprise a sequence, which anchors said polypeptide in the endoreticulum membrane and/or in the Golgi membrane of cells, more particularly of neuronal cells, more particularly of human neuronal cells, said anchoring sequence being preferably at the N-terminal end of said pro-neurite outgrowth sequence, most preferably directly linked to
25 the first amino acid at the N-terminal end of said pro-neurite outgrowth sequence.

Hence, in addition to said pro-neurite outgrowth sequence, a polypeptide of the invention may further comprise an amino acid sequence, which anchors said polypeptide in the endoreticulum membrane and/or in the Golgi membrane (preferably in the endoreticulum
30 membrane and in the Golgi membrane) of cells, more particularly of neuronal cells, more particularly of human neuronal cells (*e.g.*, the human neuroblastoma cell line SH-SY5Y cell line as described in the examples below, *e.g.*, example 4).

Said anchoring sequence preferably is at the N-terminal end of said sequence of SEQ ID NO: 6 or variant sequence A or variant sequence B. Most preferably, said anchoring

sequence is directly or indirectly, preferably directly, linked to the first amino acid at the N-terminal end of said sequence of SEQ ID NO: 6 or of said variant sequence A or of said variant sequence B.

Such an anchoring sequence may *e.g.*, be an amino acid sequence, which has the capacity
5 of anchoring the cytoplasmic domain of SEQ ID NO: 6 in the endoreticulum membrane and/or in the Golgi membrane of cells, more particularly of neuronal cells, more particularly of human neuronal cells (*e.g.*, the human neuroblastoma cell line SH-SY5Y cell line as described in the examples below, *e.g.*, example 4).

Illustrative of such an anchoring sequence is the transmembrane domain sequence of the G
10 protein of a rabies virus strain (*e.g.*, a non-apoptotic rabies virus strain), preferably the transmembrane domain sequence of the G protein of rabies virus CVS-NIV, which is of SEQ ID NO: 23.

Hence, in addition of said sequence of SEQ ID NO: 6 or of said variant sequence A or of said variant sequence B, said polypeptide may further comprise, preferably at the N-
15 terminal end of said sequence of SEQ ID NO: 6 or variant sequence A or variant sequence B, most preferably directly linked to the first amino acid at the N-terminal end of said sequence of SEQ ID NO: 6 or of said variant sequence A or of said variant sequence B:

- the sequence of the transmembrane domain of the G protein of a rabies virus strain, more particularly of a non-apoptotic rabies virus strain, or
- 20 - a sequence, which differs from said sequence of rabies virus G transmembrane domain by at least one amino acid substitution and/or deletion and/or addition, preferably by amino acid substitution(s) and/or deletion(s), more preferably by amino acid substitution(s), but which has retained the capacity of anchoring said polypeptide (more particularly the capacity of anchoring the cytoplasmic domain of SEQ ID NO: 6 -when
25 said anchoring sequence is directly linked to the first amino acid at the N-terminal end of said sequence of SEQ ID NO: 6-), in the endoreticulum membrane and/or in the Golgi membrane of cells, more particularly of neuronal cells, more particularly of human neuronal cells (*e.g.*, the human neuroblastoma cell line SH-SY5Y cell line as described in the examples below, *e.g.*, example 4).

30

Hence, in addition to said pro-neurite outgrowth sequence, a polypeptide of the invention may further comprise, preferably at the N-terminal end of said sequence of SEQ ID NO: 6 or variant sequence A or variant sequence B, most preferably directly linked to the first

amino acid at the N-terminal end of said sequence of SEQ ID NO: 6 or of said variant sequence A or of said variant sequence B:

- the sequence of the transmembrane domain of the G protein of a rabies virus strain, more particularly of a non-apoptotic rabies virus strain, or
- 5 - a sequence, which differs from said sequence of rabies virus G transmembrane domain by at least one amino acid substitution and/or deletion and/or addition, preferably by amino acid substitution(s) and/or deletion(s), more preferably by amino acid substitution(s), but which has retained the capacity of anchoring the polypeptide of SEQ ID NO: 6 (when said anchoring sequence is directly linked to the first amino acid at the N-
- 10 terminal of said sequence of SEQ ID NO: 6), in the endoreticulum membrane and/or in the Golgi membrane of cells, more particularly of neuronal cells, more particularly of human neuronal cells.

Preferred examples of such anchoring sequences notably comprise:

- 15 - the sequence of the transmembrane domain of the G protein of a rabies virus strain, more particularly of a non-apoptotic rabies virus strain, still more particularly of the CVS-NIV strain, for example the sequence of SEQ ID NO: 23 , or
- a variant sequence thereof, which is of 18 to 26 amino acids, preferably of 18 to 22 amino acids, more preferably of 22 amino acids, and which is at least 94% identical to
- 20 said sequence of SEQ ID NO: 23 over the shortest of the two sequences (*i.e.*, over the shortest of SEQ ID NO: 23 and of said variant sequence).

Such an anchoring sequence is particularly useful when it is linked to a pro-neurite outgrowth sequence that is the sequence of SEQ ID NO: 6 or said variant sequence A,

25 more particularly the sequence of SEQ ID NO: 6.

Hence, according to an embodiment of the invention, the amino acid sequence of a polypeptide of the invention comprises, or consists of the sequence of SEQ ID NO: 23 followed by (from N-terminal to C-terminal end) the sequence of SEQ ID NO: 6, wherein

30 one to four amino acids, preferably one amino acid (*e.g.*, M) is/are optionally present between said sequence of SEQ ID NO: 23 and said sequence of SEQ ID NO: 6.

Illustrative of such a polypeptide is the polypeptide of SEQ ID NO: 25.

In addition to said pro-neurite outgrowth sequence, a polypeptide of the invention may further comprise:

- a fragment of the ectodomain of the G protein of a rabies virus strain, more particularly of a non-apoptotic rabies virus strain, still more particularly of the CVS-NIV strain, and/or
- 5 - a signal peptide, this signal peptide being preferably the signal peptide of the G protein of a rabies virus strain, more particularly of a non-apoptotic rabies virus strain, still more particularly of the CVS-NIV strain.

Said ectodomain fragment and/or signal peptide may be present in said polypeptide of the invention in addition to said anchoring sequence.

- 10 Preferably, when a signal peptide is comprised in said polypeptide, said signal peptide is the first component at the N-terminal end of the polypeptide.

Preferably, when a signal peptide and an ectodomain fragment are comprised in said polypeptide, said ectodomain fragment is comprised in said polypeptide of the invention between said signal peptide and said pro-neurite outgrowth sequence, preferably between
15 said signal peptide and any possibly anchoring sequence that may be comprised in said polypeptide.

According to an embodiment of the invention, a polypeptide of the invention comprises, or consists of, from its N-terminal end to its C-terminal end:

- 20 said signal peptide (*e.g.*, SEQ ID NO: 21), said anchoring sequence (*e.g.*, SEQ ID NO: 23) and said pro-neurite outgrowth sequence.

According to another embodiment of the invention, a polypeptide of the invention comprises, or consists of, from its N-terminal end to its C-terminal end:

- 25 said signal peptide (*e.g.*, SEQ ID NO: 21), said ectodomain fragment (*e.g.*, the two amino acids GK), said anchoring sequence (*e.g.*, SEQ ID NO: 23) and said pro-neurite outgrowth sequence.

Preferably, said ectodomain fragment is one to four amino acids, more preferably of 2 amino acids.

- 30 Preferably, said ectodomain fragment is a fragment of the C-terminal end of said ectodomain, more preferably the last one to four, more particularly the last two amino acids at the C-terminal end of said ectodomain.

Hence, the amino acid sequence of a polypeptide of the invention may further comprise, (directly or indirectly, preferably directly) linked to the first amino acid at the N-terminal end of said anchoring sequence:

one to four amino acids, preferably two amino acids, more preferably one to four amino acids from the C-terminal end of the ectodomain of the G protein of a rabies virus (*e.g.*, a non-apoptotic rabies virus strain), still more preferably the last two amino acids of the C-terminal end of the ectodomain of the G protein of a rabies virus (*e.g.*, a non-apoptotic rabies virus strain), for example amino acids GK.

5 Examples of such a polypeptide notably comprise the polypeptide of SEQ ID NO: 26 and the polypeptides, which comprise the sequence of SEQ ID NO: 26.

Hence, a polypeptide of the invention may further comprise a signal peptide, preferably the signal peptide of the G protein of a rabies virus strain (*e.g.*, a non-apoptotic rabies virus strain), said peptide sequence being preferably at the N-terminal end of said polypeptide (most preferably at the very N-terminal end of said polypeptide, *i.e.*, in N-term from any anchoring sequence that may be comprised in said polypeptide).

10 Examples of such a polypeptide notably comprise the polypeptide of SEQ ID NO: 27 and the polypeptides, which comprise the sequence of SEQ ID NO: 27.

15

Examples of preferred polypeptides:

Preferred polypeptides of the invention comprise polypeptides, which are of less than 100 amino acids, more preferably of as few as amino acids as possible while still retaining a neurite outgrowth effect, more preferably of less than 90 amino acids.

20

Preferred polypeptides of the invention comprise polypeptides, which comprise, or consist of the cytoplasmic fragments of said G proteins, most preferably the cytoplasmic fragment of SEQ ID NO: 6, SEQ ID NO: 19 or SEQ ID NO: 20 (*cf.* figure 19).

25

Preferred polypeptides of the invention also comprise polypeptides, which comprise, or consist of the 11-44 sub-fragments of these cytoplasmic fragments.

Preferred polypeptides of the invention comprise polypeptides, which, in addition to said pro-neurite outgrowth sequence, comprise at least one anchoring sequence as above-defined (*e.g.*, the anchoring sequence of SEQ ID NO: 23; please see examples 4-6 below).

30

Nucleic acids, Vectors and Cells:

The invention also relates to any nucleic acid, more particularly to any DNA or RNA, which codes for a polypeptide of the invention, in accordance with the universal genetic code, taking due account of its degeneracy.

As shown in Figure 15, an illustrative nucleic acid that codes for the cytoplasmic fragment of SEQ ID NO: 6 (*i.e.*, the cytoplasmic fragment of the CVS-NIV strain) is the nucleic acid of SEQ ID NO: 5.

As shown in Figure 16, an illustrative nucleic acid that codes for the PDZ-BS of SEQ ID NO: 10 (*i.e.*, the PDZ-BS of the CVS-NIV strain) is the nucleic acid of SEQ ID NO: 9.

10 The invention also relates to any nucleic acid vector, which comprises at least one nucleic acid coding for a polypeptide of the invention. Said vector can be a transfection and/or expression vector.

Said expression vector may further comprise at least one expression signal and/or regulatory sequence upstream and/or downstream of said nucleic acid, such as at least one promoter, or at least one enhancer and at least one promoter, upstream of said nucleic acid. Said expression vector may for example be a plasmid.

Illustrative of such vectors is the plasmid available from the CNCM under deposit number I-2758.

Interestingly, lentivirus vectors, AAV vectors, adeno-virus vectors and Herpes Simplex disabled virus vectors can be used.

Alternatively or complementarily, a nucleic acid of the invention can be coupled to, or otherwise associated with, a dendrimer such as liposomes, or a cationic polymer such as DEAE-dextrane or polyethylenimine, or an inert solid nanoparticle, such as a gold particle, or any other transfection means that the person of average skill in the art may find appropriate.

The invention also relates to any cell, which comprises at least one polypeptide of the invention, and/or at least nucleic acid of the invention and/or at least one vector of the invention.

Said cell preferably is a cell, which has been genetically engineered to comprise said polypeptide and/or nucleic acid and/or vector.

Said cell can be a eukaryotic cell, preferably a mammal cell, for example a human cell or a non-human cell, most preferably a human cell.

Said cell can be a prokaryotic cell, preferably a bacterium, for example *E. coli*.

Said cell can be a virion, provided said virion does not induce any significantly deleterious or undesirable effect to the recipient subject, more particularly to a human patient.

Preferably, said cell is not a human embryonic cell.

5

Uses:

The invention more particularly relates to said polypeptide and/or nucleic acid and/or vector and/or cell, for use in inducing and/or stimulating neurite outgrowth, more particularly in the treatment and/or palliation and/or prevention of a disease, disorder or
10 condition involving an insufficient or impaired neuritogenesis, more particularly an insufficient or impaired neurite outgrowth.

Said use in a non-immunogenic use.

15 Indeed, those rabies virus G proteins, which are appropriate to the invention, can be defined as non-apoptotic G proteins.

Therefore, the polypeptide of the invention does not induce the formation of apoptotic bodies such as those that have been described in the previously-published PCT international application WO 03/048198.

20

In accordance with the invention, the polypeptide of the invention is intended as an effector of neurite outgrowth (and/or of axon and/or dendrite development), *e.g.*, for neuron differentiation from neuron progenitors or neoplastic neurons, and/or for neuron regeneration of impaired neurons (both effects being obtained through stimulation of
25 neurite outgrowth).

Therefore, the polypeptide of the invention is not an immunogenic agent or adjuvant, or at the very least it is not used as an immunogenic agent or adjuvant and it is not used under conditions which would enable said polypeptide to act as an immunogenic agent or
30 adjuvant.

More particularly, contrary to the G proteins of apoptotic rabies virus strains, such as the G proteins of the attenuated ERA strain and of the other attenuated rabies virus strains, the polypeptide of the invention does not raise a detectable humoral immune response when it is administered to neurons.

The polypeptide of the invention is not used in a pharmaceutical composition or drug, which would be an immunogenic composition or a vaccine.

- 5 Accordingly, the polypeptide of the invention is preferably not coupled to, not fused to and not associated with any antigen, more particularly with any antigen that would be a viral antigen, a tumor antigen, a cell antigen, the over-expression or alteration of which would lead to a pathology such as a neuropathology.

10 Diseases, disorders and conditions:

The invention provides polypeptides, nucleic acids, vectors and cells. These products induce and/or stimulate neuritogenesis, more particularly neurite outgrowth, still more particularly human neurite outgrowth.

- 15 A product of the invention preferably is a nucleic acid vector of the invention, more particularly a plasmid of the invention.

- More particularly, a product of the invention induces and/or stimulates neuritogenesis, more particularly neurite outgrowth from pre-mitotic neurons, neoplastic neurons, neuron
20 progenitors, as well as from impaired neurons.

Therefore, the invention relates to said product, for use as a neurogenerative and/or neuroregenerative and/or neuroprotective agent.

- A product of the invention stimulates and/or induces neurite sprouting and/or axon growth
25 and/or dendritic tree extension.

A product of the invention stimulates the activity of the growth cone. Furthermore, it prevents growth cone from collapsing upon contact with a growth collapsing agent, such as LPA or oxidative stress (*cf.* example 1 below).

- 30 A product of the invention consequently stimulates and/or induces synaptogenesis and/or neurotransmission.

A product of the invention is an agent that inhibits the proliferation of neoplastic neurons, more particularly as a neuro-differentiating agent.

A product of the invention is an agent that stimulates neuronal development and/or neuronal regeneration and/or axon growth and/or dendrite development and/or dendritic tree extension and/or neuronal plasticity and/or synaptogenesis and/or neurotransmission.

5

A product of the invention is an agent that prevents and/or inhibits and/or blocks any kind of neurotoxicity which would lead to neurite retraction and/or growth cone collapse.

A product of the invention is an agent that stimulates and/or induces neurite outgrowth and/or growth cone activity after said neurite and/or cone has been in contact with a neurotoxic agent.

10

A product of the invention is an agent that prevents and/or inhibits and/or blocks growth cone collapse and/or neurite retraction and/or axodendritic damage or lesion and/or disruption of synaptic integrity and/or loss of neuron connectivity and/or damage to nerve endings and/or neurotransmission impairment.

15

A product of the invention is a means to induce and/or stimulate neurite outgrowth, which is notably useful:

20

- in inducing neuron differentiation, for example in the treatment and/or palliation and/or prevention of a neoplasm of the nervous system, as well as
- in regenerating impaired neurons, more particularly impaired neurites, for example in the treatment and/or palliation and/or prevention of a neurodegenerative disease, disorder or condition, in the treatment and/or palliation and/or prevention of microbial infections of the neurons, or in protecting neurons from neurotoxic agents or oxidative stress.

25

Therefore, the invention relates to said product, for use in the treatment and/or palliation and/or prevention of any disease, disorder or condition which involves an insufficient or impaired neuritogenesis, more particularly an insufficient or impaired neurite outgrowth.

30

Said disease, disorder or condition is alternatively or complementarily defined as any disease, disorder or condition involving an unbalanced neuron cell cycle, wherein said neuron cell cycle is unbalanced:

- either by excessive or undesired presence of pre-mitotic neurons (more particularly, by insufficient neuron differentiation and/or by excessive or undesired re-entry of post-mitotic neurons into the neuron cell cycle, as is the case when a neoplasm develops in the nervous system),
- 5 - or by excessive or undesired neuron degeneration, more particularly excessive or undesired neurite degeneration (as is the case for a neurodegenerative disease, disorder or condition, and for certain microbial infection of the neurons).

10 A product of the invention can be used in the treatment and/or palliation and/or prevention of a disease, disorder or condition, which alters the Central Nervous System (CNS) and/or the Peripheral Nervous System (PNS), for example as a neurorestorative therapy and/or prevention and/or palliation.

15 The expression "Central Nervous System" or "CNS" is herein intended as meaning the brain and (in case of a vertebrate animal) the spinal cord.

The peripheral nervous system (PNS) is the vast network of spinal and cranial nerves linking the body to the brain and spinal cord. The PNS is subdivided into the autonomic nervous system (sympathetic NS and parasympathetic NS) and the somatic nervous system. The PNS consists of sensory neurons running from stimulus receptors to the CNS
20 and motor neurons running from the CNS to the muscle and glands.

According to an embodiment of the invention, said disease, disorder or condition is or involves a microbial infection of the nervous system, such as a bacterial and/or viral infection, more particularly a viral infection.

25 Said viral infection can for example be a Herpes Virus Simplex (HSV) infection, more particularly a HSV type 1 infection (which leads to viral encephalopathy).

Said microbial infection can be a viral infection, which does not induce neuron apoptosis, such as a rabies virus infection.

Preferably, said microbial infection is a microbial infection that induces neuron apoptosis,
30 such as poliomyelitis (*cf.* Blondel *et al.*, 2005).

According to another embodiment of the invention, said disease, disorder or condition is or involves a non-viral disease, disorder or condition, more preferably a non-bacterial and

non-viral disease, disorder or condition, still more preferably a non-microbial disease, disorder or condition.

5 According to an embodiment of the invention, said disease or disorder is or involves a neurodegenerative disease or disorder (for example, a chronic neurodegenerative disease or disorder), such as non-viral encephalopathy, Alzheimer's disease, Parkinson's disease, ALS, Huntington disease, multiple sclerosis (MS) or rare genetic disease.

10 Preferably, said neurodegenerative disease or disorder is a non-viral disease or disorder, more preferably a non-bacterial and non-viral disease or disorder, still more preferably a non-microbial disorder.

According to an embodiment of the invention, said condition is or involves a neurodegenerative condition, such as aging.

15 Preferably, said neurodegenerative condition is a non-viral condition, more preferably a non-bacterial and non-viral condition, still more preferably a non-microbial condition.

20 According to an embodiment of the invention, said disease, disorder or condition is or involves a physical or ischemic injury of the nervous system, such as seizure, stroke, trauma, epilepsy.

Preferably, said physical or ischemic injury is a non-viral disease, disorder or condition, more preferably a non-bacterial and non-viral disease, disorder or condition, still more preferably a non-microbial disease, disorder or condition.

25 According to an embodiment of the invention, said disease, disorder or condition involves the presence of a chemical neurotoxic agent and/or of an oxidative stress.

Preferably, said disease, disorder or condition is a non-viral disease, disorder or condition, more preferably a non-bacterial and non-viral disease, disorder or condition, still more preferably a non-microbial disease, disorder or condition.

30

According to an embodiment of the invention, said disease is a neoplasm, more particularly a neoplasm which comprises neoplastic neurons.

The term “neoplasm” is herein more particularly intended as a malignant neoplasm, more particularly a cancer, still more particularly a tumor or a leukaemia, even still more particularly a tumor.

5 A product of the invention does not act as an immunogenic agent. More particularly, a product of the invention does not act as an immunogenic agent, which would raise a humoral response against tumor antigens.

Preferably, a product of the invention cannot, on and of its own, act as an immunogenic agent. More particularly, a product of the invention cannot, on and of its own, act as an immunogenic agent, which would raise a humoral response against tumor antigens.

10

A product of the invention acts as an antiproliferative agent.

A product of the invention induces and/or stimulates the neurite outgrowth from neoplastic neurons, thereby inducing and/or stimulating the differentiation of neoplastic neurons into mature neurons.

15

Said neoplasm can be a neoplasm of the CNS and/or PNS, preferably a ganglioglioma, a brain tumor, a central neurocytoma, a medulloblastoma, an ependymoma, a teratoma, a neuroblastoma.

20 Preferably, said neoplasm is a non-viral neoplasm, more preferably a non-bacterial and non-viral neoplasm, still more preferably a non-microbial neoplasm

Any administration mode that the skilled person may find appropriate is encompassed by the present invention.

25 Depending on how the product of the invention is formulated, it can administered by parenteral or enteral (*e.g.*, oral) administration, preferably by parenteral administration, more preferably by parenteral injection.

Pharmaceutical composition or drug; method of treatment:

30

The invention also relates to any pharmaceutical composition or drug, which comprises at least one polypeptide of the invention and/or at least nucleic acid of the invention and/or at least one vector of the invention and/or at least one cell of the invention.

The pharmaceutical composition or drug of the invention can be used for the treatment and/or palliation and/or prevention of a disease, disorder or condition involving an insufficient or impaired neurite outgrowth as above-described in more details.

5 The pharmaceutical composition or drug of the invention is not an immunogenic composition and is not a vaccine.

10 The pharmaceutical composition or drug of the invention may further comprise at least one pharmaceutically and/or physiologically acceptable vehicle (diluent, excipient, additive, pH adjuster, emulsifier or dispersing agent, preservative, surfactant, gelling agent, as well as buffering and other stabilizing and solubilizing agent, etc.).

The pharmaceutical composition or drug of the invention does preferably not contain any immune adjuvant.

15 Most preferably, the pharmaceutical composition or drug of the invention does not comprise any antigen, more particularly any antigen that would be a viral antigen, a tumor antigen, a cell antigen, the over-expression or alteration of which would lead to a pathology such as a neuropathology.

20 The pharmaceutical composition or drug of the invention can for example be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. Preferably, it is formulated under a form suitable for parenteral administration.

25 The invention also relates to a method of treatment of a subject, more particularly of a human being, in need thereof, which comprises administering to said subject or human being at least one polypeptide of the invention and/or at least nucleic acid of the invention and/or at least one vector of the invention and/or at least one cell of the invention as above-described.

