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        Method for achieving desired glial growth factor 2 plasma levels

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(54) Title: METHOD FOR ACHIEVING DESIRED GLIAL GROWTH FACTOR 2 PLASMA LEVELS
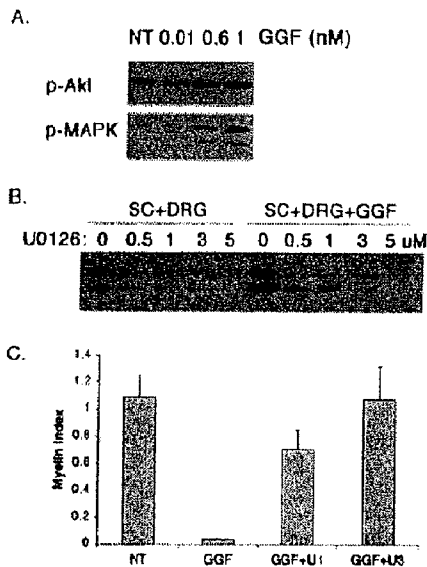
(57) Abstract: The present invention relates to administering glial growth factor 2 (GGF2) to a patient in need thereof, to achieve serum levels of GGF2 within a desired therapeutic window determined based on the disease or disorder afflicting the patient. In a particular embodiment, the patient is suffering from a disease or disorder associated with reduced levels of myelination and the GGF2 is administered to promote myelination in the patient.

Figure 1

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METHOD FOR ACHIEVING DESIRED GLIAL GROWTH FACTOR 2 PLASMA LEVELS

CROSS REFERENCE TO RELATED APPLICATIONS

5   This application claims priority under 35 USC §119(e) from U.S Provisional Application Serial No. 61/067,589, filed February 29, 2008, which application is herein specifically incorporated by reference in its entirety.

10   ## FIELD OF THE INVENTION

The present invention relates to administering glial growth factor 2 (GGF2) to a patient in need thereof, to achieve serum levels of GGF2 within a desired therapeutic window determined based on the disease or disorder afflicting the patient.

15

BACKGROUND

The reference to any prior art in this specification is not, and should not be taken as an acknowledgement or any form of suggestion that such art forms part of the common general knowledge in Australia.

20

Neuregulins (NRGs) and NRG receptors comprise a growth factor-receptor tyrosine kinase system for cell-cell signaling that is involved in organogenesis in nerve, muscle, epithelia, and other tissues (Lemke, Mol. Cell. Neurosci. 7:247-262, 1996; Burden et al., Neuron 18:847-855, 1997). The NRG family consists of three genes that encode numerous ligands

25   containing epidermal growth factor (EGF)-like, immunoglobulin (Ig), and other recognizable domains. Numerous secreted and membrane-attached isoforms function as ligands in this signaling system. The receptors for NRGs are all members of the EGF receptor (EGFR) family, and include EGFR (or ErbB1), ErbB2, ErbB3, and ErbB4, also known as HER1 through HER4, respectively, in humans (Meyer et al., Development 124:3575-3586, 1997;

30   Orr-Urtreger et al., Proc. Natl. Acad. Sci. USA 90: 1867-71, 1993; Marchionni et al., Nature 362:312-8, 1993; Chen et al., J. Comp. Neurol. 349:389-400, 1994; Corfas et al., Neuron 14:103-115, 1995; Meyer et al., Proc. Natl. Acad. Sci. USA 91:1064-1068, 1994; and Pinkas-

Kramarski et al., Oncogene 15:2803-2815, 1997).

The three NRG genes, Nrg-1, Nrg-2, and Nrg-3, map to distinct chromosomal loci (Pinkas-Kramarski et al., Proc. Natl. Acad. Sci. USA 91:9387-91, 1994; Carraway et al., Nature
5   387:512-516, 1997; Chang et al., Nature 387:509-511, 1997; and Zhang et al., Proc. Natl. Acad. Sci. USA 94:9562-9567, 1997), and collectively encode a diverse array of NRG proteins. The most thoroughly studied to date are the gene products of Nrg-1, which comprise a group of approximately 15 distinct structurally-related isoforms (Lemke, Mol. Cell. Neurosci. 7:247-262, 1996 and Peles and Yarden, BioEssays 15:815-824, 1993). The
10   first-identified isoforms of NRG-1 included Neu Differentiation Factor (NDF; Peles et al., Cell 69, 205-216, 1992 and Wen et al., Cell 69, 559-572, 1992), Heregulin (HRG; Holmes et al., Science 256:1205-1210, 1992), Acetylcholine Receptor Inducing Activity (ARIA; Falls et al., Cell 72:801-815, 1993), and the glial growth factors GGF1, GGF2, and GGF3 (Marchionni et al. Nature 362:312-8, 1993).
15