30 In the application, the term "comprising", which is synonymous with "including" or "containing", is open-ended, and does not exclude additional, unrecited element(s), ingredient(s) or method step(s), whereas the term "consisting of" is a closed term, which excludes any additional element, step, or ingredient which is not explicitly recited.

The term "essentially consisting of" is a partially open term, which does not exclude additional, unrecited element(s), step(s), or ingredient(s), as long as these additional

element(s), step(s) or ingredient(s) do not materially affect the basic and novel properties of the invention.

The term “comprising” (or “comprise(s)”) hence includes the term “consisting of” (“consist(s) of”), as well as the term “essentially consisting of” (“essentially consist(s) of”).

5 Accordingly, the term “comprising” (or “comprise(s)”) is, in the present application, meant as more particularly encompassing the term “consisting of” (“consist(s) of”), and the term “essentially consisting of” (“essentially consist(s) of”).

Each of the relevant disclosures of all references cited herein is specifically incorporated
10 by reference. The following examples are offered by way of illustration, and not by way of limitation.

EXAMPLES

15 **EXAMPLE 1: The cytoplasmic domain of the protein G of the non-apoptotic CVS-NIV rabies virus strain (cytoplasmic domain of G-CVS-NIV or of “G-survival”) induces neurite outgrowth, and the induced neurite outgrowth is highly resistant to growth cone collapsing agent (*i.e.*, LPA) and to oxidative stress (H₂O₂).**

20 Neuroregeneration and neuroprotection are common milestones in fields as large as neurotoxicity, neurodegenerative diseases, trauma-seizure-stroke, encephalopathy or even more aging at large (*cf.* Figure 1).

Immature human neurons, such as neuroblastoma cells, are able to differentiate further if
25 treated with the appropriate signaling molecules. This is the case for the SH-SY5Y cells when they are treated with the cell permeable db-cAMP.

The inventors have developed a functional neuroprotection test, which involves the use of a human neuroblastoma cell line, such as the human neuroblastoma cell line SH-SY5Y. In
30 this system, cells can be further differentiated and the outgrowth of the neurites can be monitored, which allows testing for growth cone activity stimulating effectors. Moreover, the ability of elongated neurites to fight against retraction processes can be evaluated after drug treatment such as lysophosphatidic acid (LPA) or hydrogen peroxide (H₂O₂). Data

gathered with such systems have been proved to be correlated with neurotoxicity analyses made *in vivo*.

The example below notably demonstrates that:

- 5 - the G protein of a non-apoptotic (*i.e.*, virulent) rabies virus strain, *i.e.*, the CVS-NIV strain, has a neurite outgrowth promoting effect, *i.e.*, some non-apoptotic rabies virus G proteins induce and/or stimulate neuritogenesis;
- this neurite outgrowth effect is sufficiently strong to protect neurons from growth cone collapsing drug (LPA) and oxidative stress (H₂O₂);
- 10 - it is the cytoplasmic tail of the G protein of said non-apoptotic CVS-NIV rabies virus strain, which is responsible for this neurite outgrowth effect;
- apoptotic rabies virus G proteins do not show this neurite outgrowth effect; more particularly the G protein of the ERA strain, which differs from the G protein of the CVS-NIV strain by only six amino acids (and by only two amino acids in the cytoplasmic tail)
- 15 does not show this neurite outgrowth effect;
- this is notably due to the fact that the PDZ-BS motif that is contained in the cytoplasmic tail of said non-apoptotic rabies virus G protein shows a single-point mutation compared to the one of said apoptotic rabies virus G protein: the protein G of the non-apoptotic CVS-NIV strain has amino acid Q at position 521 (position computed with respect the full
- 20 length G protein of the CVS-NIV strain, corresponding to position 41 in the cytoplasmic fragment of said G protein), whereas G-ERA has E at the same position;
- the amino acid, which is at position 491 in the full length G protein of the CVS-NIV strain (position 11 in the cytoplasmic fragment of said G protein), also contributes to this neurite outgrowth effect (amino acid H).

25

MATERIAL AND METHODS

Cells, viruses and molecular clones

- 30 SH-SY5Y is a neuroblastoma cell line, which is available from the American Type Culture Collection (ATCC; 10801 University Blvd.; Manassas, Virginia 20110-2209; U.S.A.) under deposit number CRL-2266).

The original ERA and CVS strains of rabies virus (RABV) are available from the ATCC under deposit number vr332 and vr959, respectively. The ERA and CVS strains, which have been used in this study, have been passaged in the inventors' laboratory for the last twenty years on BSR cells (a clone of Baby Hamster Kidney cells-clone 21, ATCC
5 deposit number BHK-21). These passages strains are the ERA-NIV strain and the CVS-NIV strain, respectively.

Throughout the examples and the figures of the application, CVS means CVS-NIV and ERA means ERA-NIV (G protein CNCM I-2760; SEQ ID NO: 4), unless otherwise specified.

10 The CVS-NIV strain (rRABV CVS HQ) has been deposited at the Collection Nationale de Cultures de Microorganismes (CNCM) on the 1st of April, 2009 under the terms of the Budapest Treaty (CNCM; Institut Pasteur; 25, rue du Docteur Roux; F-75724 PARIS CEDEX 15; FRANCE). The CNCM deposit number is I-4140.

The obtention and characterization of the molecular clones representative of the G protein
15 of the ERA-NIV strain, *i.e.*, of G-ERA-NIV, and of the G protein of the CVS-NIV strain, *i.e.*, of G-CVS-NIV, have been described in Préhaud *et al.* 2003.

The sequences of these G proteins are also available under accession number AF 406693 (for G-ERA-NIV) and AF 406694 (for G-CVS-NIV); *cf.* also Figures 13A and 13B.

The G protein of the CVS-NIV strain is also available from the recombinant *E. coli* strain
20 deposited on the 30th of November, 2001 at the CNCM under the terms of the Budapest Treaty. The CNCM deposit number is I-2758. This recombinant *E. coli* comprises a plasmid (plasmid pRev-TRE-G-CVS; *cf.* WO 03/048198), which inducibly expresses the G protein of the CVS-NIV strain.

The G protein of the ERA strain is also available from the recombinant *E. coli* strain
25 deposited on the 30th of November, 2001 at the CNCM under the terms of the Budapest Treaty. The CNCM deposit number is I-2760. This recombinant *E. coli* comprises a plasmid (plasmid pRev-TRE-G-ERA; *cf.* WO 03/048198), which inducibly expresses the G protein of the ERA strain.

30 Appropriate conditions for the cultivation of the recombinant *E. coli* strain containing the plasmid CNCM I-2758 coding for the G protein of the rabies virus CVS-NIV strain and for the cultivation of the recombinant *E. coli* strain containing the plasmid CNCM I-2760 coding for the G protein of the rabies virus ERA strain comprise the incubation of said

recombinant *E. coli* strain at 37°C on a standard LB-TYM growth medium (in the presence of ampicillin).

The recombinant rabies viruses (rRABV) were produced and isolated following the procedures described by Faul *et al.* 2008. rRABVs were constructed to harbor either the wild type “G survival” or “G death” sequences (G sequence of the CVS-NIV strain and of the ERA strain, respectively), or the cytoplasmic tail of G survival in an ERA genetic G gene background, or the cytoplasmic tail of G death in a CVS-NIV genetic G gene background (*cf.* Figure 2).

Recombinant rabies viruses were also produced, which derive from the CVS-NIV strain by mutations in the G coding sequence. These recombinant rabies viruses have a G protein, which differs from the G protein of the CVS-NIV strain by one or two amino acids, namely:

- by the amino acid, which in the full length G protein sequence of the CVS-NIV strain is at position 491 (position 11 in the cytoplasmic fragment thereof); and/or
- by the amino acid, which in the full length G protein sequence of the CVS-NIV strain is at position 521 (position 41 in the cytoplasmic fragment thereof).

More particularly, the following recombinant rabies virus strains were produced:

Table 2:

	Position 491 in the full length G protein of the virus (amino acid H in the G protein of the CVS-NIV strain)	Position 521 in the full length G protein of the virus (amino acid Q in the G protein of the CVS-NIV strain)
rRABV CVS LE	L	E
rRABV CVS LQ	L	Q
rRABV CVS HE	H	E

rRABV CVS LE, rRABV CVS LQ and rRABV CVS HE have been deposited at the CNCM under the terms of the Budapest Treaty on the 1st of April, 2009. The CNCM deposit numbers are I-4141, I-4142 and I-4143, respectively.

- 5 Appropriate conditions for the propagation of the virus I-4141 or I-4142 or I-4143 (recombinant rabies viruses) comprise the incubation of said virus at 37°C under 5% CO₂ with BHK-21 cells (sub-clone BSR) on a DMEM growth medium containing glucose (*e.g.*, 4.5 g/L), sodium pyruvate and glutamax (Invitrogen 31966047) and 5% FBS.

10 **High-throughput neurite outgrowth and retraction assays**

- SH-SY5Y human neuroblastoma cells are seeded on 24-well plates (Cell Bind plastic ware, Corning, USA) at a density of 40,000 cells per well in non differentiating medium [DMEMF12 (Invitrogen, U.K.) with 20% Fetal Bovine Serum plus 1%Pen:Strep and 1%
15 Glutamine], and cultured overnight at 37°C. 24h post seeding non differentiation medium is replaced with differentiating medium [Neurobasal medium (Invitrogen, U.K.) supplemented with B27 supplement (Invitrogen, U.K.), 1% P/S, 1% Glutamine and 1mM db-cAMP (dibutyryl c-AMP is membrane permeable, Sigma)], and the cells are incubated for 6h. Then, cells are mock infected, infected with rRABV at a MOI 3 in differentiating
20 medium. After 1h of incubation, cells are washed once with differentiating medium, and after adding differentiating medium they are incubated for 24h at 37°C.

For natural differentiation, the same procedure is used but db-cAMP is omitted.

- 30h post differentiation, the cells are fixed with 3% paraformaldehyde in phosphate buffered saline (PBS) for 20 min at room temperature (RT) followed by treatment for 5mn
25 with 0.1% Triton-X-100 and 50% normal goat serum (NGS) in PBS for 1h at RT. Neuronal specific anti β III tubulin Ab (Promega, France) and anti-RABV nucleocapsid Ab are used to stain the neurite processes and to reveal RABV infection respectively. Alternatively, cells are also stained with crystal violet which preserves the neurites processes.

30

Retraction assay is identical to the outgrowth assay as mentioned above, except for the addition of either 10 μ M-30 μ M or 50 μ M LPA (Sigma, USA) in differentiating medium or 75 μ M H₂O₂ (Sigma, USA), in B27 minus anti oxidant containing differentiating medium.

Cells are imaged using a Leica DM 5000B UV microscope equipped with a DC 300FX camera (x40 or x20 objectives) and analyzed using ImageJ 1.38X Software (Wayne Rasband, NIH, USA, <http://rsb.info.nih.gov/ij/>) and its plug-in NeuronJ (Meijering *et al.* 2004; <http://www.imagescience.org/meijering/software/neuronj/>). The average neurite
5 length per neuron is determined from triplicate experiments.

Detection of RABV antigens by flow cytometry

SH-SY5Y cells were differentiated by treatment with db c-AMP and infected with
10 rRABVs 6 hours post differentiation. 24 hours post infection, cells were harvested and treated for the detection by flow cytometry of either the RABV glycoprotein which reached the cytoplasmic membrane or the total amount of G protein expressed in the infected cells. The procedures followed have been described in Préhaud *et al.* 2003.

15 RESULTS and DISCUSSION

G-CVS-NIV cytoplasmic tail (*i.e.*, the cytoplasmic tail of the G protein of the CVS-NIV strain) contains a molecular signature promoting neurite outgrowth

20 In absence of infection, treatment with db-cAMP triggers elongation of neurites (Figure 3A, the two left panels). When cells are treated with db-cAMP and infected with rRABV G-CVS-NIV, they exhibit longer neurites (191%, Figure 3A, middle right panel). This result establishes that rRABV G-CVS-NIV promotes neurite outgrowth when the growth cone activity is stimulated by cAMP signaling.

25

In contrast, the length of the neurites is similar in between non infected cells and rRABV G-ERA infected cells both treated with db-cAMP (Figure 3B), which shows that G death expressing rRABV ERA has a silent phenotype for the neurite outgrowth assay. It is worth noting that the time course of the experiment (*i.e.*, 30h post differentiation and 24 hours
30 post infection) is a time point earlier than the time point where the maximum level of apoptosis is detected in rRABV ERA infected neuroblastoma cells (*i.e.*, 48h post infection).

By using rRABVs carrying the end swap of the G protein (*cf.* Figure 2) as described on Figure 3C, the inventors show that this phenotype is totally transferred by the cytoplasmic

tail only. This result firmly establishes that G-CVS-NIV cytoplasmic tail contains a molecular signature promoting neurite outgrowth when the elongation processes are initiated by the cAMP signaling pathway.

5 Furthermore, neurite outgrowth assays were performed with recombinant rabies virus, which differ from the CVS-NIV strain (rRABV G-CVS-NIV) by one or two amino acids in their respective G proteins.

More particularly, assays were performed with rRABV CVS LE, rRABV CVS LQ and rRABV CVS HE, which express a G protein that differs from G-CVS-NIV by amino acid
10 491 and/or amino acid 521 (*cf.* table 2 above). The results of these assays are illustrated by Figure 3D. In Figure 3D, all the differences with respect to the control are statistically significant ($p < 0.005$ Student's *t* test):

- rRABV G-CVS HQ *versus* N.I.: $p < 0.0001$;
- rRABV G-CVS LQ *versus* N.I.: $p = 0.0002$;
- 15 - rRABV G-CVS HE *versus* N.I.: $p = 0.0111$.

rRABV G-CVS-NIV has H and Q at positions 491 and 521 of its G protein, respectively (positions computed with respect to the full length G protein of CVS-NIV, these positions corresponding to positions 11 and 41 in the cytoplasmic fragment of this G protein, respectively).

20 rRABV CVS LQ has L and Q at these positions.
rRABV CVS HE has H and E at these positions.
rRABV CVS LE has L and E at these positions.

As illustrated by Figure 3D, rRABV CVS LQ and rRABV CVS HE still show a positive neurite outgrowth effect, although at a significantly lower extent than CVS-NIV (rRABV
25 G-CVS-NIV) [Student's *t* test analysis: rRABV CVS HQ *versus* rRABV CVS LQ: $p = 0.0156$; rRABV CVS HQ *versus* rRABV CVS HE: $p = 0.0009$].

Therefore, position 491 and position 521 of the G protein of the CVS strain (positions 11 and 41 in the cytoplasmic fragment) are both tightly involved in the neurite outgrowth
30 effect and position 521 has a predominant contribution thereto.

rRABV CVS LE did not induce any significantly positive neurite outgrowth effect compared to the control (*cf.* Figure 3C, where rRABV G-CVS Cyto Death = rRABV CVS LE).

G-CVS-NIV cytoplasmic tail is an intrinsic effector promoting neuritogenesis, which works synergistically with cAMP

5 The inventors investigated whether the expression of G-CVS-NIV cytoplasmic tail could on and of its own stimulate neuritogenesis. SH-SY5Y cells were infected with the different rRABV (*cf.* Figure 2) and the elongation of the neurites was monitored.

As observed in Figure 4A, the expression of G-CVS-NIV or G-ERA-cyto survival is sufficient to promote neurites outgrowth. These data establish that the cytoplasmic domain
10 of G-CVS-NIV contains an intrinsic molecular signature which signaled by an unknown mechanism the neurite elongation processes. When the same experiment was undertaken with or without db-cAMP, it was observed that both G-CVS-NIV and cAMP mediated neurites outgrowth work synergistically (*cf.* Figure 4B).

15 **Neuritogenesis based cytoplasmic domain of G-CVS-NIV is dependant of the molecular signature and not of the amount of expressed G protein**

The amount of rabies virus glycoprotein produced was analyzed very carefully either by monitoring the total quantity of RABV antigen produced in the infected db-cAMP treated
20 SH-SY5Y cells, or the quantity of G-protein fully processed to the cytoplasmic membrane. The two rRABVs expressing G-CVS-NIV or G-ERA have a similar amount of G-protein expressed *in toto* and at the cytoplasmic membrane (*cf.* Figure 5). Thus, the survival phenotype, *i.e.*, neurite outgrowth, is not linked to a phenomenon of quantity of available protein, but rather to specific determinant(s) present on the sequence of the cytoplasmic
25 survival peptide.

Moreover, it is interesting to note that rRABV G-ERA cyto survival express slightly less glycoprotein, but this virus still exhibits a strong survival phenotype (*cf.* Figures 3C, 4A). It means that as long as the survival determinant(s) are present inside the infected cells, the amount of the peptide is not the major caveat and the neurites outgrowth is stimulated.

30

G-CVS-NIV cytoplasmic tail confers neuroprotection against growth cone collapsing drug

LPA is a bioactive lipid acting as growth factor-like phospholipids which has been shown to exert diverse cellular functions that influence cell growth, motility, morphology and fate. On neuronal cells, LPA mediates growth cone collapsing and neurite retraction involving Rho and Rho-kinase activation which drives the re-organization of the actomyosin cytoskeleton.

The ability of the G protein cytoplasmic tail to protect against 10 μ M LPA induced neurites retraction was investigated. When db-cAMP differentiated SH-SY5Y cells are treated with LPA, retraction of the neurites is observed (*cf.* Figure 6A, left). The same effect is also observed when cells are infected with rRABV G-ERA (*cf.* Figure 6A, right).

On the contrary, the long neurites detected on db-cAMP treated and rRABV G-CVS-NIV infected cells are totally preserved against treatment with a growth cone collapsing agent (*cf.* Figure 6A, middle). This result establishes that G-CVS-NIV possess some intrinsic neuroprotective properties.

This protection is also noticed following infection with rRABV G-ERA-Cyto survival but not with rRABV G-CVS-Cyto death: *cf.* Figure 6B. Therefore, the use of the end-swap mutants firmly establishes that the neuroprotection phenotype is linked to the expression of G-CVS cytoplasmic domain.

G-CVS-NIV cytoplasmic tail neuroprotection to LPA is robust

LPA physiological range is usually around 1 μ M. The inventors used a high dose of LPA, *i.e.*, 10 μ M, to validate the robustness of the G-CVS-NIV cytoplasmic tail conferred neuroprotection.

Sub-lethal doses of LPA up to 50 μ M were tested in order to monitor the efficiency of this neuroprotection. SH-SY5Y cells were either non infected or infected with rRABV G-CVS-NIV or rRABV G-ERA-Cyto survival. 30 hours post differentiation, cells were treated with different doses of LPA (*cf.* Figure 7). Treatments of non infected cells with increasing amount of LPA drive a linear retraction of the length of the neurites (*cf.* Figure 7, left). On the contrary, cells which have been infected with rRABV G-CVS-NIV do not exhibit any obvious collapsing of their neurites whatever is the dose of the LPA (*cf.* Figure 7, middle). The same data are observed for the cells infected with rRABV G-ERA-Cyto survival for a dose of LPA up to 30 μ M (*cf.* Figure 7, right). The slight decrease observed for the highest dose of LPA (50 μ M) might be relative to the lowest amount of G protein

expressed by this rRABV. In this case, and for a very high concentration of growth cone collapsing drug, the relative amount of G protein might become a limiting factor. In any case, these data proved the very high efficiency of the G-CVS-NIV cytoplasmic tail conferred neuroprotection.

5

G-CVS-NIV cytoplasmic tail confers neuroprotection against oxidative stress

Based on the observations made above on the neuroprotection conferred by G-CVS-NIV cytoplasmic domain on LPA driven neurites retraction, the inventors asked the question whether this phenotype could be generalized to other more ubiquitous agents.

Oxidative stress represents an important pathway leading to neuronal degeneration. Oxidative stress has been implicated in many neurodegenerative diseases but also in case of acute damage to the brain such as trauma, stroke and epilepsy. Therefore, the inventors undertook a study on the neurite retraction phenotypes after hydrogen peroxide treatment. When differentiated cells are subjected to 75µM H₂O₂, their neurites shorten (*cf.* Figure 8, first pair of bars at the left). The same observation is made when the cells have been previously infected with rRABV G-ERA (*cf.* Figure 8, third pair of bars). To the contrary, no retraction of the neurites is observed when cells are infected with rRABV G-CVS-NIV (*cf.* Figure 8, second pair of bars). Thus G-CVS-NIV expression confers neuroprotection against an oxidative stress.

By using end swap mutants, we also showed that this neuroprotective property is linked to the expression of G-CVS-NIV cytoplasmic tail as described on Figure 8 (*cf.* fourth and fifth pairs of bars, relating to rRABV G-CVS-Cyto death and rRABV G-ERA-Cyto survival, respectively; *cf.* Figure 2 for the structure of these end-swap mutants).

25

The COOH terminal PDZ-BS of the cytoplasmic domain of the rabies virus G protein is critically involved in the survival phenotype fate of the infected neuronal cells.

Deletion of the last 4aa residues of the G-Cyto survival (survival G-protein-delta) was sufficient to significantly reduce the survival phenotype of the rRABV, as measured by its effect on the neurite outgrowth.

30

See for example Figure 20, which shows the neurite outgrowth measured at 8 hours post-infection, in the presence of db-cAMP, as induced by rRABV CVS-NIV Delta-PDZ-BS (CVS-NIV having amino acid H at position 491 and from which PDZ-BS has been deleted), compared to control (N.I.) and to G-CVS-NIV (CVS-NIV having amino acid H at position 491 and having its PDZ-BS, i.e., QTRL in positions 521-524) : the neurite outgrowth effect induced by rRABV CVS-NIV Delta-PDZ-BS is significantly different from the one induced by the control ($p = 0.0002$ Student's t test) and is significantly different from the one induced by rRABV CVS-NIV ($p = 0.0003$ Student's t test).

10 The inventors notably demonstrate that:

- protein G of CVS-NIV (G-CVS-NIV) is able to promote neurite outgrowth (neuritogenesis) in a system where the growth cone activity is stimulated by the c-AMP signaling pathway (delivered as an external effector);
- The cytoplasmic domain of G-CVS-NIV is responsible of this phenotype;
- 15 - The expression of the cytoplasmic tail of G-CVS-NIV is also responsible for the stimulation of the neurite outgrowth in the absence of c-AMP;
- Both G-CVS-NIV and c-AMP work as synergistic effectors;
- The phenotypes are linked to the molecular signature delivered by the cytoplasmic tail of G-CVS-NIV, more particularly by those amino acids which are at positions 491 and 521
- 20 in the full length G-CVS-NIV protein (positions 11 and 41 in the cytoplasmic fragment thereof), still more particularly by the amino acid, which is at position 521 in the full length G-CVS-NIV protein (position 41 in the cytoplasmic fragment thereof), and which is part of the PDZ-BS;
- The survival phenotype conferred by the G-CVS-NIV cytoplasmic tail is highly resistant
- 25 to treatments by growth cone collapsing agent (*i.e.*, LPA);
- The survival phenotype conferred by the G-CVS-NIV cytoplasmic tail is also resistant to oxidative stress (H_2O_2).

EXAMPLE 2: The cytoplasmic domain of G-CVS-NIV confers neuroprotection against HSV-1 cytophatic effect

Herpes simplex virus type I encephalitis (HSVE) is the most common sporadic fatal central nervous system infection in western countries and manifest throughout the year in

patients of all ages. HSVE develops when herpes simplex virus type 1 infects brain tissues in a lytic:necrotic manner.

The ability of the G protein cytoplasmic tail to protect against the cytopathic effect of HSV-1 *in cellulo* was investigated.

5

MATERIAL AND METHODS

Neurite outgrowth

10 As described in example 1 above.

HSV-1 cytopathic effect determination

rRABV were produced as described in example 1 above.

15 rRABV was propagated in BSR cells (*cf.* Faul *et al.* 2008)

HSV-1 strain KOS (*cf.* Skare *et al.* 1975) was propagated in U373MG cells (ATCC HTB 17).

SH-SY5Y cells were mock infected or infected with rRABV in differentiating medium
20 minus db-c-AMP for 6 hours. Then, HSV-1 was added at a multiplicity of infection of 3 (MOI 3) and the neurites outgrowth phenotypes were determined 24 hours later. In order to establish the effect on neurite outgrowth of the sole HSV-1 infection, SH-SY5Y cells were differentiated by db-c-AMP in differentiating medium for 6 hours and the HSV-1 infection (MOI 3) was then realized.

25 Neurite outgrowth phenotype was determined as well 24hours later.

RESULTS and DISCUSSION

SH-SY5Y neuroblastoma cells, which have been differentiated by treatment with db c-
30 AMP, show a drastic retraction of their neurites (up to 79 %, *cf.* Figure 9A) after HSV-1 infection, which can eventually lead to the death of the cells.

To the contrary, when cells have been previously infected with rRABV G-CVS-NIV or rRABV G-ERA-Cyto Survival (*cf.* example 1 above), they exhibit neurite outgrowth (*cf.* Figure 9B) and are protected against extensive neurite retraction (*cf.* Figure 9C).

EXAMPLE 3: The cytoplasmic domain of G-CVS-NIV exhibits anti-proliferative properties for neuroblastoma cells

- 5 Neuroblastoma is the second most common solid tumor of childhood accounting for more than 13% of cancer death in children for the United States. Prognosis depends on the clinical stage of the disorder and the age of the child. Generally, most patients with neuroblastoma are treated with therapeutic approaches that include surgery, radiation and cytotoxic chemotherapy.
- 10 Since human malignant neuroblastoma is characterized by poor differentiation and uncontrolled proliferation of immature neuroblasts, pro-differentiative drugs such as all-trans-retinoic acid (ATRA) at high doses have been used. The inventors have demonstrated that RABV G-CVS-NIV cytoplasmic tail is able to promote neurite outgrowth on SH-SY5Y human neuroblastoma cells (*cf.* the examples above), *i.e.*, to
- 15 induce the differentiation of these cells. Therefore, the inventors wanted to answer the question of whether this differentiation property was efficient enough to control the proliferation of such cells in culture.

MATERIAL AND METHODS

20

MTT assay

The assay is described by Sargent 2003. Briefly, cells, which have been mock infected or infected with rRABV for 48h in differentiating medium without db c-AMP, are stained

25 with the MTT solution for 3.5 hours at 37°C with 5% CO₂. The mixture of medium and staining solution is then removed, the MTT crystals are dissolved with the dissolvent for one hour at room temperature under constant agitation. The assay is read at an OD of 550nm. Each condition represents a n of 8. Statistical analysis was done with ANOVA tests.

30

Flow cytometry

Cell counting and morphological changes were assessed by side and forward light scattering (SSC and FSC, respectively) as described in Préhaud *et al.* 2003. Each point

represents the cells gathered during 1 minute at low speed. Flow cytometry analyses were gated on a viable population in order to exclude dead or necrotic cells which represent less than 1% of the cell population at the 48h time point.

5 Neurite outgrowth

As described in example 1 above.

ATRA treatment

10

All trans retinoic acid (ATRA) treatment of the neuroblastoma cells was undertaken as already described by Préhaud *et al.* 2005.

RESULTS and DISCUSSION

15

In a first instance, SH-SY5Y cells were either non-treated or treated with ATRA at 5 μ M or 10 μ M in the culture medium. Cell proliferation was either assayed by flow cytometry for viable cells or by cell counting via an MTT assay. The data are presented on Figures 10A and 10B, respectively.

20

In each case, ATRA treatment of the cells leads to a slower growth of the neuroblasts with a decrease in proliferation varying in between 27% up to 55% depending on the test used to monitor cell growth. Thus, SH-SY5Y cells are effectively responding to the effect of anti-proliferative drugs.

25

Therefore, SH-SY5Y cells were infected either with rRABV G-CVS-NIV or rRABV G-ERA and the proliferation of the cells (established by a MTT assay) as well as the outgrowth of the neurites were measured (*cf.* Figures 11A and 11B). We found that the cell growth was inversely correlated to the neurite outgrowth, establishing that the neurosurvival property of G-CVS-NIV is associated by an intrinsic anti-proliferative

30

phenotype on neuroblasts.

Neuroblastoma cells were infected in a second series of experiments with either the parental rRABV G-CVS-NIV or rRABV G-ERA viruses as well as the end swap mutants

(rRABV G-CVS-Cyto death or rRABV G-ERA-Cyto survival; *cf.* example 1 above and Figure 2). Cell growth was measured by flow cytometry and MTT assay (*cf.* Figures 12A and 12B, respectively). The data showed that in each case when the cells are infected with neurites outgrowth promoting G-cyto survival tail viruses, the proliferation of the neuroblasts is affected varying from a lag in between 18.3% to 23%. Thus, G-Cyto survival possesses an intrinsic property to slow down neuroblasts cell growth.

EXAMPLE 4: experimental demonstration that pro-survival properties of Cyto-G are conserved in the expression vector

10

This example demonstrates that, when it is expressed in the cell in the absence of viral infection (polypeptides delivered at first as an expression vector), the RABV cytoplasmic domain of the G protein of a non-apoptotic rabies virus strain (Cyto-G) conserves the pro-survival properties (neuritogenesis and protection against retraction).

15 This example further investigates whether said survival properties depend upon the size and upon the anchorage into the cytoplasmic or endoplasmic membrane.

Mutagenesis was undertaken by using oligonucleotides-PCR based mutagenesis procedures successfully used to generate the rRABVs of example 1 (QuickChange
20 Lightning site-directed mutagenesis kit, Agilent Technologies; Stratagene product division; Catalog number 210518-12; kit used in accordance with the manufacture's recommendations; U.S. Patent Nos. 7,176,004; 7,132,265; 7,045,328; 6,734,293; 6,489,150; 6,444,428; 6,391,548; 6,183,997; 5,948,663; 5,932,419; 5,866,395; 5,789,166; 5,545,552, and patents pending).

25 The oligonucleotides were designed for introducing respectively *NheI* and *XmaI* sites at the extremities of the constructs.

The mutagenic primers were:

GFullATG:

30 GGCCGCTAGCATGGTTCCTCAGGCTCTCCTGTTT (SEQ ID NO: 28)

GCytoATG:

GGCCGCTAGCATGAGAAGAGTCAATCGATCAGAACCT (SEQ ID NO: 29)

GendSTOP:

GGCCCCCGGGTCACAGTCTGGTCTGACCCCCACT (SEQ ID NO: 30)

mutTM.Cyto:

CCCCTTCTGGTTTTTCCATTGTGTTTTGGGGGGAAGTATGTATTACTGAGT
(SEQ ID NO: 31)

- 5 The mutants were screened by sequencing the inserts. By using this methodology, the following three constructs (G-Full; G-[SP-(2aa)-TM-Cyto]; G-Cyto) were notably obtained:

G-Full: accession number AF 406694 (G protein of CVS-NIV)

10 MVPQALLFVPLLVFPLCFGKFPIYTIPDKLGPWSPIDIHHLSCPNNLVVEDEGCTNL
SGFSYMELKVGYILAIKMNGFTCTGVVTEAETYTNFVG YVTTTTFKRKHF RPTPDA
CRAAYNWKMAGDPRYEESLHNPYPDYHWLRTVKTTKESLVIISPSVADLDPYDR
SLHSRVFPSGKCPGVAVSSTYCSTNHDIWMPENPRLGMSCDIFTNSRGKRASK
GSETCGFVDERGLYKSLKGACKLKL CGVLGLRLMDGTWVAMQTSNETKWCPPD
15 QLVNLHDFRSDEIEHLVVEELVRKREECLDALESIMTTKSVSFRRLSHLRKLVPGF
GKAYTIFNKTLM EADAHYKSVRTWNEILPSKGCLRVGGRCHPHVNGVFFNGIILG
PDGNVLIP EMQSSLLQQHMELLE SSVIPLVHPLADPSTVFKDGD EADFVEVHLPD
VHNQVSGVDLGLPNWGKYVLLSAGALTALMLIIFLMTCCRRVNRSEPTQHNLRG
TGREVS VTPQSGKIISSWESHKSGGQTRL (SEQ ID NO: 2)

20

G-[SP-(2aa)-TM-Cyto]:

G-[SP-(2aa)-TM-Cyto] is a construct comprising (from N- to C-term):

- the signal peptide of a G protein (*e.g.*, the signal peptide of CVS-NIV; SEQ ID NO: 21);
- 2 amino acids (*e.g.*, from the ectodomain of a G protein, such as from G-CVS-NIV);
- 25 - the transmembrane domain of the G protein of a non-apoptotic rabies virus strain (*e.g.*, the transmembrane domain of G-CVS-NIV; SEQ ID NO: 23);
- the cytoplasmic domain of the G protein of a non-apoptotic rabies virus strain (*e.g.*, the cytoplasmic domain of G-CVS-NIV; SEQ ID NO: 6).

The sequence of G-[SP-(2aa)-TM-Cyto] that was used in the present example is:

30 MVPQALLFVPLLVFPLCFGGKYVLLSAGALTALMLIIFLMTCCRRVNRSEPTQHN
LRGTGREVS VTPQSGKIISSWESHKSGGQTRL (SEQ ID NO: 27)

G-Cyto:

G-Cyto is a construct comprising (from N- to C-term): an amino acid (*e.g.*, M) and the cytoplasmic domain of the G protein of a non-apoptotic rabies virus strain (*e.g.*, the cytoplasmic domain of G-CVS-NIV; SEQ ID NO: 6).

The sequence of the G-Cyto that was used in the present example is:

- 5 MRRVNRSEPTQHNLRGTGREVSVTPQSGKIISWESHKSGGQTRL (SEQ ID NO: 23).

The sequences are shown on Figure 22.

The sequence alignment is shown on Figure 23.

- 10 A schematic representation of the three constructs is shown in Figure 24.

Inserts were removed by *NheI* and *XmaI* digestion and were cloned in SAP dephosphorylated pCI-Neo derivative (Promega, France) by standard cloning procedures. *E. coli* XL1-blue cells (Stratagene, USA) were transformed with the plasmid constructs.

- 15 Plasmid DNA was extensively purified on Purelink columns (Invitrogen, U.K.) and the inserts were sequenced in order to verify the integrity of the sequence inserted in the plasmid. Plasmid clones were used to nucleofect human neuroblastoma cells [SH-SY5Y cells (ATCC CRL-2266), or Ntera cl2D1 cells (ATCC CRL-1973)] using the Amaxa GmbH electroporation technology (Amaxa® nucleofector® kit V Catalog # VCA 1003
- 20 Lonza Germany following the manufacturer's recommendations). The expression of the G proteins was assayed by flow cytometry as described in example 1.

For transient expression, the biological assays were undertaken 24h post nucleofection.

- For stable expression, both nucleofected cell lines [SH-SY5Y or Ntera cl2D1] were
- 25 treated with G418 at 800µg/ml over three weeks. Cell clones resistant to G418 were isolated and frozen. The control cell line consists of cells, which have been nucleofected with pCI-Neo alone without any insert.

- The neurite outgrowth assays and retraction assays were performed as described in
- 30 example 1.

Illustrative results are shown in Figures 25-28.

Figure 25 illustrates the expression of a polypeptide comprising the cytoplasmic domain of a non-apoptotic rabies virus strain, more particularly the expression:

- of the full length G protein of said rabies virus strain,
 - of a polypeptide, which comprises the transmembrane and cytoplasmic domains of said G protein, but which does not comprise the ectodomain of said G protein, and
 - of a polypeptide, which comprises the cytoplasmic domain of said G protein, but which does not comprise the ectodomain and the transmembrane domain of said G protein, compared to the expression measured with the control plasmid.
- As already illustrated, the G level expression is different and the biological phenotypes are not correlated directly with the level of expression (see below and Figure 5).

The transient expression of the cytoplasmic domain of the G protein of a non-apoptotic rabies virus strain -in the absence of viral infection- induces and/or stimulates neurite outgrowth from human neuroblastoma cells (Figure 26). A transiently-expressed polypeptide comprising the transmembrane domain in addition to the cytoplasmic domain (but which does not comprise the ectodomain) of said G protein is more efficient in inducing and/or stimulating neurite outgrowth than the transiently-expressed full length G protein or than the transiently-expressed cytoplasmic domain of said G protein (cytoplasmic domain without the ectodomain and without the transmembrane domain) (Figure 26).

The stable expression of the cytoplasmic domain of the G protein of a non-apoptotic rabies virus strain, or of polypeptides or proteins comprising said domain -in the absence of viral infection- is feasible in human neuroblastoma cells (Figure 27). A stably-expressed polypeptide comprising the transmembrane domain in addition to the cytoplasmic domain (but which does not comprise the ectodomain) of said G protein is more efficient in inducing and/or stimulating neurite outgrowth than the stably-expressed full length G protein or than the stably-expressed cytoplasmic domain of said G protein (cytoplasmic domain without the ectodomain and without the transmembrane domain) (Figure 27). The stably-expressed cytoplasmic domain of said G protein (cytoplasmic domain without the ectodomain and without the transmembrane domain) is as efficient in inducing and/or stimulating neurite outgrowth as the stably-expressed full length G protein (Figure 27).

The expression of a polypeptide comprising the cytoplasmic domain of a non-apoptotic rabies virus strain -in the absence of viral infection- confers resistance to a neurotoxic agent (*e.g.*, the growth cone collapsing drug LPA; (*cf.* Figure 28, showing results obtained with the G-(SP-[2a]-TM-Cyto) construct).

- 5 In the same experimental conditions, this batch of LPA is inducing growth cone collapsing as illustrated in Figures 6A, 6B, 7.

The transmembrane domain of G-(SP-[2a]-TM-Cyto) notably allows the polypeptide expressed by this construct to be anchored in the Golgi membrane and/or the
10 endoreticulum membrane of the cells, thereby increasing the efficiency of the expressed polypeptide in inducing and/or stimulation neurogeneration, neuroregeneration and neuroprotection.

Hence, coupling the cytoplasmic domain of the G protein of a non-apoptotic rabies virus strain to such a membrane-anchoring sequence increases the neurogeneration,
15 neuroregeneration and neuroprotection effects induced and/or stimulated by said cytoplasmic domain.

EXAMPLE 5: experimental demonstration that the cytoplasmic domain of a non-apoptotic rabies virus strain possess intrinsic properties to drive neuronal precursor
20 **(EC cells) to differentiation to mature neurons (commitment)**

The cytoplasmic domain of a non-apoptotic rabies virus strain, such as Cyto-G (*cf.* example 4 above; *e.g.*, SEQ ID NO: 6 or 24), promotes the outgrowth of the neurites by such promoting the differentiation of the neuroblastoma cell line SH-SY5Y (*cf.* example 4
25 above).

Example 5 brings the experimental demonstration that this property reveals a wider ability of said cytoplasmic domain to promote and to orientate the commitment of the cell line toward the neuronal differentiation.

Production of pure post-mitotic human neurons from the embryonic carcinoma cell line
30 NTERA 2cl-D1 (ATCC CRL-1973) has been described in the art, *e.g.*, in Préhaud *et al.* 2005.

Constructs expressing the cytoplasmic domain of the G protein of a non-apoptotic rabies virus strain, as described in example 4 above, were used, *e.g.*:

- the G-Full construct containing the G protein of a non-apoptotic rabies virus strain (G protein of SEQ ID NO: 2) as an insert,
- the G-Cyto construct containing the cytoplasmic domain of a non-apoptotic rabies virus strain (cytoplasmic domain of SEQ ID NO: 6) as an insert, and
- 5 - the G-(SP)-[2aa]-TM-Cyto construct, containing the signal peptide of a non-apoptotic rabies virus strain (signal peptide of SEQ ID NO: 21), two amino acids (from the C-terminal end of the ectodomain of the G protein of a non-apoptotic rabies virus strain, *e.g.*, GK), the transmembrane domain of the G protein of a non-apoptotic virus strain (transmembrane domain of SEQ ID NO: 23) and the
- 10 cytoplasmic domain of a non-apoptotic rabies virus strain (cytoplasmic domain of SEQ ID NO: 6).

The ability of the G-expressing constructs to induce the differentiation of NTera 2-D1 cells into neurons was investigated.

Stable G-expressing NTera 2-D1 cells were processed by following the ATRA-neurosphere like protocol as described Préhaud *et al.* 2005.

After the last antimitotic treatment and the two replicates, cells were seeded either for accounting neurite tips (5 days post last replicate) or neuronal network architecture (50 days post last replicate).

20 Illustrative results are shown in Figures 29-31.

The expression of a polypeptide comprising the cytoplasmic domain of the G protein of a non-apoptotic rabies virus strain –in the absence of viral infection- induces and/or stimulates the production of mature post-mitotic human neurons by differentiation from neuroblastoma cells: *cf.* Figure 29, illustrating the results obtained with the expression:

- of the full length G protein of a non-apoptotic rabies virus strain,
- of a polypeptide comprising the transmembrane domain and the cytoplasmic domain (but which does not comprise the ectodomain) of said G protein,
- of the cytoplasmic domain of said G protein (cytoplasmic domain without the
- 30 ectodomain and without the transmembrane domain),

compared to the control plasmid.

The polypeptide, which comprised the transmembrane domain in addition to the cytoplasmic domain of the G protein of a non-apoptotic rabies virus strain (but which does not comprise the ectodomain of said G protein) is more efficient in inducing and/or

stimulating said differentiation of post-mitotic human neurons than the full length G protein or than the cytoplasmic domain of said G protein (cytoplasmic domain without the ectodomain and without the transmembrane domain); *cf.* Figure 30.

- 5 Further to inducing and/or stimulating the production of mature post-mitotic human neurons by differentiation from neuroblastoma cells, the expression of a polypeptide comprising the cytoplasmic domain of the G protein of a non-apoptotic rabies virus strain -in the absence of viral infection- induces and/or stimulates the organisation of a network of mature post-mitotic human neurons with long axons (*cf.* Figure 31).

10

EXAMPLE 6: experimental demonstration that the cytoplasmic domain of a non-apoptotic rabies virus strain is able to promote regeneration of injured neurons

- 15 Injured neurons may have the possibility to regenerate depending on their origins and on the local environment. For example, neurons from the mammalian central nervous system have very limited regenerative capacity, even though lesion to a peripheral process results in increased regeneration of dorsal root ganglion neurons. It is clearly established that intrinsic regeneration signals influence the success of proper regeneration, some of them involving specific kinase pathway (Hammarlund *et al.* 2009).

- 20 The cytoplasmic domain of a non-apoptotic rabies virus strain, such as the cytoplasmic domain of SEQ ID NO: 6 contained in the Cyto-G construct (*cf.* example 4 above), exhibits at least two interesting properties as far as regenerative medicine is concerned.

First, it promotes neurite outgrowth, which means that said cytoplasmic domain can stimulate the neuronal growth cone.

- 25 Secondly, it preserves neurites from retraction after treatment with LPA or H₂O₂, which means that said cytoplasmic domain can stimulate or reinforce molecular locks avoiding the collapsing of the growth cone.

- 30 In order to bring the experimental demonstration that these properties drive the regeneration of an injured neuron, the phenomenon was investigated in a cellular model. The NT2-N cell line (human neuronal cells), stably expressing the G-[SP-(2aa)-TM-Cyto] construct of example 4 (construct containing SEQ ID NO: 27 as an insert), was chosen. The pCI-Neo-NT2-N was used as control.

The NT2-N cell line was derived from differentiated Ntera-2cl.D1 (NT2/D1; ATCC CRL-1973) cells as described in Préhaud *et al.* 2005, Cheung *et al.* 1999 and Paquet-Durand *et al.* 2003.

5 For scratch-induced assays, cells were seeded on poly-D-Lysin-laminin coated cell
+(Sarstedt, Germany) 12 wells plastic ware, and were grown for two days in order to
recover completely after trypsinisation. The medium was changed 10h before scratching.
Individual wounds were made with an injection needle (26GX1/2", 12-4.5). At least 10
scratching were made on each individual well. Cells were fixed with PFA (4%) 3 days
10 post wounding and stained with crystal violet solution. Cells are imaged using a Leica DM
5000B microscope equipped with a DC 300FX camera (x20 objective) and analysed using
ImageJ 1.38X Software (Wayne Rasband, NIH, USA, <http://rsb.info.nih.gov/ij/>) and its
plug-in NeuronJ. The average percentage of neuron in regeneration is determined from 8
experiments.

15

Illustrative results are shown in Figures 32-33.

The expression of a polypeptide, which comprises the cytoplasmic domain of the G
protein of a non-apoptotic rabies virus strain, induces and/or stimulates the regeneration of
wounded mature post-mitotic human neurons (*cf.* Figure 32, illustrating the results
20 obtained with the expression of a polypeptide comprising the transmembrane and
cytoplasmic domains of the G protein of a non-apoptotic rabies virus strain).

The neurite regeneration thus induced after wounding is drastic, more particularly when
the polypeptide comprise the transmembrane and the cytoplasmic domains of said G
protein, but does not comprise the ectodomain of said G protein (*cf.* Figure 33, illustrating
25 the results obtained with the expression of a polypeptide comprising the transmembrane
and cytoplasmic domains of the G protein of a non-apoptotic rabies virus strain).

EXAMPLE 7:

30 An infectious rRABV cDNA was generated from the CVS-N2c rabies virus strain
(Morimoto *et al.* 1999), a fixed pathogenic and non-apoptotic RABV.

The infectious rRABV cDNA thus generated (cN2C) is described in Schnell *et al.* 2010.