The Nrg-2 gene was identified by homology cloning (Chang et al., Nature 387:509-512, 1997; Carraway et al., Nature 387:512-516, 1997; and Higashiyama et al., J. Biochem. 122:675-680, 1997) and through genomic approaches (Busfield et al., Mol. Cell. Biol. 17:4007-4014, 1997). NRG-2 cDNAs are also known as Neural- and Thymus-Derived
20   Activator of ErbB Kinases (NTAK; Genbank Accession No. AB005060), Divergent of Neuregulin (Don-1), and Cerebellum-Derived Growth Factor (CDGF; PCT application WO 97/09425). Experimental evidence shows that cells expressing ErbB4 or the ErbB2/ErbB4 combination are likely to show a particularly robust response to NRG-2 (Pinkas-Kramarski et al., Mol. Cell. Biol. 18:6090-6101, 1998). The Nrg-3 gene product (Zhang et al., *supra*) is
25   also known to bind and activate ErbB4 receptors (Hijazi et al., Int. J. Oncol. 13:1061-1067, 1998).

An EGF-like domain is present at the core of all forms of NRGs, and is required for binding and activating ErbB receptors. Deduced amino acid sequences of the EGF-like domains
30   encoded in the three genes are approximately 30-40% identical (pairwise comparisons). Moreover, there appear to be at least two sub-forms of EGF-like domains in NRG-1 and NRG-2, which may confer different bioactivities and tissue-specific potencies.

Cellular responses to NRGs are mediated through the NRG receptor tyrosine kinases EGFR, ErbB2, ErbB3, and ErbB4 of the epidermal growth factor receptor family (Busfield et al., 1997, Mol Cell Biol. 17:4007-14; Carraway et al., 1997, Nature 387:512-6; Chang et al., 1997, Nature 387:509-12). High-affinity binding of all NRGs is mediated principally via

5   either ErbB3 or ErbB4 (Ferguson et al., 2000, EMBO J. 19:4632-43). Binding of NRG ligands leads to dimerization with other ErbB subunits and transactivation by phosphorylation on specific tyrosine residues (Honegger et al., 1990, Mol Cell Biol. 10:4035-44; Lemmon and Schlessinger, 1994, Trends Biochem Sci. 19:459-63; Heldin, 1995, Cell. 80:213-23; Hubbard et al., 1998, J Biol Chem. 273:11987-90). In certain experimental

10   settings, nearly all combinations of ErbB receptors appear to be capable of forming dimers in response to the binding of NRG-1 isoforms. ErbB2, however, appears to be a preferred dimerization partner that may play an important role in stabilizing the ligand-receptor complex.

15   GGF2 has been shown to promote proliferation, differentiation and protection of Schwann cells (Goodearl et al., 1993, J Biol Chem. 268:18095-102; Minghetti et al., 1996 J Neurosci Res. 43:684-93). Expression of NRG-1, ErbB2, and ErbB4 is also necessary for trabeculation of the ventricular myocardium during mouse development (Meyer and Birchmeier 1995, Nature 378:386-90; Gassmann et al., 1995, Nature 378:390-4; Kramer et

20   al., 1996, Proc Natl Acad Sci USA 93:4833-8). GGF2 has also been shown to promote proliferation and protection of cardiomyocyte cells (Zhao et al., 1998, J Biol Chem 273:10261-10269). GGF2-mediated neuroprotection has also been demonstrated in animal models of stroke, although parameters relating to dosing remain undefined.

25   The present invention advances the use of GGF2 with respect to therapeutic applications by presenting guidance as to methods for GGF2 administration that optimize therapeutic benefit, while limiting adverse effects. The present invention defines target therapeutic windows for GGF2 serum concentration levels that are specified with respect to particular disease conditions.

30

SUMMARY

The present invention relates to administering GGF2 to a patient in need thereof to achieve a

serum plasma level of GGF2 within a target therapeutic window determined to be effective in
the treatment of a disease or disorder.   In accordance with the present invention, GGF2 may
be administered in a pharmaceutical composition.

5   In accordance with the present invention, a method for avoiding inhibition of Schwann cell
        myelination following administration of glial growth factor 2 (GGF2) in a subject is
        presented, said method comprising: providing a subject in need of neuron myelination;
        providing GGF2 in a pharmaceutically acceptable carrier;   administering the GGF2 to the
        subject; and, determining that the amount of GGF2 is less than the amount that inhibits
10   Schwann cell myelination.

        In another embodiment, the present invention relates to a method for promoting myelination
        in a patient afflicted with a disease or disorder associated with reduced levels of myelination,
        the method comprising: selecting the patient afflicted with a disease or disorder associated
15   with reduced levels of myelination; administering glial growth factor 2 (GGF2) to the patient
        in an amount of about 500 ng of GGF2 per kg of body weight; whereby myelination is
        promoted.