The gene coding for G-vir (the G protein of a non-apoptotic rabies virus strain, such as the G protein of SEQ ID NO: 2) has been integrated in this RABV cDNA. The infectious virus has been recovered as described in example 1.

- 5 The recombinant virus has been injected to groups of 8 six-week-old female C57Bl6 mice by intramuscular route with the number of virus particles of rRABV G-vir to trigger fatal encephalitis in 80% of mice. Number of mice and experiments have been carefully evaluated to minimize the animal contribution.
- 10 Disease progression has been monitored by scoring clinical signs and mortality (as described in Camelo *et al.* 2001). In the course of infection, groups of two or three mice have been perfused, and brains have been removed, snap frozen and stored at -80°C before being processed for RNA extraction (half brain) and immunohistochemistry or multiplex arrays (second half brain) in order to measure the neuroinvasiveness of the virus.
- 15 This model allows to further analyze different parameters of the brain innate immune response triggered by the rRABV strain.

BIBLIOGRAPHIC REFERENCES

5 Blondel *et al.*, Poliovirus, pathogenesis of poliomyelitis, and apoptosis, CTMI, 2005, 289, 25-56.

Camelo *et al.* 2001 “*Selective role for the p55 Kd TNF- α receptor in immune unresponsiveness induced by an acute viral encephalitis*”; J. Neuroimmunol. 113: 95–108.

10 Cheung *et al.* 1999 ; BioTechniques 26: 946-954.

Faul *et al.* 2008; Virology 382: 226-238.

15 Guigoni and Coulon 2002, Journal of NeuroVirology 8: 306-317.

Hammarlund *et al.* 2009 “*Axon regeneration requires a conserved MAP kinase pathway*” Science 323: 802-806.

20 Kumar *et al.* 2007; Nature 448: 39-43 and associated Supplementary Information (“*Methods*”).

Lay *et al.* 2003; Ann. N. Y. Acad. Sci. 1010: 577-581.

25 Loh *et al.* 2008; Cell Death and Differentiation 15: 283-298.

Meijering *et al.* 2004; Cytometry Part A 58A: 167-176; and <http://www.imagescience.org/meijering/software/neuronj/>

30 Morimoto *et al.* 1999, Journal of Virology 73(1): 510-518.

Paquet-Durand *et al.* 2003 Brain Res. Dev. Brain Res. 142: 161-167.

Préhaud *et al.* 1988; Journal of Virology 62(1): 1-7.

Préhaud *et al.* 2003; Journal of Virology 77(19): 10537-10547.

Préhaud *et al.* 2005; Journal of Virology 79(20): 12893-12904.

5

Sargent 2003; Recent Results in Cancer Research, vol. 161, Springer-Verlag Berlin Heidelberg, pages 13-25

Sarmiento *et al.* 2005; Journal of NeuroVirology 11: 571-581.

10

Schnell *et al.* 2010; Nature Reviews Microbiology 8(1): 51-65.

Skare *et al.* 1975; J. Virol. 15:726-732 (Structure and function of herpesvirus genomes. I. Comparison of five HSV-1 and two HSV-2 strains by cleavage of their DNA with *Eco R I* restriction endonuclease).

15

Ugolini 1995; The Journal of Comparative Neurology 356: 457-480.

Ugolini 2008; Dodet B, Fooks AR, Müller T, Tordo N, and the Scientific & Technical Department of the OIE (eds): Towards the Elimination of Rabies in Eurasia. Dev. Biol. Basel, Karger, vol. 131, pp. 493-506.

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WO 03/048198; PCT international application in the name of Institut Pasteur, Inv. Lafon *et al.*, published on 12 June 2003; as well as its national/regional counterpart applications.

25

Each of the relevant disclosures of all references cited herein is specifically incorporated by reference.

CLAIMS

1. A product selected from:

5 **- a polypeptide, which comprises:**

 - the **sequence** of SEQ ID NO: 6,

 or

 - a sequence, which:

 - is of 34 to 54 amino acids,

10 - is at least 94% identical to said sequence of SEQ ID NO: 6 over the
entire length of the shortest of the two sequences,

 - comprises a PDZ-BS sequence, wherein said PDZ-BS sequence is:

x_1 is any amino acid except E (preferably Q), and

x_2 is T or S or I (preferably not I, more preferably T), and

15 x_3 is any amino acid (preferably R), and

x_4 is L or V (preferably L)

(SEQ ID NO: 14),

said sequence being referred to as **variant sequence A**,

 or

20 - a sequence, which:

 - is of 44 amino acids,

 - is at least 94% identical to said sequence of SEQ ID NO: 6,

 - comprises a PDZ-BS sequence, wherein said PDZ-BS sequence is:

x_1 is E, and

25 x_2 is T or S or I (preferably not I, more preferably T), and

x_3 is any amino acid (preferably R), and

x_4 is L or V (preferably L)

(SEQ ID NO: 13 with $x_1 = E$),

 and

30 - does not comprise the amino acid L at position 11,

said sequence being referred to as **variant sequence B**,

 or

 - a **fragment** of said sequence of SEQ ID NO: 6 or of said variant sequence A
or of said variant sequence B, wherein said fragment is of at least 34 amino

acids and has retained the PDZ-BS sequence of said sequence of SEQ ID NO:
6 or of said variant sequence A or of said variant sequence B, respectively,

or

- a **nucleic acid** coding for said polypeptide,

5 or

- a **vector** comprising said nucleic acid,

or

- a **cell** comprising said polypeptide and/or said nucleic acid and/or said vector,

or

10 - a **pharmaceutical composition or drug**, comprising at least one of said polypeptide,
nucleic acid, vector and cell,

said product being for use in the treatment and/or palliation and/or prevention of a disease
or disorder affecting the nervous system by stimulation and/or induction of neurite
outgrowth and/or neurite spouting and/or axon growth and/or dendritic tree extension from
15 neurons of said nervous system, wherein said product is used as an active principle for
said stimulation and/or induction.

2. The product of claim 1, which is used to stimulate and/or induce synaptogenesis and/or
neurotransmission and/or neuronal plasticity.

20

3. The product of any one of claims 1-2, which is used as a neurogenerative and/or
neuroregenerative and/or neuroprotective agent .

4. The product of any one of claims 1-3, wherein said use is in a non-immunogenic use
25 and/or said product is not an immunogenic agent or adjuvant

5. The product of any one of claims 1-4, wherein the PDZ-BS sequence of said variant
sequence A is QTRL (SEQ ID NO: 10).

30 6. The product of any one of claims 1-5, wherein said variant sequence A is of 44 amino
acids and has the amino acid H or L, preferably H, at position 11.

7. The product of any one of claims 1-6, wherein the PDZ-BS sequence of said variant
sequence B is ETRL (SEQ ID NO: 12).

8. The product of claim 7, wherein said variant sequence B has the amino acid H at position 11.
- 5 9. The product of any one of claims 1-8, wherein said fragment is the fragment 11-44 from the sequence of SEQ ID NO: 6.
- 10 10. The product of any one of claims 1-9, wherein said polypeptide is or comprises the cytoplasmic fragment of the G protein of a rabies virus strain, or a sub-fragment of such a cytoplasmic fragment.
11. The product of claim 10, wherein said rabies virus strain is the strain deposited at the CNCM under I-4140, I-4142 or I-4143.
- 15 12. The product of any one of claims 1-11, wherein said polypeptide further comprises an amino acid sequence, which anchors said polypeptide in the endoreticulum membrane and/or in the Golgi membrane of cells, more particularly of neuronal cells, more particularly of human neuronal cells, said anchoring sequence being preferably at the N-terminal end of said sequence of SEQ ID NO: 6 or variant sequence A or variant sequence
- 20 B, most preferably directly linked to the first amino acid at the N-terminal end of said sequence of SEQ ID NO: 6 or of said variant sequence A or of said variant sequence B.
13. The product of any one of claims 1-12, wherein said polypeptide further comprises, preferably at the N-terminal end of said sequence of SEQ ID NO: 6 or variant sequence A
- 25 or variant sequence B, most preferably directly linked to the first amino acid at the N-terminal end of said sequence of SEQ ID NO: 6 or of said variant sequence A or of said variant sequence B:
- the sequence of the transmembrane domain of the G protein of a rabies virus strain, more particularly of a non-apoptotic rabies virus strain, or
 - 30 - a sequence, which differs from said sequence of rabies virus G transmembrane domain by at least one amino acid substitution and/or deletion and/or addition, preferably by amino acid substitution(s) and/or deletion(s), more preferably by amino acid substitution(s), but which has retained the capacity of anchoring the polypeptide of SEQ ID NO: 6 (when said anchoring sequence is directly linked to the first amino acid at the N-

terminal of said sequence of SEQ ID NO: 6), in the endoreticulum membrane and/or in the Golgi membrane of cells, more particularly of neuronal cells, more particularly of human neuronal cells.

5 14. The product of claim 12 or 13, wherein said anchoring sequence is:

- the sequence of SEQ ID NO: 23, or
 - a variant sequence thereof, which is of 18 to 26 amino acids, preferably of 18 to 22 amino acids, more preferably of 22 amino acids, and which is at least 94% identical to said sequence of SEQ ID NO: 23 over the shortest of the two sequences (*i.e.*, over the
- 10 shortest of SEQ ID NO: 23 and of said variant sequence).

15 15. The product of any one claims 12-14, wherein said polypeptide comprises said sequence of SEQ ID NO: 6 or said variant sequence A, more particularly said sequence of SEQ ID NO: 6.

15

16. The product of claim 15, wherein the amino acid sequence of said polypeptide consists of the sequence of SEQ ID NO: 23 followed by (from N-terminal to C-terminal end) the sequence of SEQ ID NO: 6, wherein one to four amino acids, preferably one amino acid (*e.g.*, M) is/are optionally present between said sequence of SEQ ID NO: 23 and said

20 sequence of SEQ ID NO: 6.

17. The product of any one of claims 12-16, wherein said polypeptide is or comprises the sequence of SEQ ID NO: 25.

25 18. The product of any one of claims 12-17, wherein the amino acid sequence of said polypeptide further comprises, (directly or indirectly, preferably directly) linked to the first amino acid at the N-terminal end of said anchoring sequence:

one to four amino acids, preferably two amino acids, more preferably one to four amino acids from the C-terminal end of the ectodomain of the G protein of a rabies virus (*e.g.*, a non-apoptotic rabies virus strain), still more preferably the last two amino acids of the C-terminal end of the ectodomain of the G protein of a rabies virus (*e.g.*, a non-apoptotic rabies virus strain), for example amino acids GK.

30

19. The product of claim 18, wherein said polypeptide is or comprises the sequence of SEQ ID NO: 26.

20. The product of any one of claims 12-19, wherein said polypeptide further comprises a
5 signal peptide, preferably the signal peptide of the G protein of a rabies virus strain (*e.g.*, a non-apoptotic rabies virus strain), said peptide sequence being preferably at the N-terminal end of said polypeptide (most preferably at the very N-terminal end of said polypeptide, *i.e.*, in N-term from any anchoring sequence that may be comprised in said polypeptide).

10

21. The product of claim 20, wherein said polypeptide is or comprises the sequence of SEQ ID NO: 27.

22. The product of any one of claims 1-21, wherein said polypeptide consists of less than
15 100 amino acids.

23. The product of any of claims 1-22, wherein said neurons are neurons that are impaired by neurodegeneration.

20 24. The product of any one of claims 1-23, wherein said disease or disorder is a neurodegenerative disease or disorder, such as non-viral encephalopathy, Alzheimer's disease, Parkinson's disease, ALS, Huntington's disease, an age-related neurodegenerative disease or disorder, MS or rare genetic disease.

25 25. The product of claim 23 or 24, wherein said disease or disorder is MS.

26. The product of any one of claims 1-22, wherein said neurons are neurons that are impaired by a microbial infection of the nervous system and/or by a physical or ischemic injury of the nervous system and/or by a chemical neurotoxic agent and/or by an oxidative
30 stress.

27. The product of any one of claims 1-22, 26, wherein said disease or disorder involves a microbial infection of the nervous system, such as poliomyelitis.

28. The product of any one of claims 1-22, 26, wherein said disease or disorder is a physical or ischemic injury of the nervous system, such as seizure, stroke, trauma, epilepsy.

5 29. The product of any one of claims 1-22, 26, wherein said disease or disorder involves the presence of a chemical neurotoxic agent and/or of an oxidative stress.

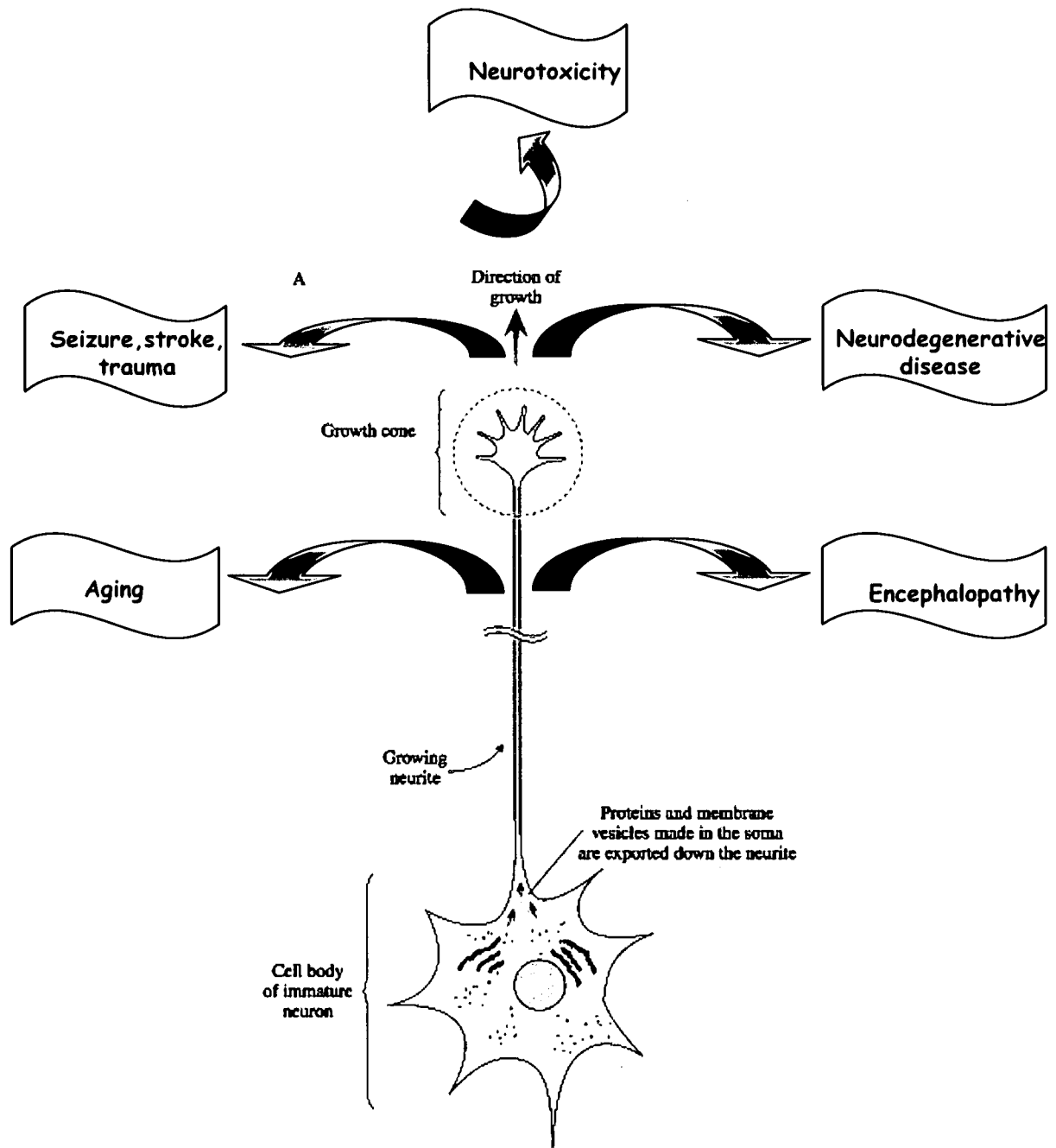
30. The product of any one of claims 1-22, wherein said neurons are pre-mitotic neurons or neuron progenitors.

10

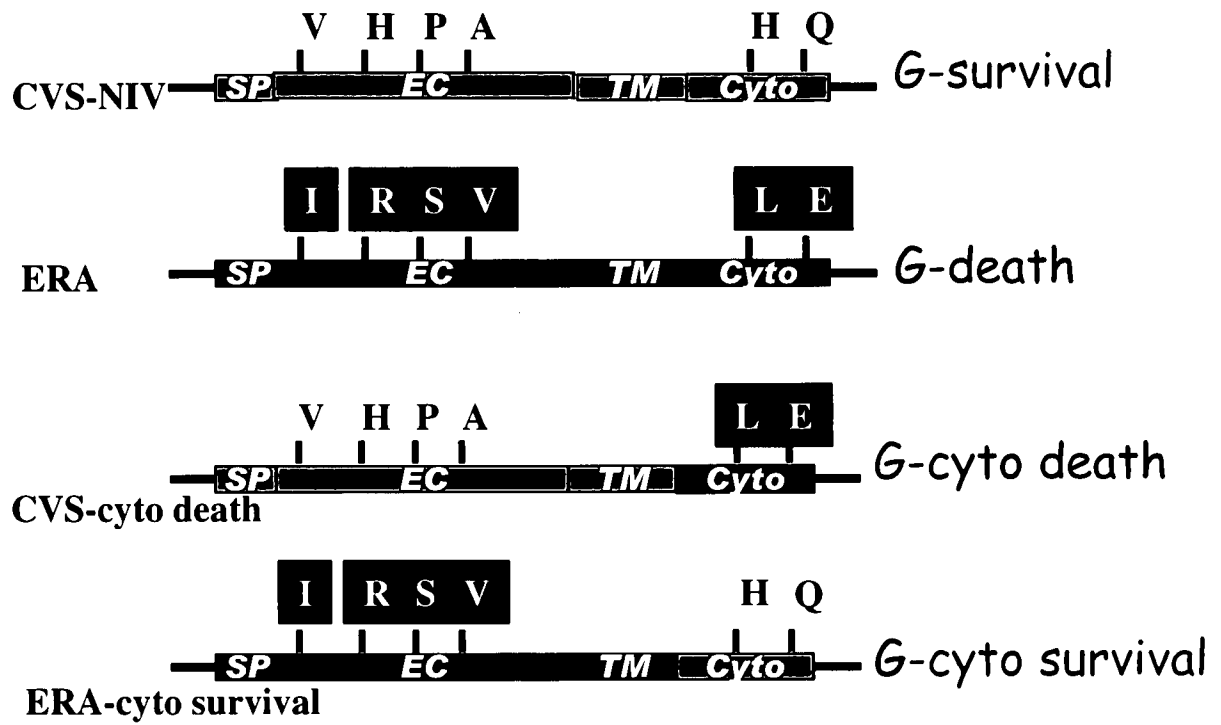
31. The product of any one of claims 1-22, 30, which is used as a neuro-differentiating agent.

15 32. The product of any one of claims 1-22, 30, 31, wherein said disease is a tumor of the CNS and/or PNS, such as a ganglioglioma, a brain tumor, a central neurocytoma, a medulloblastoma, an ependynoma, a teratoma, a neuroblastoma.

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**FIGURE 1**

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**FIGURE 2**

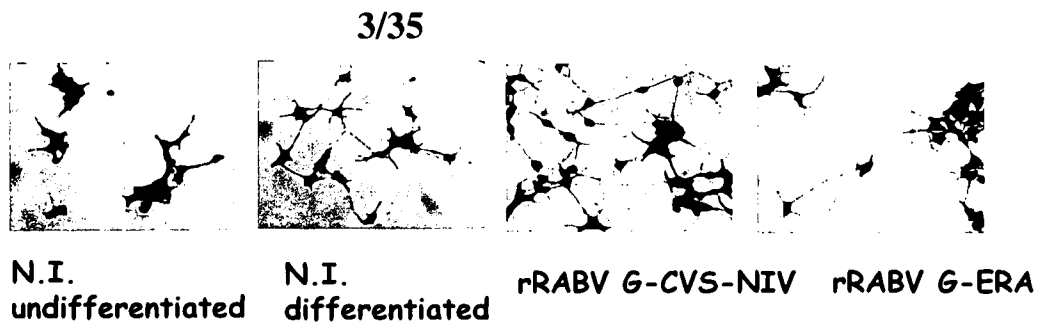


FIGURE 3A

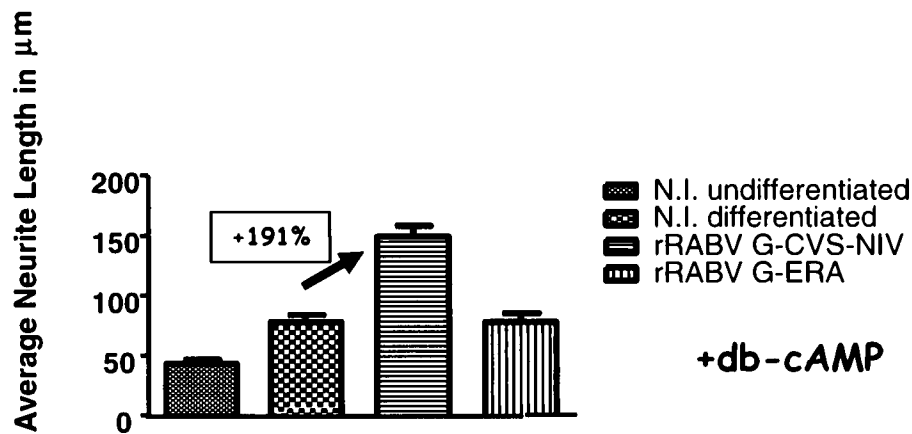


FIGURE 3B

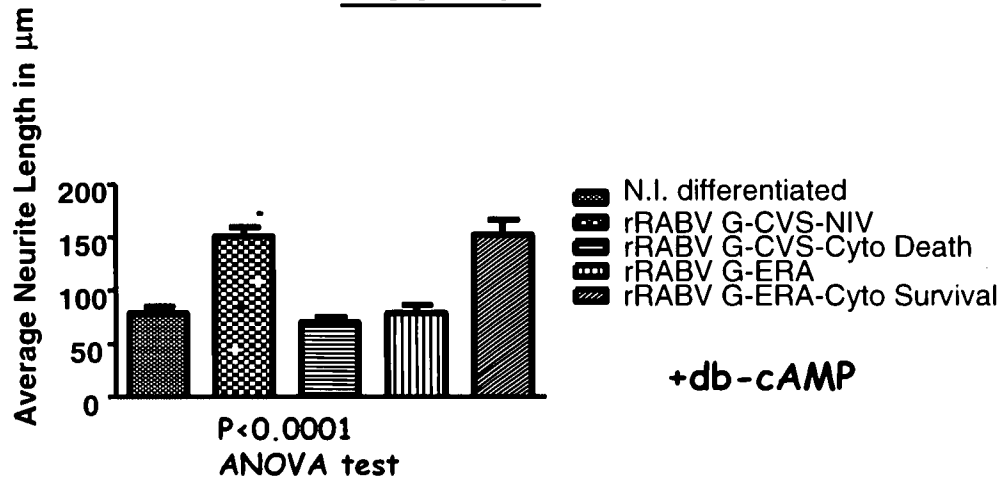
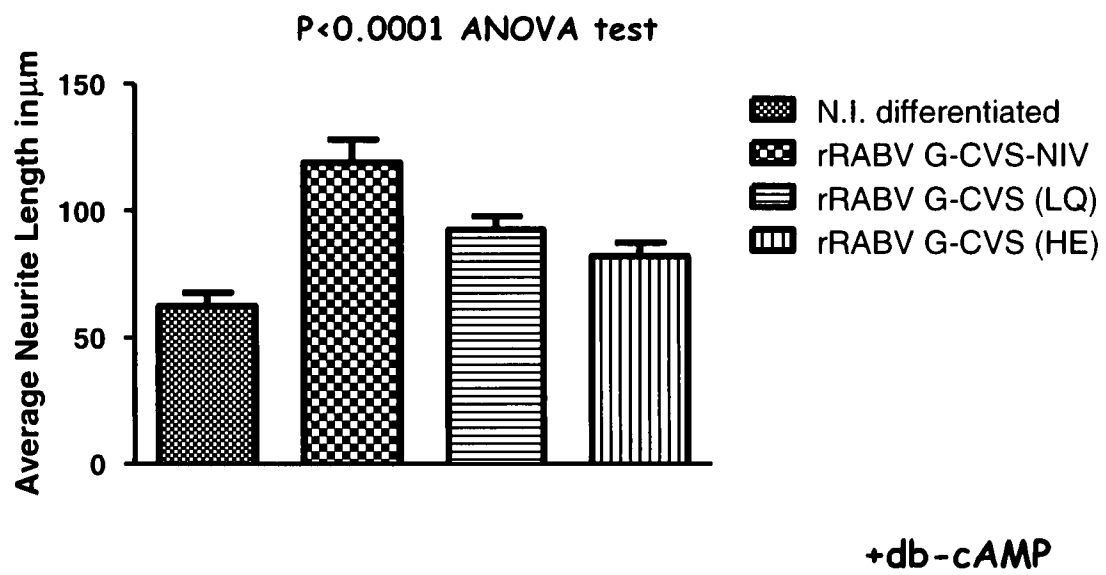
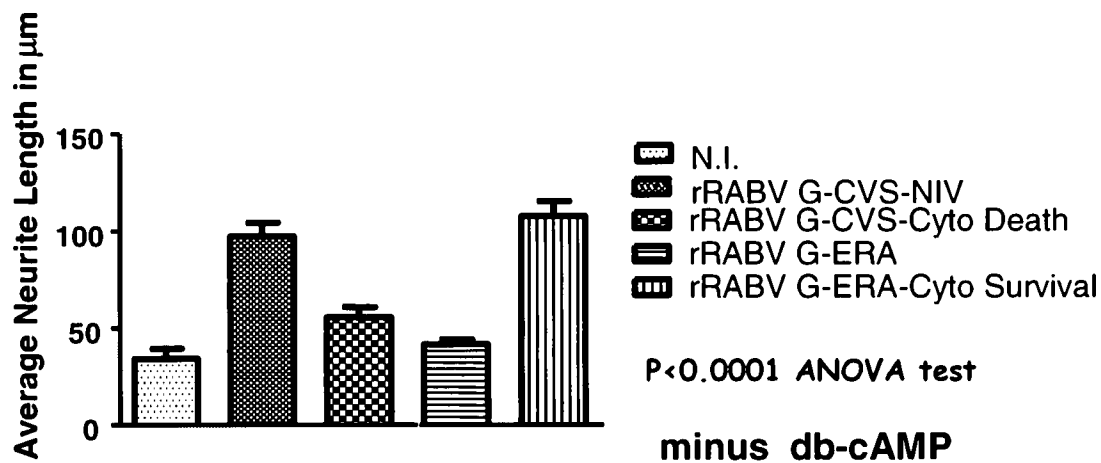
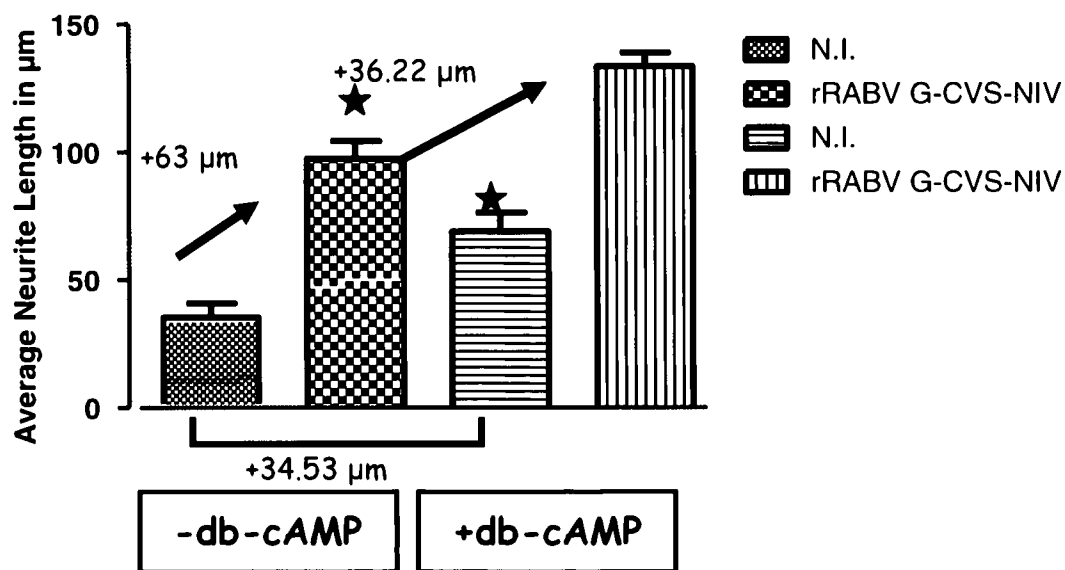


FIGURE 3C

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**FIGURE 3D**

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**FIGURE 4A**

★ Means are significantly different, Student's t test ($P 0.0067$)

FIGURE 4B

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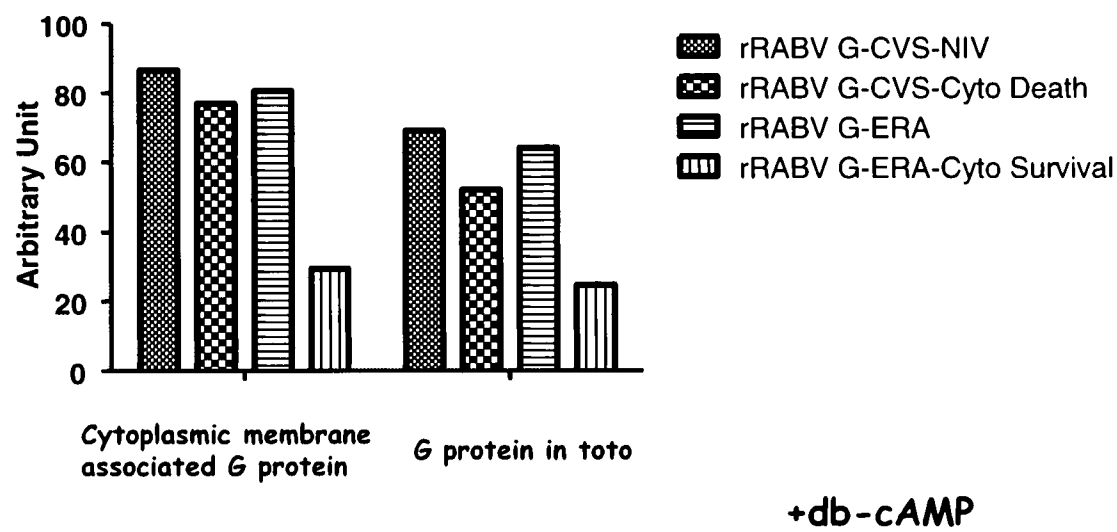
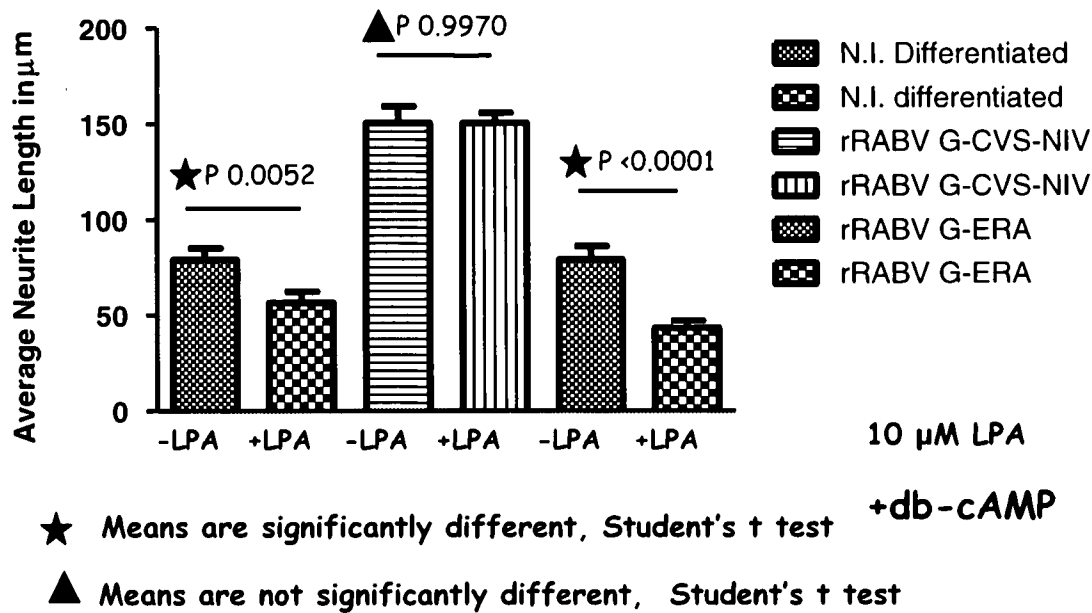
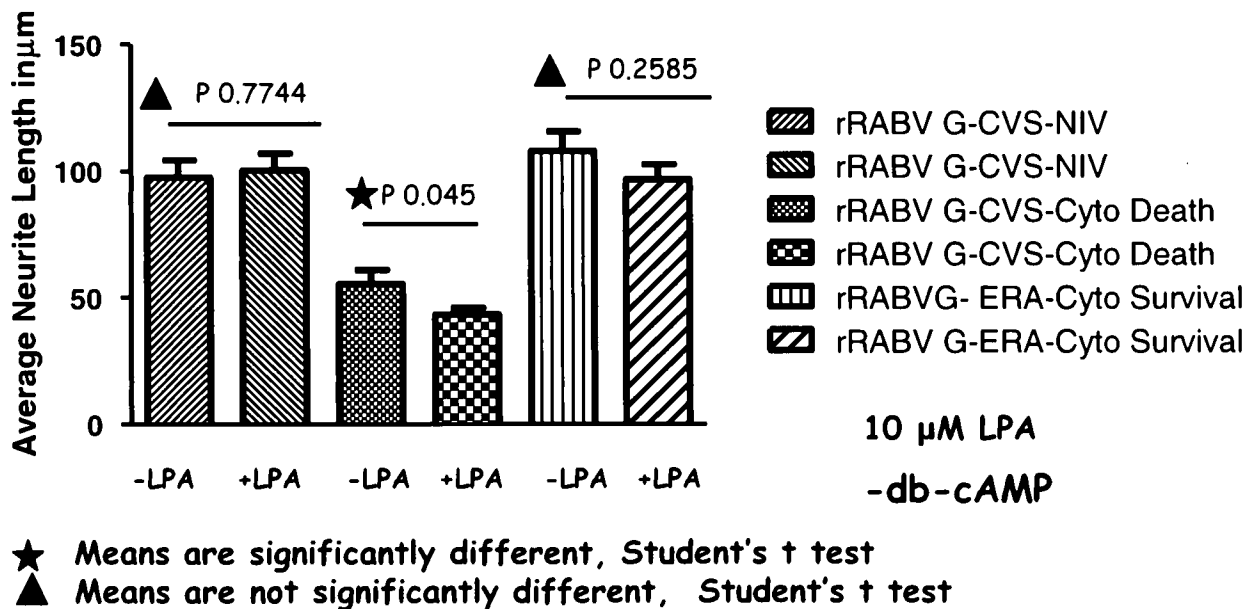
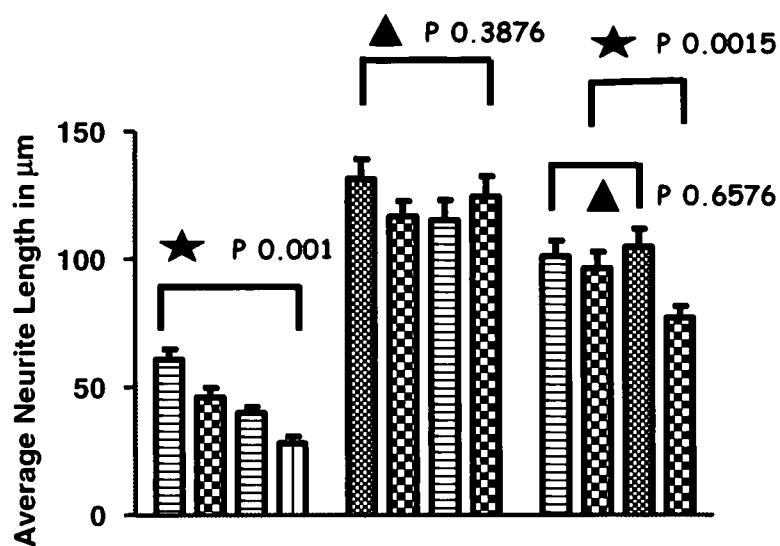


FIGURE 5

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**FIGURE 6A****FIGURE 6B**

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- N.I. -LPA
- ▣ N.I. +LPA 10 μM
- ▤ N.I. +LPA 30 μM
- ▥ N.I. +LPA 50 μM
- ▦ rRABV G-CVS-NIV -LPA
- ▧ rRABV G-CVS-NIV +LPA 10 μM
- ▨ rRABV G-CVS-NIV +LPA 30 μM
- ▩ rRABV G-CVS-NIV +LPA 50 μM
- rRABV G-ERA-Cyto Survival -LPA
- rRABV G-ERA-Cyto Survival +LPA 10 μM
- ▬ rRABV G-ERA-Cyto Survival +LPA 30 μM
- ▭ rRABV G-ERA-Cyto Survival +LPA 50 μM

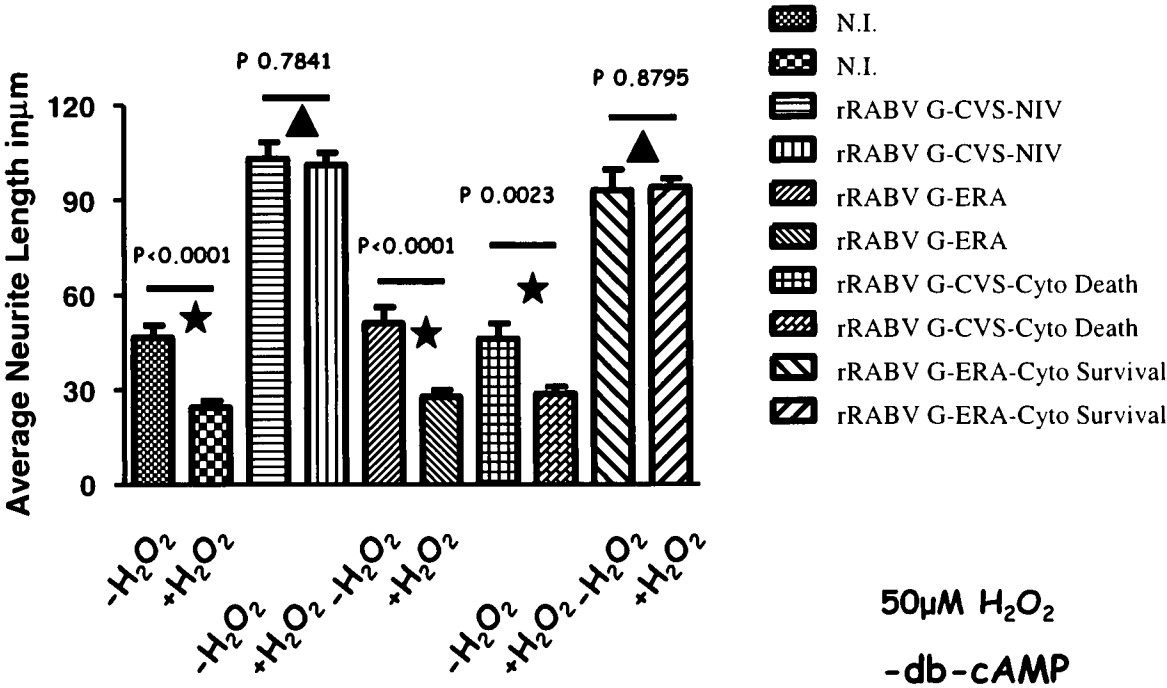
★ Means are significantly different, ANOVA test

-db-cAMP

▲ Means are not significantly different, ANOVA test

FIGURE 7

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▲ Means are not significantly different, Student's t test

★ Means are significantly different, Student's t test

FIGURE 8

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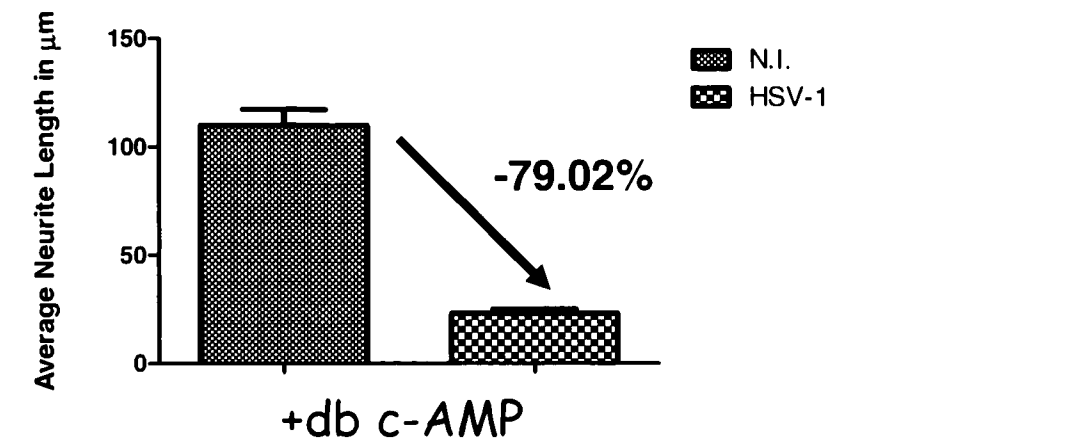