        In yet another embodiment, the present invention relates to a method for promoting
20   myelination in a patient afflicted with a disease or disorder associated with reduced levels of
        myelination, the method comprising: selecting a patient afflicted with a disease or disorder
        associated with reduced levels of myelination; and, administering glial growth factor 2
        (GGF2) to the patient at an amount that achieves a plasma level of about 0.01 nM GGF2.

25   In a further embodiment, the present invention relates to a method for broadening the
        therapeutic dose range for GGF2 when GGF2 is used to facilitate myelination, the method
        comprising:
        selecting a subject with a disease or disorder associated with reduced levels of myelination;
        administering GGF2 and a Mek1/Erk pathway inhibitor to the patient, and, whereby GGF2-
30   mediated myelination is occurs at higher doses of GGF2 than would occur in the absence of
        administering the Mek1/Erk pathway inhibitor.

In another embodiment, the present invention relates to a method for determining if an amount of GGF2 is a therapeutically effective amount for promoting myelination, the method comprising:

providing a subject receiving GGF2 therapy; and measuring c-Jun protein levels in the

5   subject, whereby an increase in c-Jun relative to baseline c-Jun levels indicates that the amount of GGF2 is near a maximum threshold of therapeutic efficacy for promoting myelination.

In a particular embodiment of the invention, GGF2 is administered to a mammal using a

10   dosing regimen directed to achieving a narrow target therapeutic window of plasma GGF2 concentrations.

As indicated herein, GGF2 is known to be able to promote proliferation, differentiation and protection of Schwann cells. GGF2 has also been shown to promote remyelination and

15   reduce symptoms in animal models of multiple sclerosis including experimental autoimmune encephalomyelitis. Under some circumstances (e.g., at high concentrations of GGF2), however, GGF2 can prevent myelination of neurons co-cultured with Schwann cells.

The data presented herein demonstrate that GGF2 is indeed capable of promoting myelination

20   of peripheral nerves but teach that precise dosing of GGF2 to a mammal in need thereof is required to achieve the desired GGF2-mediated promoted myelination of peripheral nerves. As taught herein, GGF2 is administered so as to be within a therapeutic window of plasma GGF2 concentrations in order to promote myelination. In the absence of the results presented herein, there is no appreciation of the narrow therapeutic window of plasma GGF2

25   concentrations required to promote myelination in a mammal in need thereof.

The data presented herein also demonstrate that GGF2 is sufficient to promote myelination and rescue the myelination defect on CRD-Nrg1-deficient axons. At high concentrations, however, GGF2 inhibits myelination in an Erk-dependent manner. The present results

30   demonstrate that GGF2 is capable of both promoting and inhibiting myelination depending on the concentration presented to the Schwann cells.

Accordingly, the present invention relates to the surprising discovery that a hitherto

unrealized positive correlation exists between GGF2-mediated PI3-kinase pathway activation

and promotion of myelination and a negative correlation exists between GGF2-mediated

Mek1/Erk pathway activation and promotion of myelination. Alternatively stated, the present

5   inventors discovered that administration of GGF2 can be finely tuned to promote myelination .

by assessing activation levels of these pathways. In accordance with the present invention, a

target therapeutic window for GGF2 with regard to promoting myelination in a subject is an

amount of GGF2 that promotes PI3-kinase pathway activation (assayed, for example, by

detecting phosphorylated Akt) in the absence of detectable Mek1/Erk pathway activation

10   (assayed, for example, by detecting phosphorylated Erk).

The formulations and compositions of the present invention exhibit a specific, desired release

profile that maximizes the therapeutic effect while minimizing adverse side effects. The

desired release profile may be described in terms of the maximum plasma concentration of

15   the drug or active agent (C_{max}) and the plasma concentration of the drug or active agent at a

specific dosing interval (C_{tau}). A ratio of C_{max} to C_{tau}. (C_{max}:C_{tau}) may be calculated from the

observed C_{max} and C_{tau}. A dosing interval ($_{tau}$) is the time since the last administration of the

drug or active agent. In the present application, the dosing interval ($_{tau}$) may be, for example,

twelve (12) hours, in which case C_{tau} is the concentration of the drug or active agent at twelve

20   (12) hours from the last administration.

Additionally, the formulations and compositions of the present invention exhibit a desired

release profile that may be described in terms of the maximum plasma concentration of the

drug or active agent at steady state (C_{maxSS}) and the minimum plasma concentration of the

25   drug or active agent at steady state (C_{minSS}). Steady state is observed when the rate of

administration (absorption) is equal to the rate of elimination of the drug or active agent. A

ratio of C_{maxSS} to C_{minSS} (C_{maxSS}: C_{minSS}) may be calculated from the observed C_{maxSS} and

C_{minSS}. In addition, the formulations and compositions of the present invention exhibit a

desired release profile that may be described in terms of the average maximum plasma

30   concentration of the drug or active agent at steady state (C_{avSS}).