FIGURE 9A

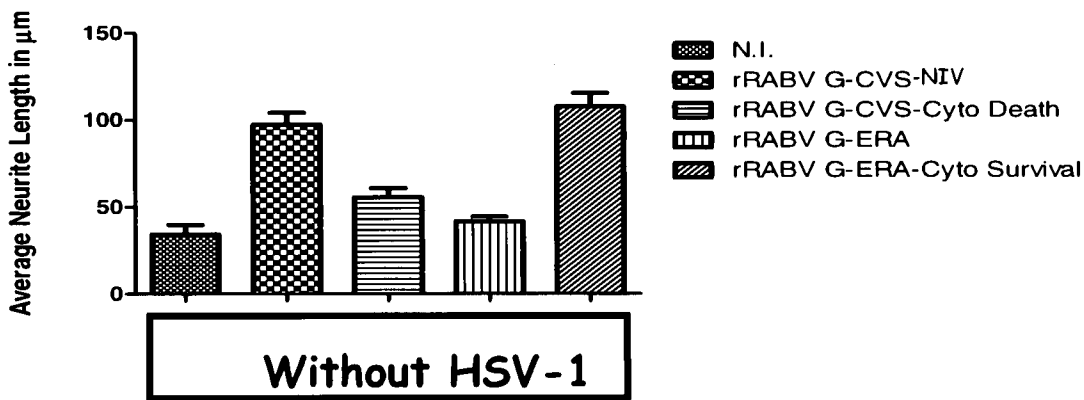


FIGURE 9B

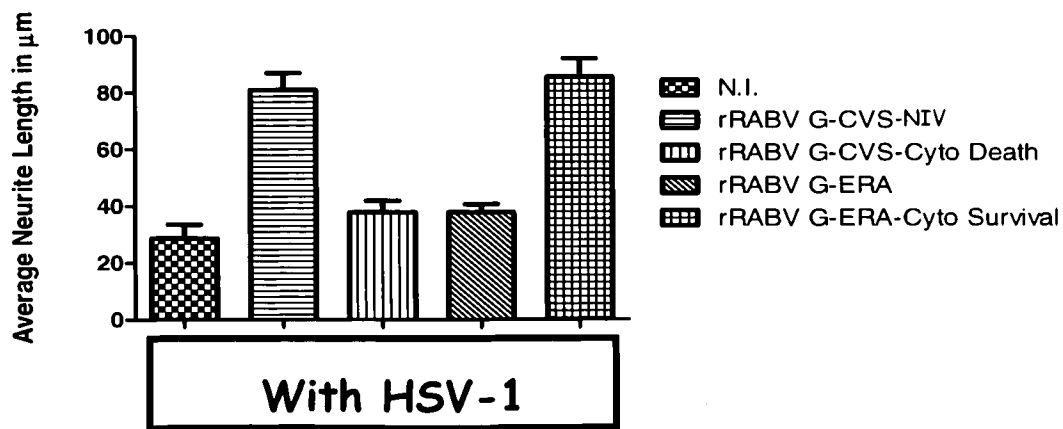


FIGURE 9C

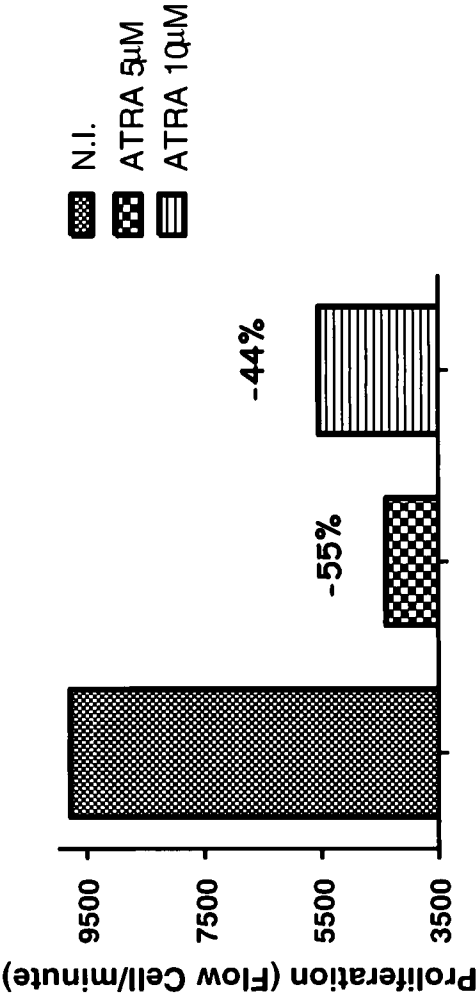


FIGURE 10A

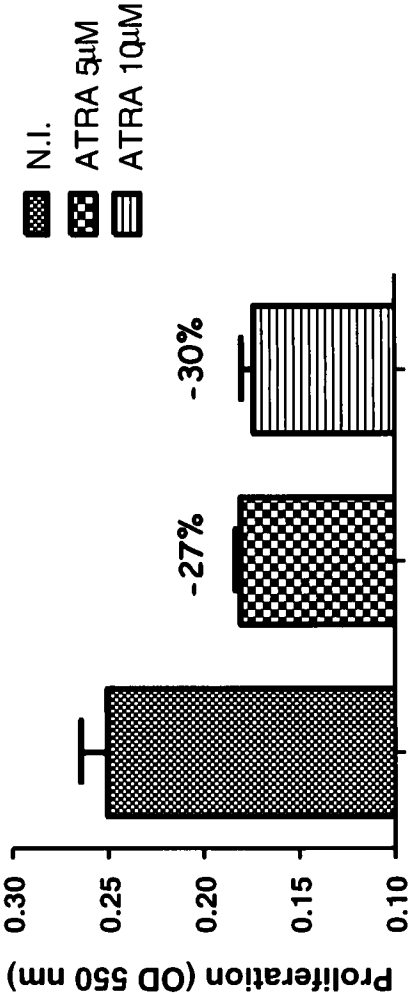


FIGURE 10B

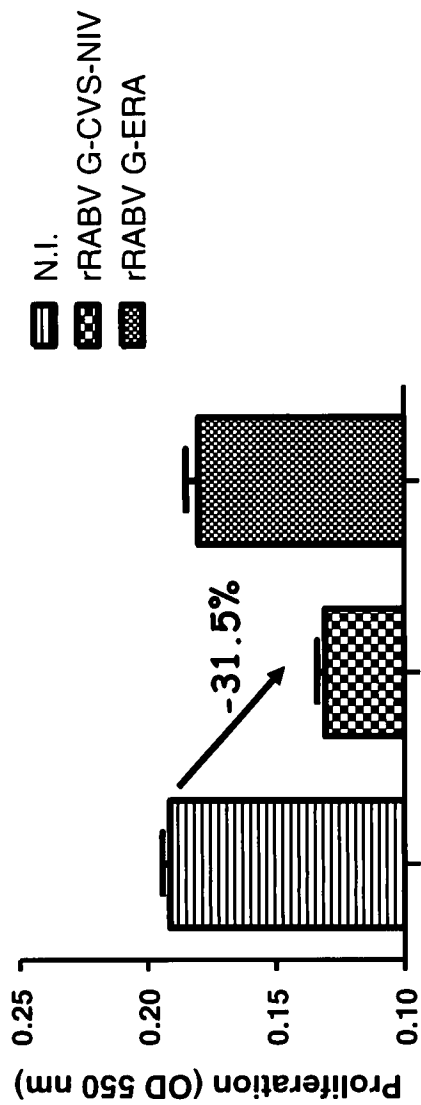


FIGURE 11A

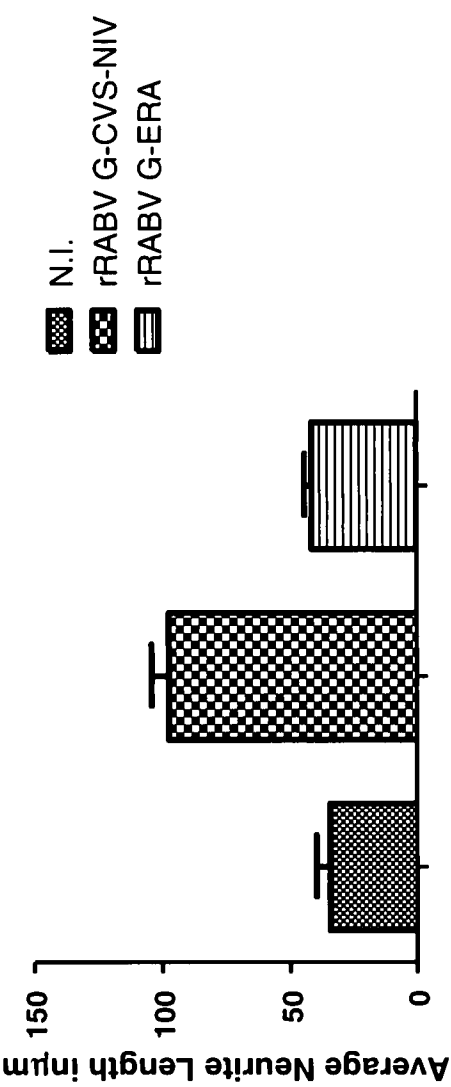


FIGURE 11B

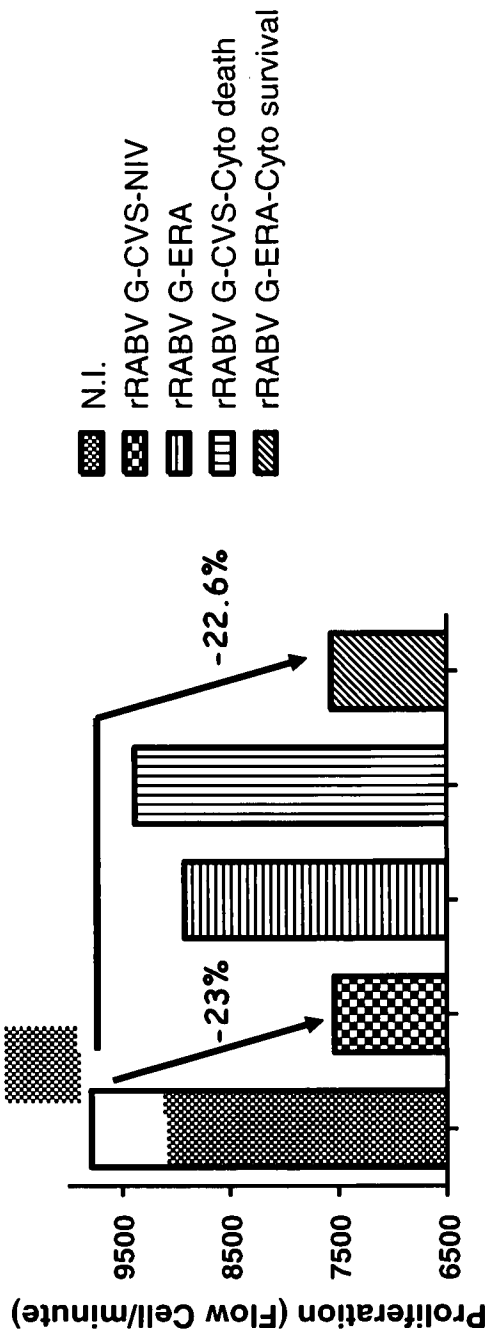


FIGURE 12A

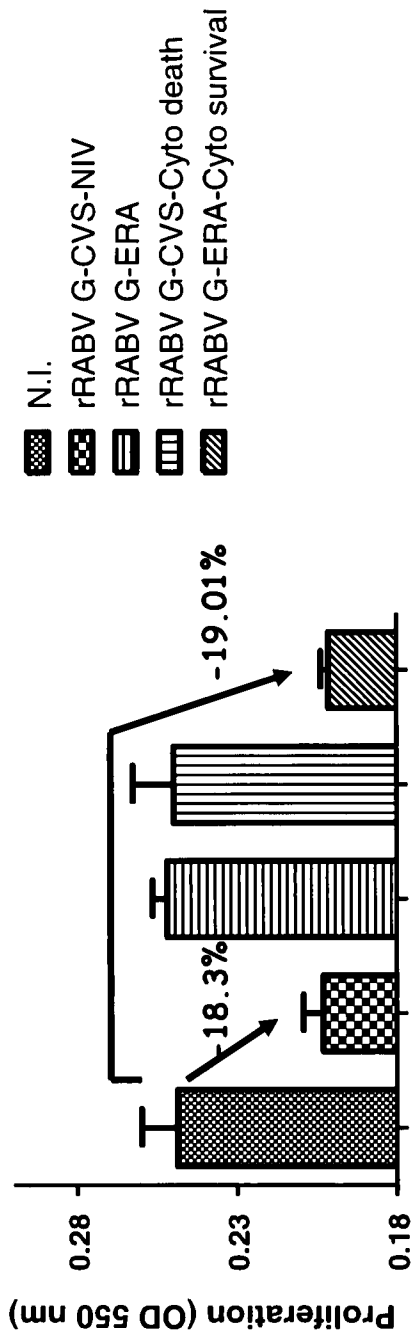


FIGURE 12B

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Nucleotide sequence encoding the **protein G of the CVS-NIV strain** of Rabies virus (sequence accession number AF 406694, which includes the ATG start codon and the TGA stop codon):

```

1 atgggttcctc aggcctctcct gtttgtaccc cttctggttt ttccattgtg ttttgggaaa
61 ttccctathtt acacgatacc agacaagctt ggtccctgga gcccgataga catacatcac
121 ctacagctgcc caaacaattt ggtagtggag gacgaaggat gcaccaacct gtcagggttc
181 tcctacatgg aacttaaagt tggatacatc ttagccataa aaatgaacgg gttcacttgc
241 acaggcgcttg tgacggaggc tgaacactac actaacttcg ttggttatgt cacaaccacg
301 ttcaaaaagaa agcatttccg cccaacacca gatgcatgta gagccgcgta caactggaag
361 atggccggtg accccagata tgaagagtct ctacacaatc cgtaccctga ctaccactgg
421 cttcgaactg taaaaaccac caaggagtct ctcggttatca tatctccaag tgtggcagat
481 ttggacccat atgacagatc ccttctactc aggggtcttc ctagcgggaa gtgcccagga
541 gtacgggtgt cttctaccta ctgctccact aaccacgatt acaccatttg gatgcccag
601 aatccgagac tagggatgtc ttgtgacatt ttaccaata gtacagggaa gagagcatcc
661 aaagggaagt agacttcggt cttttagatg gaaagaggcc tatataagtc tttaaaagga
721 gcatgcaaac tcaagttatg tggagtctta ggacttagac ttatggatgg aacatgggtc
781 gcgatgcaaa catcaaatga aaccaaagg tgcctcccg atcagttggt gaacctgcac
841 gactttcgct cagacgaaat tgagcacctt gttgtagagg agttggtagg gaagagagag
901 gagtgtctgg atgcactaga gtccatcatg acaaccaagt cagtgaagtt cagacgtctc
961 agtcatttaa gaaaacttgt ccctgggttt ggaaaagcat ataccatatt caacaagacc
1021 ttgatggaag ccgatgctca ctacaagtca gtcagaactt ggaatgagat cctcccttca
1081 aaagggtgtt taagagttgg ggggaggtgt catcctcatg tgaacggggt gtttttcaat
1141 ggtataatat taggacctga cggcaatgtc ttaatcccag agatgcaatc atccctctc
1201 cagcaacata tggagtgtgt ggaatcctcg gttatcccc ttgtgcaccc cctggcagac
1261 ccgtctaccg ttttcaagga cggtgacgag gctgaggatt ttgttgaagt tcaccttccc
1321 gatgtgcaca atcaggtctc aggaagttgac ttgggtctcc cgaactgggg gaagtatgta
1381 ttactgagtg caggggccct gactgccttg atgttgataa ttttcctgat gacatgttgt
1441 agaagagtca atcgatcaga acctacgcaa cacaatctca gagggacagg gagggaggtg
1501 tcagtcactc cccaaagcgg gaagatcata tcttcatggg aatcacacaa gagtgggggt
1561 cagaccagac tgtga

```

SEQ ID NO: 1

Sequence of the protein G of the CVS-NIV strain of Rabies virus (virulent strain); 524aa (sequence accession number AF 406694):

```

MVPQALLFVPLLVPFLCFGKFPITYTIPDKLGPWSPIDIHHLSCPNNLVVEDEGCTNLSGFSYMEKVGYILAIKMNGFTCT
GVVTEAETYTNFVGYYVTTTFKRKHFRPTPDACRAAYNWKMAGDPRIEESLHNPYPDYHWLRTVKTTKESLVIISPSVADLD
PYDRSLHSRVFPSPGKCPGVAVSSTYCSTNHDTIWMFPENPRLGMSCDIFTNSRGKRASKGSETCGFVDERGLYKSLKGACK
LKLCGVLGLRLMDGTWVAMQTSNETKWCPDQLVNLHDFRSDIEHLVVEELVRKREELDALESIMTTKSVSFRLSHLR
KLVPFGFKAYTIFNKTLMEADAHYKSVRTWNEILPSKGCRLRVGGRCHPHVNGVFPNGIILGPDGNVLIPEMQSSLLQQHME
LLESSVILVHPLADPSTVFKDGEAEDFVEVHLDPVHNQVSGVDLGLPNWGKYVLLSAGALTALMLIIFLMTCCRNVNRS
EPTQHNLRGTGREVSVTPQSGKISSWESHKSGGQTRL

```

SEQ ID NO: 2

FIGURE 13A

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Nucleotide sequence encoding the **protein G of the ERA strain** of Rabies virus (sequence accession number AF 406693, which includes the ATG start codon and the TGA stop codon):

```

1 atgggttcctc aggcctctcct gtttgtaccc cttctgggtt ttcattgtg ttttgggaaa
61 ttccctatctt acacgatacc agacaagcct ggtccctgga gcccgattga catacatcac
121 ctcagctgcc caaacaattt gatagtggag gacgaaggat gcaccaacct gtcagggttc
181 tcctacatgg aacttaaagt tggatacatc ttagccataa aaatgaacgg gttcacttgc
241 acaggcggtg tgacggagggc tgaaacctac actaacttcg ttggttatgt cacaaccacg
301 ttcaaaaagaa agcattttccg cccaacacca gatgcatgta gagccgcgta caactggaag
361 atggcccggtg accccagata tgaagagtct ctacacaatc cgtaccctga ctaccgctgg
421 cttcgaactg taaaaaccac caaggagtct ctcgttatca tatctccaag tgtggcgagt
481 ttggacccat atgacagatc ccttctactc aggggtcttcc ctacggggaa gtgctcagga
541 gtagcggtgt cttctaccta ctgctccact aaccacgatt acaccatttg gatgcccgag
601 aatccgagac tagggatgtc ttgtgacatt ttaccaata gtagagggaa gagagtatcc
661 aaagggagtg agacttgccg ctttgtagat gaaagaggcc tatataagtc tttaaaagga
721 gcatgcaaac tcaagttatg tggagttcta ggacttagac ttatggatgg aacatgggtc
781 gcgatgcaaa catcaaatga aaccaaattg tgccctccc atcagttggt gaacctgcac
841 gactttcgct cagacgaaat tgagcacctt gttgtagagg agttgggtcag gaagagagag
901 gagtgtctgg atgcactaga gtccatcatg acaaccaagt cagtgaattt cagacgtctc
961 agtcatttaa gaaaacttgt ccctgggttt ggaaaagcat ataccatatt caacaagacc
1021 ttgatggaag ccgatgctca ctacaagtca gtcagaactt ggaatgagat cctcccttca
1081 aaagggtgtt taagagttgg ggggaggtgt catcctcatg tgaacggggt gtttttcaat
1141 ggtataatat taggacctga cggcaatgtc ttaatcccag agatgcaatc atccctcctc
1201 cagcaacata tggagttgtt ggaatcctcg gttatcccc ttgtgcaccc cctggcagac
1261 ccgtctaccg ttttcaagga cggtgacgag gctgaggatt ttgttgaagt tcaccttccc
1321 gatgtgcaca atcaggtctc aggagttgac ttgggtctcc cgaactgggg gaagtatgta
1381 ttactgagtg caggggccct gactgccttg atgttgataa ttttctgat gacatgttgt
1441 agaagagtca atcgatcaga acctacgcaa ctcaatctca gagggacagg gagggaggtg
1501 tcagtcactc cccaaagcgg gaagatcata tcttcatggg aatcacacaa gagtgggggt
1561 gagaccagac tgtga

```

SEQ ID NO: 3

Sequence of the protein G of the ERA strain of Rabies virus (attenuated strain); 524aa (sequence accession number AF 406693):

```

MVPQALLFVPLLVPPLCFGKFPITYTIPDKLGPWSPIDIHHLSCPNNLIVEDEGCTNLSGFSYME LKVGYILAIKMNGFTCT
GVVTEAETYTNFVGYYTTTFKRKHFRPTPDACRAAYNWKMAGDP RYEESLHNPYPDYRWLRTRVKTTKESLVIISPSVADLD
PYDRSLHSRVFPSPGKCSGVAVSSTYCSTNHDYTIWMPENPRLGMSCDIFTNSRGKRVSKGSETCGFVDERGLYKSLKGACK
LKL CGVLGLRLMDGTWVAMQTSNETKWCPDQLVNLHDFRSD EIEHLVVEELVRKREECLDALESIMTTKSVSFRRLSHLR
KLVPGF GKAYTIFNKTLM EADAHYKSVRTWNEILPSKGCLRVGG RCHPHVNGVFFNGIILGPDGNVLIP EMQSSLLQQHME
LLESSVIPLVHPLADPSTVFKDGDEAEDFVEVHL PDVHNQVSGVDLGLPNWKGKYL LLSAGALTALMLIIFLMTCCRNVNRS
EPTQLNLRGTGREVSVTPQSGKIISSWESHKSGGETRL

```

SEQ ID NO: 4

FIGURE 13B

G-CVS-NIV	1	MVPQALLFVPLLVPFLCFCGKFFIYTI	PDKLGWPSPIDIHHLSCPNNLVVEDEGCTNLSGF
G-ERA	1	MVPQALLFVPLLVPFLCFCGKFFIYTI	PDKLGWPSPIDIHHLSCPNNLVVEDEGCTNLSGF
		*****	*****
G-CVS-NIV	61	SYMELKVGYLAIKMNNGFTCTGVVTEAETY	TNFGYVTTTFKRKHFRPTPDACRAAYNWK
G-ERA	61	SYMELKVGYLAIKMNNGFTCTGVVTEAETY	TNFGYVTTTFKRKHFRPTPDACRAAYNWK
		*****	*****
G-CVS-NIV	121	MAGDPREESLHNPYPDYHWRVT	TKTESLVIISPSVADLDPYDRSLHSRVFPGKCPG
G-ERA	121	MAGDPREESLHNPYPDYHWRVT	TKTESLVIISPSVADLDPYDRSLHSRVFPGKCSG
		*****	*****
G-CVS-NIV	181	VAVSSTYCSSTNHDYTIWMPENPR	LGMSCDIFTNSRGKRAKSGSETCGFVDERGLYKSLKG
G-ERA	181	VAVSSTYCSSTNHDYTIWMPENPR	LGMSCDIFTNSRGKRVKSGSETCGFVDERGLYKSLKG
		*****	*****
G-CVS-NIV	241	ACKLKLGVGLRLMDGTWVAMQTSNET	KWCPPDQLVNLHDFRSDIEIHLVVEELVRKRE
G-ERA	241	ACKLKLGVGLRLMDGTWVAMQTSNET	KWCPPDQLVNLHDFRSDIEIHLVVEELVRKRE
		*****	*****
G-CVS-NIV	301	ECLDALESIMTTKSVSFRRLSHLRKL	VPFGKAYTIFNKTLMEADAHYKSVRTWNEILPS
G-ERA	301	ECLDALESIMTTKSVSFRRLSHLRKL	VPFGKAYTIFNKTLMEADAHYKSVRTWNEILPS
		*****	*****
G-CVS-NIV	361	KGCLRVGGRCHPHVNGVFFNGIILGPD	GNVLIPMQSSLLQQHMELLESSVPLVHPLAD
G-ERA	361	KGCLRVGGRCHPHVNGVFFNGIILGPD	GNVLIPMQSSLLQQHMELLESSVPLVHPLAD
		*****	*****
G-CVS-NIV	421	PSTVFKDGDEAEFVEVHLPDVHNQV	SGVDLGLPNWGKYVLLSAGALTALMLIIFLMTCC
G-ERA	421	PSTVFKDGDEAEFVEVHLPDVHNQV	SGVDLGLPNWGKYVLLSAGALTALMLIIFLMTCC
		*****	*****
G-CVS-NIV	481	RRVNRSEPTQNLRGTGREVS	VTPOSGKIISWESHKSGGQTRL SEQ ID NO: 2
G-ERA	481	RRVNRSEPTQNLRGTGREVS	VTPOSGKIISWESHKSGGETRL SEQ ID NO: 4
		*****	*****

FIGURE 14

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Nucleotide sequence encoding the **cytoplasmic domain of the G protein of the CVS-NIV strain** of Rabies virus (fragment 1441-1572 of the full-length G coding sequence):

```
agaagagtca atcgatcaga acctacgcaa cacaatctca gagggacagg gagggaggtg
tcagtcactc cccaaagcgg gaagatcata tttcatggg aatcacacaa gagtgggggt
cagaccagac tg                               SEQ ID NO: 5
```

Sequence of the cytoplasmic domain of the G protein of the CVS-NIV strain of Rabies virus (very last 44 C-terminal aa of the G protein, i.e. fragment 481-524 of the full-length G protein):

```
RRVNRSEPTQHNLRGTGREVSVTPQSGKISSWESHKSGGQTRL      SEQ ID NO: 6
```

Nucleotide sequence encoding the **cytoplasmic domain of the G protein of the ERA strain** of Rabies virus (fragment 1441-1572 of the full-length G coding sequence):

```
agaagagtca atcgatcaga acctacgcaa ctcaatctca gagggacagg gagggaggtg
tcagtcactc cccaaagcgg gaagatcata tttcatggg aatcacacaa gagtgggggt
gagaccagac tg                               SEQ ID NO: 7
```

Sequence of the cytoplasmic domain of the G protein of the ERA strain of Rabies virus (very last 44 C-terminal aa of the G protein, i.e. fragment 481-524 of the full-length G protein):

```
RRVNRSEPTQLNLRGTGREVSVTPQSGKISSWESHKSGGETRL      SEQ ID NO: 8
```

FIGURE 15

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Nucleotide sequence encoding the **PDZ-BS motif of the protein G of the CVS-NIV strain** of Rabies virus (fragment 1561-1572 of the full-length G coding sequence):

cagaccagac tg

SEQ ID NO: 9

PDZ-BS motif of the protein G of the CVS-NIV strain of Rabies virus (very last 4 C-terminal aa of the G protein, *i.e.*, fragment 521-524 of the full-length G protein):

QTRL

SEQ ID NO: 10

Nucleotide sequence encoding the **PDZ-BS motif of the protein G of the ERA strain** of Rabies virus (fragment 1561-1572 of the full-length G coding sequence):

gagaccagac tg

SEQ ID NO: 11

PDZ-BS motif of the protein G of the ERA strain of Rabies virus (very last 4 C-terminal aa of the G protein, *i.e.*, fragment 521-524 of the full-length G protein):

ETRL

SEQ ID NO: 12

Generic sequence of the PDZ-BS motif of a rabies virus G protein:

$x_1 - x_2 - x_3 - x_4$, wherein:

x_1 is any amino acid, and

x_2 is T or S or I, and

x_3 is any amino acid, and

x_4 is L or V.

SEQ ID NO: 13

Sequence of the PDZ-BS motif of a non-apoptotic rabies virus G protein:

$x_1 - x_2 - x_3 - x_4$, wherein:

x_1 is any amino acid except E (x_1 being preferably Q), and

x_2 is T or S or I (preferably T), and

x_3 is any amino acid (preferably R), and

x_4 is L or V (preferably L).

SEQ ID NO: 14

FIGURE 16

G-NIV
G-Gif

```
1 MVPQALLFVPLLVFPLCFGKFPITYTIPDKLGPWSPIDIHHLSCPNNLVVEDEGCTNLSGFF
1 MVPQVLLFVLLLGFSLCFGKFPITYTIPDKLGPWSPIDIHHLRCPNNLVVEDEGCINLSGFF
  *** ** * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
61 SYMELKVGYILAIKMNGFTCTGVVTEAETYTNFVGYYVTTTFKRKHFRPTPDACRAAYNWK
61 SYMELKVGYISAIKVNNGFTCTGVVTEAETYTNFVGYYVTTTFKRKHFRPTPDACRAAYNWK
  *** ** * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
121 MAGDPRYEESLHNPYPDYHHLRTVKTTKESLVIISPSVADLDPYDRSLHSRVFPFGKCPG
121 MAGDPRYEESLQNPYPDYHHLRTVRTTKESLIIISPSVTDLPYDKSLHSRVFPFGKCSG
  *** ** * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
181 VAVSSTYCSNHDYTIWMPENPRLGMSCDIFTNSRGKRASKGSETCGFVDERGLYKSLKG
181 ITVSSTYCSNHDYTIWMPENPRPGTPCDIFTNSRGKRASNGNKTCGFVDERGLYKSLKG
  *** ** * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
241 ACKLKLCGVLGLRLMDGTWVAMQTSNETKWCPDQLVNLHDFRSDIEIHLVVEELVRKRE
241 ACRLKLCGVLGLRLMDGTWVAMQTSDETKWCSPDQLVNLHDFRSDIEIHLVVEELVKKRE
  *** ** * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
301 ECLDALESIMTTKSVSFRRLSHLRKLVPGFGKAYTIFNKTLM EADAHYKSVRTWNEILPS
301 ECLDTLESIMTTKSVSFRRLSHLRKLVPGFGKAYTIFNKTLM EADAHYKSVRTWNEIIPS
  *** ** * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
```

FIGURE 17 (start)

```

361 KGCLRVGGRCHPHVNGVFFNGIILGPDGNVLIPEMQSSLLQQHMELLESSVPLVHPLAD
361 KGCLKVGGRCHPHVNGVFFNGIILGPDDRVLIPEMQSSLLRQHMELLESSVPLMHPLAD
    **** ***** ***** ***** ***** ***** ***** *****
421 PSTVFKDGEAEDFVEVHLPDVHNQVSGVDLGLPNWGKYVLLSAGALTALMLIIFLMTCC
421 PSTVFKEGDEAEDFVEVHLPDVYKQISGVDLGLPNWGKYVLMTAGAMIGLVLI FSLMTWC
    ***** ***** * ***** ***** ***** ***** * * * * *
481 RRVNRSEPTQHNLRGTGREVSVTPQSGKIISSWESHKSGGQTRL SEQ ID NO: 2
481 RRANRPESKQRSFGGTGGNVSVTSQSGKVI PSWESYKSGGEIRL SEQ ID NO: 15
    ** ** * * * * * * * * * * * * * * * * * * * * * *

```

FIGURE 17 (end)

CLUSTAL 2.0.10 multiple sequence alignment

```

G_ACA57830_
G_AAC34683_
G_ABV24348_
G-NIV
MVPQVLLFVPLLGFSLCFGKFPYITIPDELGPWSPIDIHHLSCPNNLVVEDEGCTNLSEF 60
MVPQVLLFVPLLGFSLCFGKFPYITIPDKLGPWSPIDIHHLSCPNNLVVEDEGCTNLSEF 60
MVPQVLLFVPLLGFSLCFGKFPYITIPDKLGPWSPIDIHHLRCPNNLVVEDEGCTNLSEF 60
MVPQALLFVPLLVFPPLCFGKFPYITIPDKLGPWSPIDIHHLSCPNNLVVEDEGCTNLSEF 60
***.*** ** *.*****;***** ***** ***** *

G_ACA57830_
G_AAC34683_
G_ABV24348_
G-NIV
SYMELKVGYSIAIKVNGFTCTGVVTEAEITYTNFVGYYVTTTFKRKHFRPTPDACRAAYNWK 120
SYMELKVGYSIAIKVNGFTCTGVVTEAEITYTNFVGYYVTTTFKRKHFRPTPDACRAAYNWK 120
SYMELKVGYSIAIKVNGFTCTGVVTEAEITYTNFVGYYVTTTFKRKHFRPTPDACRAAYNWK 120
SYMELKVGYSIAIKVNGFTCTGVVTEAEITYTNFVGYYVTTTFKRKHFRPTPDACRAAYNWK 120
***** ***;***** ***** ***** *****

G_ACA57830_
G_AAC34683_
G_ABV24348_
G-NIV
MAGDPRYEESLHNPYPDYHHLRVTRTTKESLIIISPSVTDLDPYDKSLHSRVFPFGKCSG 180
MAGDPRYEESLHNPYPDYHHLRVTRTTKESLIIISPSVTDLDPYDKSLHSRVFPFGKCSG 180
MAGDPRYEESLQNPYPDYHHLRVTRTTKESLIIISPSVTDLDPYDKSLHSRVFPFGKCSG 180
MAGDPRYEESLHNPYPDYHHLRVTVKTTKESLVIISPSVADLDPYDRSLHSRVFPFGKCPG 180
*****;*****;*****;*****;*****;*****;*****.***.

G_ACA57830_
G_AAC34683_
G_ABV24348_
G-NIV
ITVSSSTYCSSTNHDYTIWMPEDPRPRTPCNIFTNSRGKRASKGNKTCGFVDERGLYKSLKG 240
ITVSSSTYCSSTNHDYTIWMPENPRPRTPCDIFTNSRGKRASKGNKTCGFVDERGLYKSLKG 240
ITVS-TYCSSTNHDYTIWMPEDPRPGTPCDIFTNSRGKRASKGNKTCGFVDERGLYKSLKG 239
VAVSSTYCSSTNHDYTIWMPENPRLGMSCDIFTNSRGKRASKGSETCGFVDERGLYKSLKG 240
:.*.*****;.*.*****;.*.*****;.*.*****;.*.*****;.*.*****

```

FIGURE 18 (start)

G_ACA57830	ACRLKCGVLGLRLMDGTWVAMQTSDETKWCPPDQLVNLHDFHSDEIEHLVVEELVKKRE	300
G_AAC34683	ACRLKCGVLGLRLMDGTWVAMQTSDETKWCPPDQLVNLHDFRSDEIEHLVVEELVKKRE	300
G_ABV24348	ACRLKCGVLGLRLMDGTWVAMQTSDETKWCSPDQLVNLHDFRSNEIEHLVVEDLVKKRE	299
G_NIV	ACKLKCGVLGLRLMDGTWVAMQTSNETKWCPPDQLVNLHDFRSDEIEHLVVEELVRKRE	300
	:**:*****:*****:*****:*****:*****:*****:***:***	
G_ACA57830	ECLDALESIMTTKSVSFRRLSHLRKLVPGFGKAYTIFNKTLMEADAHYKSVRTWNEIIPS	360
G_AAC34683	ECLDALESIMTTKSVSFRRLSHLRKLVPGFGKAYTIFNKTLMEADAHYKSVRTWNEIIPS	360
G_ABV24348	ECLDTLESIMTTKSVSFRRLSHLRKLVPGFGKAYTIFNKTLMEADVHYKSVRTWNEIIPS	359
G_NIV	ECLDALESIMTTKSVSFRRLSHLRKLVPGFGKAYTIFNKTLMEADAHYKSVRTWNEIIPS	360
	:**:*****:*****:*****:*****:*****:*****:***:***	
G_ACA57830	KGCLKVGGRCHPHVNGVFFNGIILGPDGHVLIPEMQSSLLQQHMELLKSSVIPLMHPLAD	420
G_AAC34683	KGCLKVGGRCHPHVNGVFFNGIILGPDGHVLIPEMQSSLLQQHMELLKSSVIPLMHPLAD	420
G_ABV24348	KGCLKVGGRCHPHVNGVFFNGIILGPDGDRVLIPEMQSSLLRQHMELESSVIPLMHPLAD	419
G_NIV	KGCLRVGGRCHPHVNGVFFNGIILGPDGDNVLIPEMQSSLLQQHMELESSVIPLVHPLAD	420
	:**:*****:*****:*****:*****:*****:*****:***:***	
G_ACA57830	PSTVFKEGDEAEDFVEVHLPDVYKRIISGVDLGLPNWGKYVLMTAGAMIGLVLI FSLMTWC	480
G_AAC34683	PSTVFKEGDEAEDFVEVHLPDVYKQISGVDLGLPNWGKYVLMTAGAMIGLVLI FSLMTWC	480
G_ABV24348	PSTVFKEGDEAEDFVEVHLPDVYKKISGVDLGLPNWGKYVLMTAGAMIGLVLI FSLMTWC	479
G_NIV	PSTVFKDGDDEAEDFVEVHLPDVHNVQSGVDLGLPNWGKYVLLSAGALTALMLIIFLMTCC	480
	:**:*****:*****:*****:*****:*****:*****:***:***	
G_ACA57830	RRANRPESKQSFSGGTGRNVSVTSQSGKVI PSWESYKSGGEIRL	524
G_AAC34683	RRANRPESKQSFSGGTGRNVSVTSQSGKVI PSWESYKSGGEIRL	524
G_ABV24348	RRANRPESKQSFSGGTGGNVSVTSQSGKVI PSWESYKSGGEIRL	523
G_NIV	RRVNRSEPTQHNLRGTGREVSVTPQSGKIISWESHKSGGQTRL	524
	:**:*****:*****:*****:*****:*****:*****:***:***	

FIGURE 18 (end)

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Cytoplasmic fragment of the G protein of the rabies virus
CVS-NIV strain (SEQ ID NO: 6):

RRVNRSEPTQ**H**NLRGTGREVSVTPQSGKIISWESHKSGG**Q**TRL

Cytoplasmic fragment of the G protein of the rabies virus
CVS-NIV LQ strain (SEQ ID NO: 19):

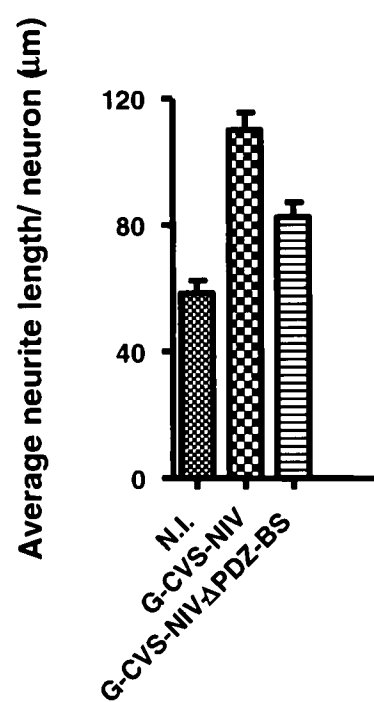
RRVNRSEPTQ**L**NLRGTGREVSVTPQSGKIISWESHKSGG**Q**TRL

Cytoplasmic fragment of the G protein of the rabies virus
CVS-NIV strain HE (SEQ ID NO: 20):

RRVNRSEPTQ**H**NLRGTGREVSVTPQSGKIISWESHKSGG**E**TRL

FIGURE 19

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**FIGURE 20**

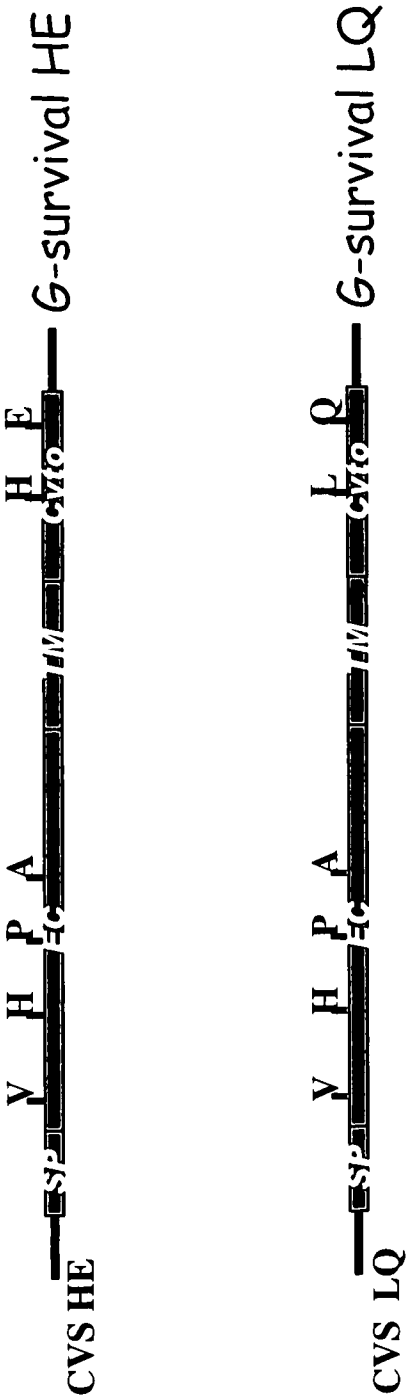


FIGURE 21

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G-CVS domains**G full length:**

MVPQALLFVPLLVPPLCFGKFPITYTIPDKLGPWSPIDIHHLSCPNNLVVEDEGCTNLSGFSYMELKVGYILAIKMNGFTCT
 GVVTEAETYNFVGYVTTTFKRKHFRPTPDACRAAYNWKMAGDPRIEESLHNPYPDYHWLRTVKTKESESLVISPVSADLD
 PYDRSLHSRVFSPGKCPGVAVSSTYCSNHDYTIWMPENPRLGMSCDIFTNSRGKRASKGSETCGFVDERGLYKSLKGACK
 LKLCGVLGRLMDGTWVAMQTSNETKWCPDQLVNLHDFRSEIEHLVVEELVRKREECLDALESIMTTKSVSFRRLSHLR
 KLVPGFGKAYTIFNKTLM EADAHYKSVRTWNEILPSKGCLRVGGRCHPHVNGVFFNGIILGPDGNVLIPEMQSSLLQQHME
 LLESSVIPLVHPLADPSTVFKDGD EADFVEVHLDPVHNQVSGVDLGLPNWGKYVLLSAGALTALMLIIFLMTCCRVRNRS
 EPTQHNLRGTGREVSVTPQSGKIISSWESHKSGGQTRL (SEQ ID NO: 2)

Signal peptide (SP):

MVPQALLFVPLLVPPLCFG (SEQ ID NO: 21)

Ectodomain (EC):

KFPITYTIPDKLGPWSPIDIHHLSCPNNLVVEDEGCTNLSGFSYMELKVGYILAIKMNGFTCTGVVTEAETYNFVGYVTTT
 FKRKHFRPTPDACRAAYNWKMAGDPRIEESLHNPYPDYHWLRTVKTKESESLVISPVSADLDPYDRSLHSRVFSPGKCPGV
 AVSSTYCSNHDYTIWMPENPRLGMSCDIFTNSRGKRASKGSETCGFVDERGLYKSLKGACKLKLCGVLGRLMDGTWVAM
 QTSNETKWCPDQLVNLHDFRSEIEHLVVEELVRKREECLDALESIMTTKSVSFRRLSHLRKLVPGFGKAYTIFNKTLM E
 ADAHYKSVRTWNEILPSKGCLRVGGRCHPHVNGVFFNGIILGPDGNVLIPEMQSSLLQQHME LLESSVIPLVHPLADPSTV
 FKDGD EADFVEVHLDPVHNQVSGVDLGLPNWGK (SEQ ID NO: 22)

Transmembrane domain (TM):

YVLLSAGALTALMLIIFLMTCC (SEQ ID NO: 23)

Cytoplasmic domain (Cyto):

RRVNRSEPTQHNLRGTGREVSVTPQSGKIISSWESHKSGGQTRL (SEQ ID NO: 6)

Amino acids coded by construct inserts:**Amino acid M + SEQ ID NO: 6 =**

MRRVNRSEPTQHNLRGTGREVSVTPQSGKIISSWESHKSGGQTRL (SEQ ID NO: 24)

Transmembrane domain of SEQ ID NO: 23 + cytoplasmic domain of SEQ ID NO: 6 =

YVLLSAGALTALMLIIFLMTCCRVRNRS EPTQHNLRGTGREVSVTPQSGKIISSWESHKSGGQTRL (SEQ ID NO: 25)

Two amino acids from the C-terminal end of the ectodomain of G-CVS-NIV (amino acids GK) + transmembrane domain of SEQ ID NO: 23 + cytoplasmic domain of SEQ ID NO: 6 =

GKYVLLSAGALTALMLIIFLMTCCRVRNRS EPTQHNLRGTGREVSVTPQSGKIISSWESHKSGGQTRL (SEQ ID NO: 26)

Signal peptide of SEQ ID NO: 21 + two amino acids from the C-terminal end of the ectodomain of G-CVS-NIV (amino acids GK) + transmembrane domain of SEQ ID NO: 23 + cytoplasmic domain of SEQ ID NO: 6 =

MVPQALLFVPLLVPPLCFGGKYVLLSAGALTALMLIIFLMTCCRVRNRS EPTQHNLRGTGREVSVTPQSGKIISSWESHKSGGQTRL (SEQ ID NO: 27)