In an embodiment of the invention directed to a patient in need of remyelination, target peak

serum levels of GGF2 are about 0.01 nM.

In an embodiment of the invention directed to a patient in need of remyelination, target peak serum levels of GGF2 are at or about any of the following values, or range between the following values from about 0.001 to 0.01 ng/ ml; 0.01 to 0.1 ng/ ml; 0.1 to 1.0 ng/ ml; 1.0 to 5   10 ng/ ml; 10 to 100 ng/ ml; or 100 to 1000 ng/ ml. In a particular embodiment, the target peak serum level is about 1.0 ng/ ml.

In an embodiment of the invention directed to a patient who has had a stroke, target peak serum levels of GGF2 are at or about any of the following values, or range between the 10   following values from about 0.00001 to 0.0001 ng/ ml; 0.0001 to 0.001 ng/ ml; 0.001 to 0.01 ng/ ml; 0.001 to 0.01 ng/ ml; 0.01 to 0.1 ng/ ml; 0.1 to 1.0 ng/ ml; 1.0 to 10 ng/ ml; 10 to 100 ng/ ml; 100 to 1000 ng/ ml; 1000 to 10000 ng/ ml; or 10000 to 100000 ng/ ml. In a particular embodiment, the target peak serum level is about 0.2 micrograms/ml.

15   In an embodiment of the invention directed to a patient who has neuropathy, target peak serum levels of GGF2 are at or about any of the following values, or range between the following values from about 0.001 to 0.01 ng/ ml; 0.01 to 0.1 ng/ ml; 0.1 to 1.0 ng/ ml; 1.0 to 10 ng/ ml; 10 to 100 ng/ ml; or 100 to 1000 ng/ ml. In a particular embodiment, the target peak serum level is about 6.25 ng/ml.
20

In an embodiment of the invention directed to a patient who has heart failure, target peak serum levels of GGF2 are at or about any of the following values, or range between the following values from about 0.001 to 0.01 ng/ ml; 0.01 to 0.1 ng/ ml; 0.1 to 1.0 ng/ ml; 1.0 to 10 ng/ ml; 10 to 100 ng/ ml; or 100 to 1000 ng/ ml. In a particular embodiment, the target 25   peak serum level is about 6.8 micrograms/ml.

In accordance with the present invention, pharmaceutical compositions comprising GGF2 may be administered via different routes known to those skilled in the art. Any appropriate route of administration may be employed, for example, intravenous, parenteral, 30   subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol, oral, or topical (e.g., by applying an adhesive patch carrying a formulation capable of crossing the dermis and entering the bloodstream) administration. Oral administration is envisioned to include

sustained release oral dosage forms comprising GGF2. A GGF2 pharmaceutical composition, as described herein, can be used to treat individuals affected with neurological disorders wherein said pharmaceutical composition maximizes the therapeutic effect, while minimizing adverse side effects.

5

In a first embodiment of the present invention, GGF2 is administered to a mammal afflicted with a neurological disorder associated with demyelination, wherein the GGF2 is administered in a dosing regimen to achieve and maintain a narrow target therapeutic window of plasma GGF2 concentrations. As taught herein, precise dosing of GGF2 is necessary in

10   order to achieve serum plasma levels of GGF2 required for therapeutic efficacy with respect to inducing myelination in a subject in need thereof. Examples of demyelinating disorders for which suitable dosing of GGF2 is necessary in order to achieve therapeutic efficacy include Guillain-Barre Syndrome, chronic inflammatory demyelinating polyneuropathy, peripheral demyelination due to traumatic injury, multiple sclerosis, optic neuritis, central

15   demyelination due to traumatic injury, transverse myelitis, progressive multifocal leukoencephalopathy, Devic's disease (neuromyelitis optica), acute disseminated encephalomyelitis, adrenoleukodystrophy and adrenoleukoneuropathy.

In a second embodiment of the present invention, GGF2 is administered to a mammal

20   afflicted with a cardiac muscle disorder, such as congestive heart failure, myocardial infarction, reperfusion injury, chemical, viral or idiopathic cardiotoxicity, arrhythmias, wherein the GGF2 is administered in a dosing regimen to achieve a target therapeutic window of plasma GGF2 concentrations.

25   In a third embodiment of the present invention, GGF2 is administered to a mammal that has suffered a stroke, spinal cord injury or traumatic brain injury, wherein the GGF2 is administered in a dosing regimen to achieve a target therapeutic window of plasma GGF2 concentrations.

30   It will be appreciated that for any of the applications detailed herein, GGF2 may be administered in any suitable form, or as a component in a pharmaceutical composition and via any means, all of which are described herein and/or understood in the art.

8

Accordingly, the present invention is directed to identification of a target therapeutic window with respect to a therapeutically effective plasma level of GGF2. The target therapeutic window varies depending of the disease or disorder afflicting the patient and the desired activity conferred by achieving the appropriate therapeutically effective GGF2 plasma level.

5

A method for selecting individuals based on presentation of symptoms is also encompassed herein. Also encompassed is a method for selecting individuals based on responsiveness to achieving the therapeutically effective GGF2 plasma level, as indicated for each application, is also encompassed herein.

10

In addition to the methods of treatment set forth above, the present invention extends to the use of any of the compounds of the invention for the preparation of medicaments or as medicaments that may be administered for such treatments, as well as to such compounds for the treatments disclosed and specified.

15

The present invention also encompasses a pharmaceutical composition comprising GGF2 or an EGFL domain and a Mek1/Erk pathway inhibitor and its use in the treatment of a patient afflicted with a disease or disorder associated with reduced levels of myelination.

20

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A-C show (A) GGF2-induced Akt and MAPK activation in Schwann cell-DRG neuron co-cultures. Schwann cell-DRG co-cultures under myelinating condition were treated GGF (0.6

25 μM) and 20 minutes later, Akt and MAPK activation levels were assessed by Western blot analysis. (B) Inhibition of GGF2-induced MAPK activation by U0125. Co-cultures were pretreated with increasing doses of U0125 for 30 minutes then stimulated with GGF2. Control cultures were left untreated. MAPK activation was assessed 20 minutes later. (C) Inhibition of GGF2-induced MAPK activation by U0125 (1 and 3 μM) reverses the inhibitory effect of GGF2

30 on myelination. Co-cultures were co-treated with GGF2 and U0125 (1 and 3 μM) under myelinating conditions. Ten to twelve days later, cultures were fixed and immunostained for MBP to assess the level of myelination.

Figure 2 shows GGF2 promotes myelination at low concentrations. Co-cultures were treated with GGF2 at concentrations ranging from 0.5 to 1000 pM (0.0005 to 1 nM) under myelinating conditions. Ten to twelve days later, myelination was assessed by MBP immunostaining. More particularly, the GGF2 concentrations from left to right are as follows: NT, 0.5 pM, 1pM, 3 pM,

5   10 pM, 30 pM, 300 pM, 600 pM, and 1,000 pM, respectively. Ten to twelve days later, myelination was assessed by MBP immunostaining.

Figure 3A-F show that an inhibitory effect of GGF on myelination is mediated by the

10   Mek1/Erk activation. (A) Schwann cell DRG co-cultures were treated with GGF (0.01, 0.6, and 1 nM) and 45 minutes later the cell lysates were prepared and levels of active Erk (p-Erk) and Akt (p-Akt) were determined by Western blot analysis. At 1 nM (boxed), GGF induced activation of both Erk and Akt. (B) Inhibition of GGF-induced Erk activation in co-cultures. Schwann cell-DRG co-cultures were pre-treated with U0126 for 30 minutes then GGF (0.6

15   nM) was added in the continuous presence of U0126. After 45 minutes the cell lysates were prepared and level of p-Erk and p-Akt were determined. Treatment with U0126 inhibited both endogenous and GGF-induced Erk activation without affecting Akt activation. (C) Images of MBP$^+$ myelin segments formed in co-cultures treated with GGF or GGF+U0126 (1 nM). Treatment with U0126 abolished the inhibitory effect of GGF and induced myelination.

20   Control cultures were maintained without any treatment (NT). Scale bar: 100 μm. Quantification of the result is shown in (D). (E) Inhibition of endogenous Erk activity in co-cultures promotes myelination. Co-cultures were treated with increasing concentration of U0126 (0.5, 1 and 3 nM) under myelinating condition and 11 days later, myelination was analyzed as above. A significant increase in myelination was observed in cultures treated

25   with U0126. Error bars indicate ± SE (p < 0.001). (F) Inhibition of GGF-induced Erk activation is accompanied by a decrease in c-Jun and an increase in Krox20 expression. Co-cultures were maintained under myelinating condition in the presence of GGF or GGF+U0126 (0.5, 1 and 3 nM) for 11 days and the cell lysates were analyzed for MBP, c-Jun and Krox 20 expression. Actin level served as a loading control. GGF-induced c-Jun

30   expression was down-regulating with the treatment with U0126. Level of Krox 20 protein appeared increased in cultures treated with U0126.