FIGURE 22

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CLUSTAL 2.0.12 multiple sequence alignment

```

Gfull      MVPQALLFVPLLVFPLCFGKFPITYTIPDKLGPWSPIDIHHLSCPNNLVVEDEGCTNLSGF 60
GSP2aaTMCyto MVPQALLFVPLLVFPLCFG----- 19
GCyto      -----

Gfull      SYMELKVGYILAICKMNGFTCTGVVTEAETYTNFVGyvTTTTFKRKHFRPTPDACRAAYNWK 120
GSP2aaTMCyto -----
GCyto      -----

Gfull      MAGDPRYEESLHNYPDYHWLRTVKTTKESLVIISPSVADLDPYDRSLHSRVFSPGKCPG 180
GSP2aaTMCyto -----
GCyto      -----

Gfull      VAVSSTYCSTNHDTIWMPEPNRLGMSCDIFTNSRGKRASKGSETCGFVDERGLYKSLKG 240
GSP2aaTMCyto -----
GCyto      -----

Gfull      ACKLKLCGVLGLRLMDGTWVAMQTSNETKWCPPDQLVNLHDFRDEIEHLVVEELVRKRE 300
GSP2aaTMCyto -----
GCyto      -----

Gfull      ECLDALESIMTTKSVSFRRLSHLRKLVPFGKAYTIFNKTLMEADAHYKSVRTWNEILPS 360
GSP2aaTMCyto -----
GCyto      -----

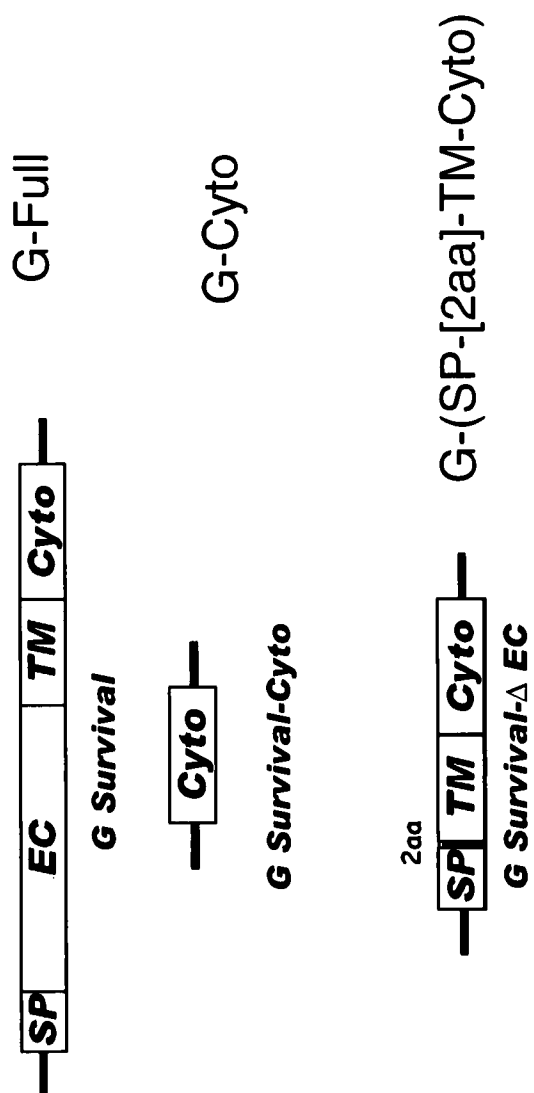
Gfull      KGCLRVGGRCHPHVNGVFFNGIILGPDGNVLIPMQSSLLQQHMELLESSVIPLVHPLAD 420
GSP2aaTMCyto -----
GCyto      -----

Gfull      PSTVFKDGDEAEDFVEVHLPDVHNQVSGVDLGLPNWGKYVLLSAGALTALMLIIFLMTCC 480
GSP2aaTMCyto -----GKYVLLSAGALTALMLIIFLMTCC 43
GCyto      -----M 1

Gfull      RRVNRSEPTQHNLRGTRGVSVTPQSGKIISWESHKSGGQTRL 524 SEQ ID NO: 2
GSP2aaTMCyto RRVNRSEPTQHNLRGTRGVSVTPQSGKIISWESHKSGGQTRL 87 SEQ ID NO: 27
GCyto      RRVNRSEPTQHNLRGTRGVSVTPQSGKIISWESHKSGGQTRL 45 SEQ ID NO: 24
*****

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FIGURE 23



(SP Signal peptide, EC Extracellular domain, TM Transmembrane domain, Cyto Cytoplasmic domain)

FIGURE 24

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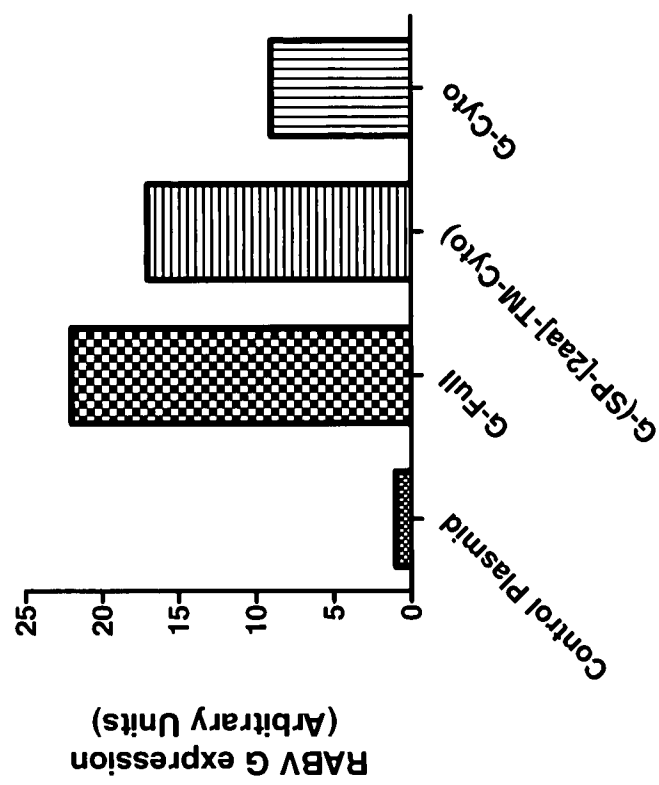


FIGURE 25

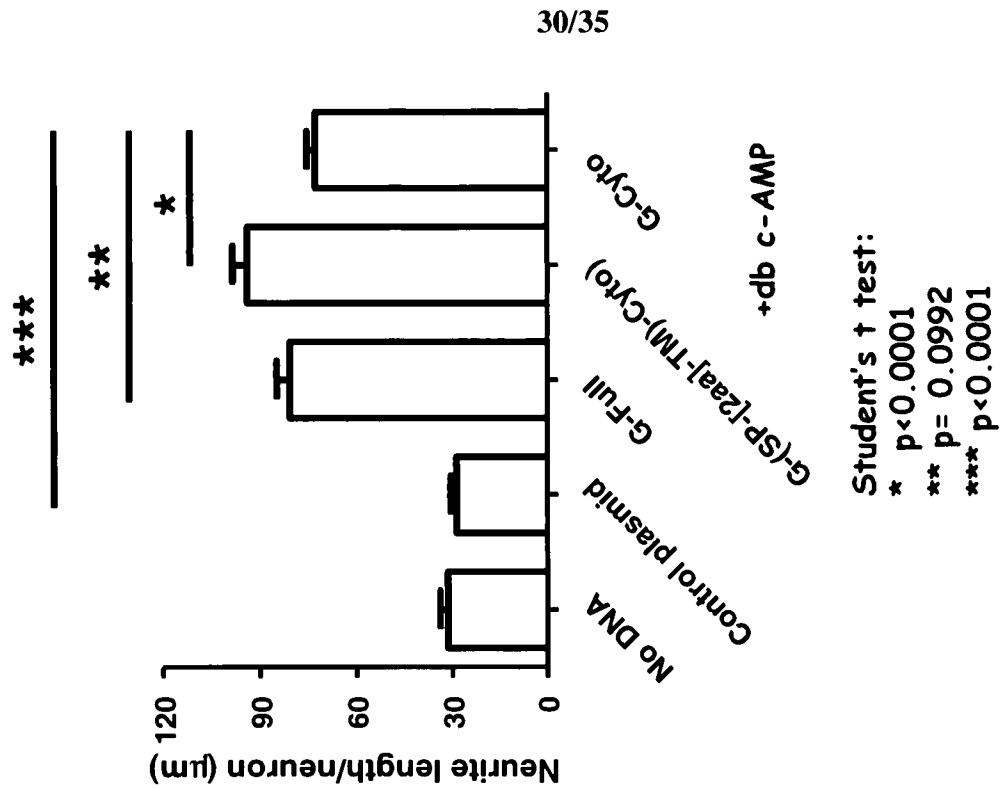


FIGURE 27

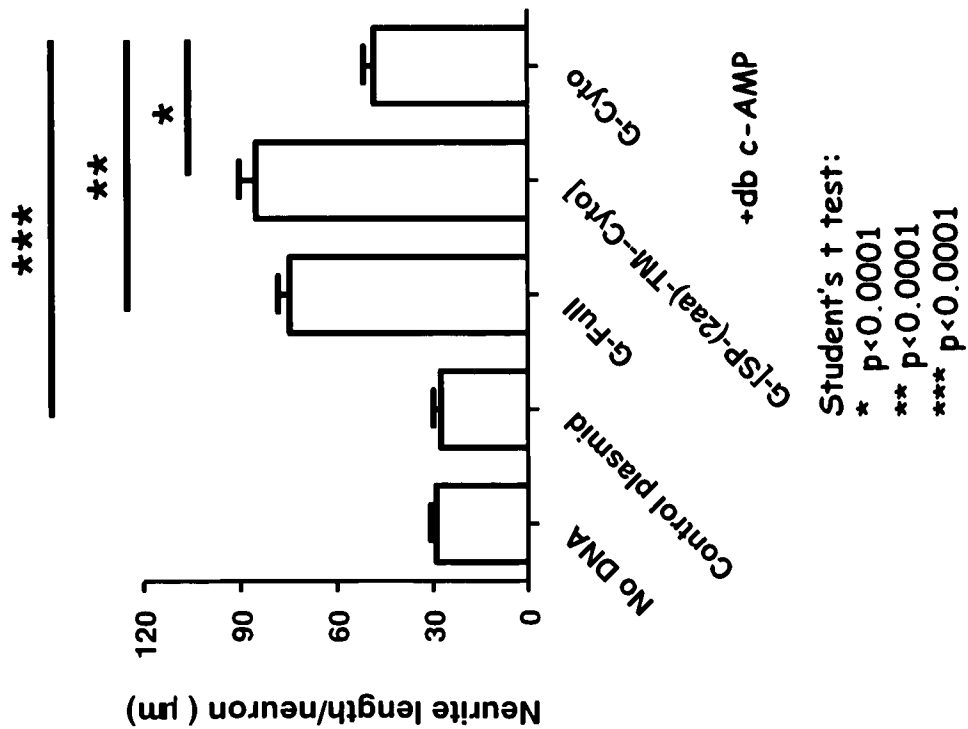


FIGURE 26

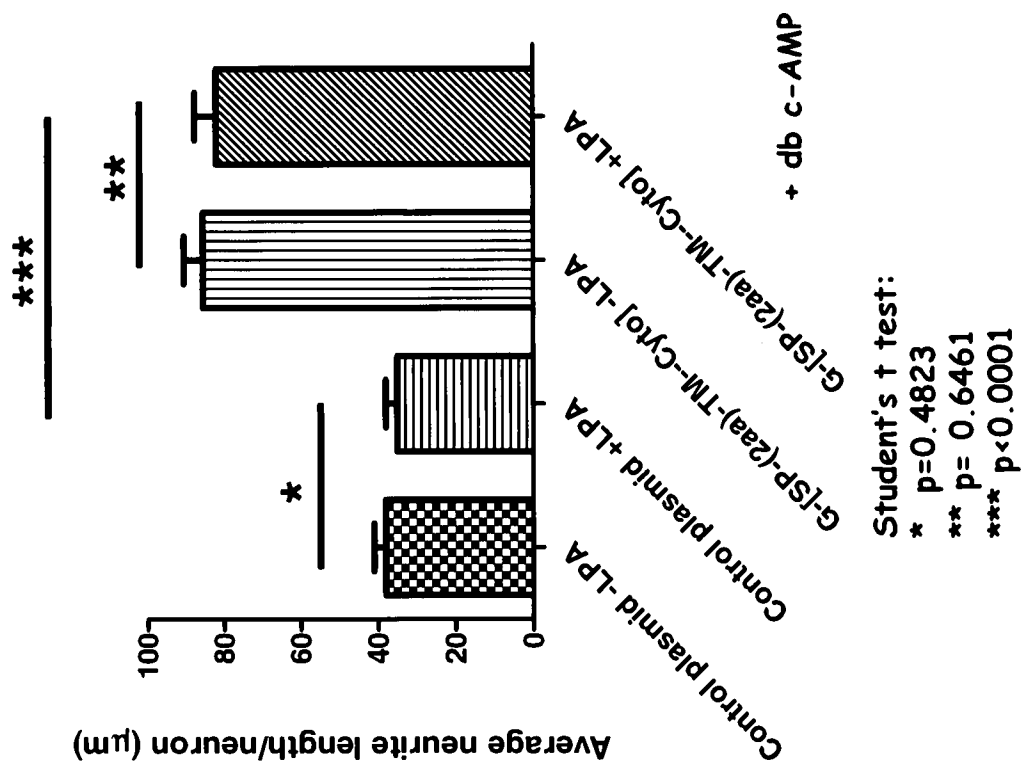


FIGURE 28

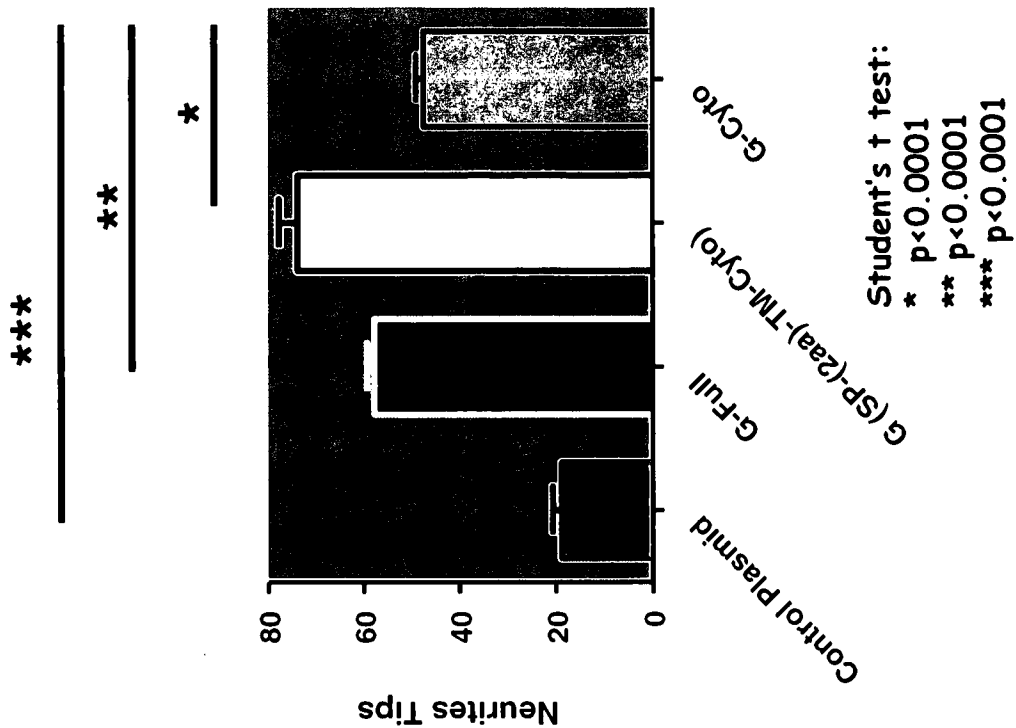


FIGURE 30

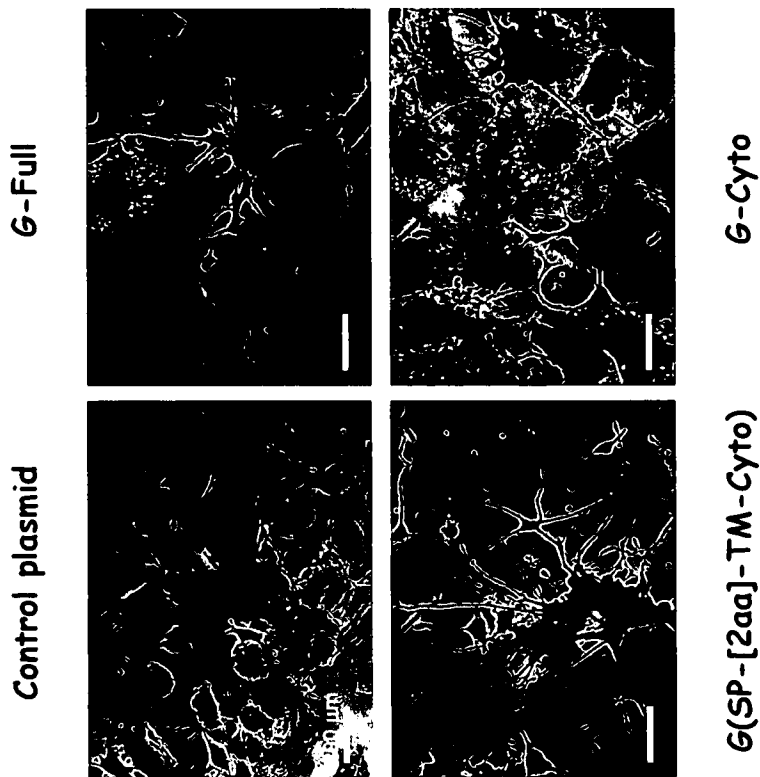


FIGURE 29

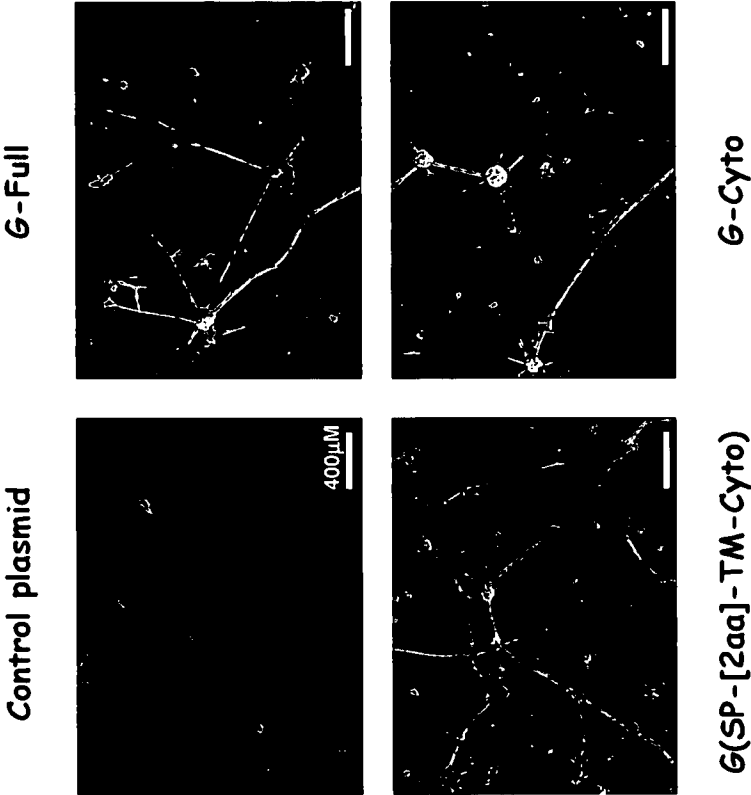
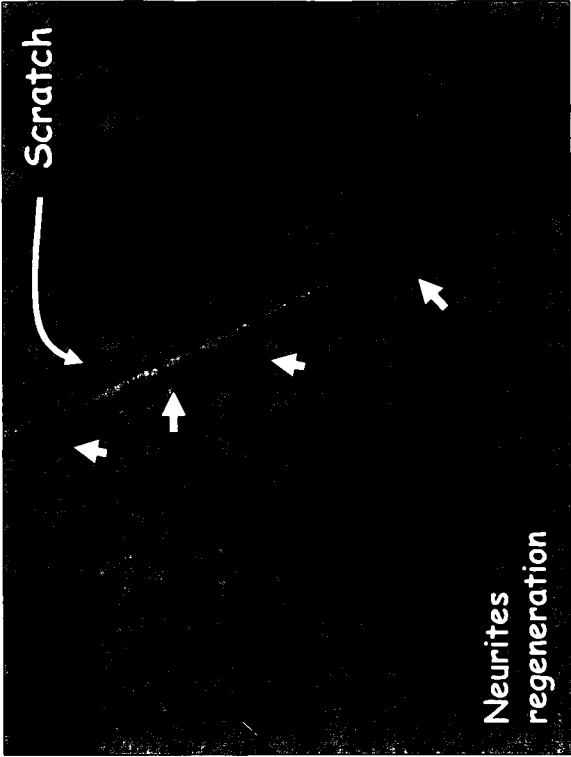


FIGURE 31

G(SP-[2aa]-TM-Cyto)



Control plasmid



FIGURE 32

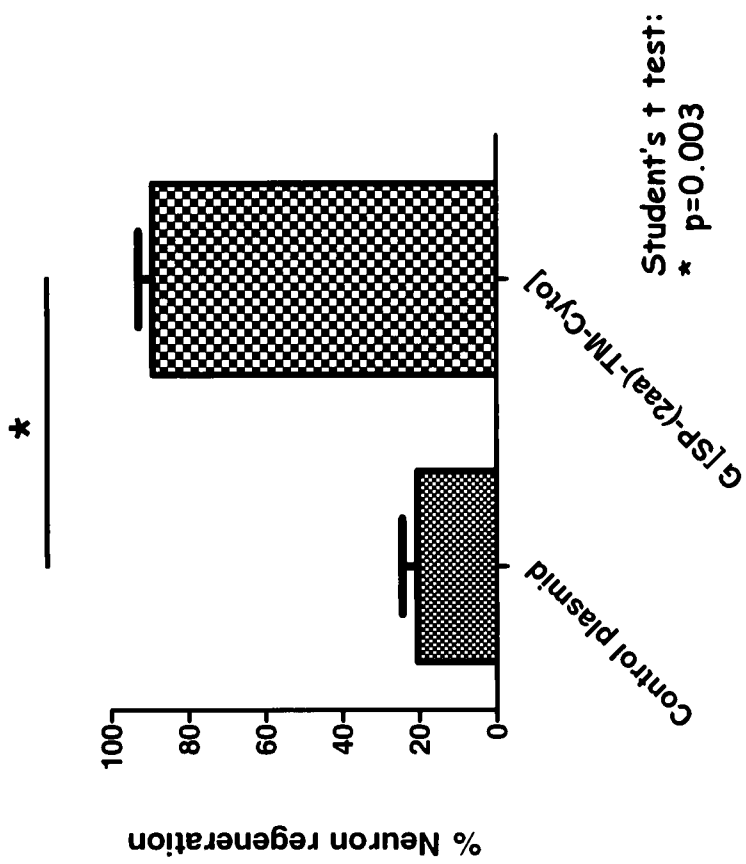


FIGURE 33

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2010/000967

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N15/47 C12N15/86 C07K14/145 A61K38/17 A61K48/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PRÉHAUD CHRISTOPHE ET AL: "Glycoprotein of nonpathogenic rabies viruses is a key determinant of human cell apoptosis." JOURNAL OF VIROLOGY OCT 2003, vol. 77, no. 19, October 2003 (2003-10), pages 10537-10547, XP002532332 ISSN: 0022-538X cited in the application	1,5,6, 9-20
Y	page 10538, left-hand column, paragraph 5 * abstract	2-4, 23-32
X	-& DATABASE GENBANK 12 September 2003 (2003-09-12), PREHAUD,C.J.; LAY,S.J.: "Rabies virus strain CVS glycoprotein gene, complete cds" XP002532334 Database accession no. AF406694 the whole document ----- -/--	1,5,6, 9-20

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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Date of the actual completion of the international search

10 June 2010

Date of mailing of the international search report

18/06/2010

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2010/000967

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/048198 A2 (PASTEUR INSTITUT [FR]; LAFON MONIQUE [FR]; PREHAUD CHRISTOPHE [FR]; LA) 12 June 2003 (2003-06-12) cited in the application claims 1-34; figure 11; sequence 2 -----	1,6-8, 18-20
X	WO 2004/031390 A1 (OXFORD BIOMEDICA LTD [GB]; MAZARAKIS NICHOLAS [GB]; AZZOUZ MIMOUN [GB]) 15 April 2004 (2004-04-15) page 56 - page 57; claims 1-50; sequences 1,2 -----	1,6-8, 18-20
Y		2-4, 23-32
X	US 2004/076613 A1 (MAZARAKIS NICHOLAS [GB] ET AL) 22 April 2004 (2004-04-22) * abstract; claims 1-31; example 10; sequences 3,13 page 311 -----	1,6-8, 18-20
Y		2-4, 23-32
X	WO 2006/078272 A2 (UNIV JEFFERSON [US]; SCHNELL MATTHIAS [US]; DIETZSCHOLD BERNHARD [US]) 27 July 2006 (2006-07-27) page 87; figures 7,9; sequences 32,34 -----	1,5,6, 9-20
X	KLINGEN YVONNE ET AL: "Double-labeled rabies virus: live tracking of enveloped virus transport." JOURNAL OF VIROLOGY JAN 2008, vol. 82, no. 1, January 2008 (2008-01), pages 237-245, XP002532333 ISSN: 1098-5514 * abstract; figure 1 -----	1,6-8, 18-20
A	DIETZSCHOLD B ET AL: "New approaches to the development of live attenuated rabies vaccines" HYBRIDOMA AND HYBRIDOMICS, vol. 21, no. 2, 1 April 2002 (2002-04-01), pages 129-134, XP002323000 MARY ANN LIEBERT, NEW YORK, NY, US ISSN: 1536-8599 * abstract -----	1,6-8, 18-20

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2010/000967

Patent document cited in search report		Publication date		Patent family member(s)		Publication date
WO 03048198	A2	12-06-2003	AU	2002364431 A1		17-06-2003
			CA	2364150 A1		07-06-2003
WO 2004031390	A1	15-04-2004	AU	2003271883 A1		23-04-2004
			EP	1551974 A1		13-07-2005
			JP	2006502240 T		19-01-2006
US 2004076613	A1	22-04-2004	NONE			
WO 2006078272	A2	27-07-2006	US	2008311147 A1		18-12-2008