Figure 4A-C shows that GGF promotes myelination at low concentration. (A) Schwann cells were treated with varying concentrations of GGF ranging from 0.0003 to 10 nM) and 20 minutes later the cell lysates were prepared and the levels of Erk and Akt activation were analyzed by Western blot (top) and densitometric analysis (bottom). An increase in Akt activation appeared at lower concentration range (boxed) compared to Erk activation. (B) Co-cultures were treated with different concentrations of GGF (0.0005, 0.001, 0.003, 0.01, 0.03, 0.3, 0.6, and 1nM) for 11 days under myelinating condition then fixed and immunostained for MBP and DAPI. Images of the control and cultures treated with 0.01nM of GGF are shown along with the quantification of the result (right). A clear biphasic effect of GGF is shown that promotes myelination at low concentrations (0.0005 to 0.01 nM) while inhibiting the process at higher (0.3 nM and above) concentration. (C) Low concentration of GGF (0.01 nM) significantly increased myelination on CRD-Nrg1$^{+/-}$ neurons (p=0.003). Error bars show $\pm$ SEM. Data were analyzed by one-way ANOVA (*: $p < 0.001$)

Figure 5A-D shows the nucleic and amino acid sequences of full length GGF2.

Figures 6-11 show the nucleic and amino acid sequences of epidermal growth factor like (EGFL) domains 1-6.

Figure 12 shows a table relating to neuregulin nomenclature.

DETAILED DESCRIPTION OF THE INVENTION

The data presented herein demonstrated that in order to promote myelination of peripheral nerves, GGF2 must be administered to a mammal using a dosing regimen directed to achieving a therapeutic window of, e.g., plasma GGF2 concentrations or GGF2 doses.

Definitions

The terms used herein have meanings recognized and known to those of skill in the art, however, for convenience and completeness, particular terms and their meanings are set forth below

Throughout this specification and the claims, unless the context requires otherwise, the word "comprise" and its variations, such as "comprises" and "comprising," will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

As used herein "about" means a stated value plus or minus another amount; thereby

establishing a range of values. In certain preferred embodiments "about" indicates a range relative to a base (or core or reference) value or amount plus or minus up to 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, .75%, .5%, .25% or .1%.

5   By "epidermal growth factor-like domain" or "EGF-like domain" is meant a polypeptide motif encoded by the NRG-1, NRG-2, or NRG-3 gene that binds to and activates ErbB2, ErbB3, ErbB4, or combinations thereof, and bears a structural similarity to the EGF receptor-binding domain as disclosed in Holmes et al., Science 256:1205-1210, 1992; U.S. Pat. No. 5,530,109; U.S. Pat. No. 5,716,930; U.S. Ser. No. 08/461,097; Hijazi et al., Int. J. Oncol.

10   13:1061-1067, 1998; Chang et al., Nature 387:509-512, 1997; Carraway et al., Nature 387:512-516, 1997; Higashiyama et al., J Biochem. 122:675-680, 1997; and WO 97/09425). See Figures 10-15 for nucleic and amino acid sequences of epidermal growth factor like (EGFL) domains 1-6.

15   By "neuregulin" or "NRG" is meant a polypeptide that is encoded by an NRG-1, NRG-2, or NRG-3 gene or nucleic acid (e.g., a cDNA), and binds to and activates ErbB2, ErbB3, or ErbB4 receptors, or combinations thereof.

By "neuregulin-1," "NRG-1," "heregulin," "GGF2," or "p185erbB2 ligand" is meant a

20   polypeptide that binds directly to or transactivates the ErbB2 receptor and is encoded by the p185erbB2 ligand gene described in U.S. Pat. No. 5,530,109; U.S. Pat. No. 5,716,930; and U.S. Pat. No. 7,037,888, the contents of each of which are incorporated herein by reference. See Figures 9A-D for the nucleic and amino acid sequences of full length GGF2. See Figure 12 for a table pertaining to neuregulin nomenclature.

25

Polypeptides encoded by the NRG-1, NRG-2, and NRG-3 genes possess EGF-like domains that allow them to bind to and activate ErbB receptors. Holmes et al. (Science 256:1205-1210, 1992) have shown that the EGF-like domain alone is sufficient to bind and activate the p185erbB2 receptor. Accordingly, any polypeptide product encoded by the NRG-1, NRG-2,

30   or NRG-3 gene, e.g., a polypeptide having an EGF-like domain encoded by a neuregulin gene or cDNA (e.g., an EGF-like domain, as described in U.S. Pat. No. 5,530,109; U.S. Pat. No. 5,716,930;
U.S. Pat. No. 7,037,888, U.S. Pat. No. 7,135,456, and U.S. Pat. No. 7,319,019 ; or an EGF-

like domain as disclosed in WO 97/09425) may be used in the methods of the invention to achieve a therapeutic window wherein an efficacious serum plasma level of GGF2 is achieved.

5   It must also be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art. Although any methods

10   and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the present invention, particular methods, devices, and materials are now described.

"Local administration" means direct administration by a non-systemic route at or near the site

15   of affliction or disorder.

The terms "patient" and "subject" are used herein to refer to all animals, including mammals. Examples of patients or subjects include humans, cows, dogs, cats, goats, sheep, and pigs.

20   The term "pharmaceutically acceptable salts, esters, amides, and prodrugs" as used herein refers to those carboxylate salts, amino acid addition salts, esters, amides, and prodrugs of the compounds of the present invention which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of patients without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio, and

25   effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds of the invention.

The term "prodrug" refers to compounds that are rapidly transformed in vivo to yield the parent compounds of the above formula, for example, by hydrolysis in blood. A thorough

30   discussion is provided in T. Higuchi and V. Stella, "Pro-drugs as Novel Delivery Systems," Vol. 14 of the A.C.S. Symposium Series, and in Bioreversible Carriers in Drug Design, ed. Edward B. Roche, American Pharmaceutical Association and Pergamon Press, 1987, both of which are incorporated herein by reference.

The term "salts" refers to the relatively non-toxic, inorganic and organic acid addition salts of compounds of the present invention. These salts can be prepared in situ during the final isolation and purification of the compounds or by separately reacting the purified compound

5   in its free base form with a suitable organic or inorganic acid and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, nitrate, acetate, oxalate, valerate, oleate, palmitate, stearate, laurate, borate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate mesylate, glucoheptonate, lactobionate and laurylsulphonate salts, and the like. These may include

10   cations based on the alkali and alkaline earth metals, such as sodium, lithium, potassium, calcium, magnesium, and the like, as well as non-toxic ammonium, tetramethylammonium, tetramethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like. (See, for example, S. M. Barge et al., "Pharmaceutical Salts," J. Pharm. Sci., 1977, 66:1-19 which is incorporated herein by reference.).

15

A "therapeutically effective amount" is an amount sufficient to decrease the symptoms associated with a medical condition or infirmity, to normalize body functions in disease or disorders that result in impairment of specific bodily functions, or to provide improvement in one or more of the clinically measured parameters of a disease. Preferably, improvement in

20   symptoms associated with the disease associated with a demyelinating disease, for example, including walking speed, lower extremity muscle tone, lower extremity muscle strength, or spasticity. As related to the present application, a therapeutically effective amount is an amount sufficient to reduce the pain or spasticity associated with the neurological disorder being treated, or an amount sufficient to result in improvement of sexual, bladder or bowel

25   function in subjects having a neurological disorder which impairs nerve conduction; or which hinders normal sexual, bladder or bowel functions.

"Treatment" refers to the administration of medicine or the performance of medical procedures with respect to a patient, to ameliorate the clinical condition of the patient,

30   including a decreased duration of illness or severity of illness, or subjective improvement in the quality of life of the patient or a prolonged survival of the patient.

As used herein, the term "target therapeutic window" refers to the dose range or serum concentration range that achieves the desired therapeutic results. With regard to GGF2, in a particular embodiment, the target therapeutic window refers to an amount of GGF2 sufficient to induce Schwann cell myelination in a subject, which amount is less than the amount

5   sufficient to inhibit myelination in a subject. In a surprising discovery, the present inventors identified the target therapeutic window for GGF2 with respect to its ability to promote myelination by determining the relative levels of PI3-kinase pathway activation and Mek1/Erk pathway activation. More particularly, the present inventors discovered the hitherto unrealized positive correlation between GGF2-mediated PI3-kinase pathway

10   activation and promotion of myelination and a negative correlation between GGF2-mediated Mek1/Erk pathway activation and promotion of myelination. Alternatively stated, the present inventors discovered that administration of GGF2 can be finely tuned to promote myelination by assessing activation levels of these pathways. A target therapeutic window for GGF2 with regard to promoting myelination in a subject is defined as an amount of GGF2 that promotes

15   PI3-kinase pathway activation (assayed, for example, by detecting phosphorylated Akt) in the absence of detectable Mek1/Erk pathway activation (assayed, for example, by detecting phosphorylated Erk). Detection of phosphorylated Akt and phosphorylated Erk can be achieved using standard assays known in the art, including ELISA, Western (immuno) blot, immunocytochemistry, in vitro kinase assay, LC/MS (liquid chromatography/mass

20   spectrometry), MaldiTOF MS (Matrix Assisted Laser Desorption /Ionization- Time of Flight mass spectrometry) or other protein systems known to the field such as Luminex

One skilled in the art would appreciate that other intracellular markers of PI3-kinase pathway activation and Mek1/Erk pathway activation are known and are used in accordance with the

25   present invention. In accordance with the present invention, other indicators of PI3-kinase pathway activation and Mek1/Erk pathway activation can be used to determine the therapeutic window in which GGF2 promotes myelination.

In addition, the compounds of the present invention can exist in unsolvated as well as

30   solvated forms with pharmaceutically acceptable solvents such as water, ethanol, and the like. In general, the solvated forms are considered equivalent to the unsolvated forms for the purposes of the present invention.

"MAP Kinase Inhibitors"

A non-limiting list of MAP kinase inhibitors that may be used in the present invention includes: Arctigenin, which potently inhibits the activity of MKK1 in vitro with an IC50 value of 1 nM and thus inhibits the phosphorylation and activation of MAP kinases ERK1/2,

5   p38 kinase and JNK and their activities in Raw264.7 cells treated with LPS; PD 98059, which is a potent, selective and cell-permeable inhibitor of MAP kinase-kinase (also known as MAPK/ERK kinase or MEK) that inhibits phosphorylation of MAP kinase by MAP kinase-kinase but does not inhibit MAP kinase itself. The IC50 values for PD 98059-induced effects are in the 1-20 μM range for many assays; SB202190, which is a highly selective,

10   potent and cell permeable inhibitor of p38 MAP kinases that binds within the ATP pocket of the active kinase with a Kd of 38 nM as measured in recombinant human p38 and selectively inhibits the p38alpha and beta isoforms (IC50 values are 50 and 100 nM for p38alpha/SAPK2alpha and p38beta2/SAPK2beta respectively); SB203580,which is a highly selective and cell permeable inhibitor of p38 mitogen-activated protein kinase with IC50

15   values of 50 and 500 nM for p38/SAPK2a and p38/SAPK2b respectively and also inhibits the phosphoinositide-dependent protein kinase 1 (PDK1) at 10-fold higher concentrations (IC50 ~3-5 μM) (Displays 100-500-fold selectivity over Lck, GSK3b and Akt/PKB); SL 327, which is a selective inhibitor of MEK1 and MEK2 with IC50 values of 180 and 220 nM, respectively. It blocks hippocampal LTP in vitro and is brain penetrant in vivo, blocking fear

20   conditioning and learning in rats, and producing neuroprotection in mice, following systemic administration; SP600125, which is a selective inhibitor of c-Jun N-terminal kinase (JNK). It competitively and reversibly inhibits JNK1, 2 and 3 (IC50 = 40-90 nM) and has been shown to have less inhibitory potency on ERK2, p38b and a range of other kinases and is known to be active in vivo; and U0126, which is a selective inhibitor of the mitogen-activated protein

25   kinase kinases, MEK-1 and MEK-2, with a 100-fold higher potency than PD 98059 and is a weak inhibitor of PKC, Raf, ERK, JNK, MEKK, MKK-3, MKK-4/SEK, MKK-6, Abl, Cdk2 and Cdk4 and inhibits AP-1 transactivation in cell-based reporter assays.

Other inhibitors that are currently in FDA Phase trial include the farnesyl transferase

30   inhibitors (FTIs). Zarnestra ® (R115777, tipifarnib), for example, is the FTI that is furthest along in development. A phase II trial of patients with previously treated metastatic breast cancer tested two different dosing schedules: continuous and intermittent. The objective response rates in the 2 groups were 10% and 14%, with an additional 15% and 9% who had

stable disease for at least 6 months. The major side effects observed were bone marrow suppression and neuropathy, both of which were less in the intermittent dosing group than the continuous. Several phase I studies of zarnestra and other FTIs have been performed in combination with cytotoxic chemotherapy and have demonstrated the safety of these

5   combination regimens. Phase II trials in breast cancer are underway, including one using zarnestra in combination with an aromatase inhibitor. FDA approval for zarnestra use in acute myeloid leukemia (AML) is pending phase III data, as the FDA committee voted against accelerated approval for zarnestra based on data from a single-armed phase II trial.

10   With regard to Zarnestra®, for Phase I clinical trials, Zarnestra® is administered at 400 mg administered orally twice daily for two weeks; for Phase II clinical trials, Zarnestra® is administered at 300 mg administered orally twice daily for the first 21 days of each 28-day cycle; for Phase III clinical trials, Zarnestra® is administered at 600 mg administered orally twice daily for the first 21 days of each 28-day cycle.

15

The Raf inhibitors comprise another types of inhibitors that are currently in FDA Phase trials. Sorafenib (BAY 43-9006), for example, is the first compound to target not only the Raf/MEK/Erk signaling pathway, but also the VEGFR and PDGFR pathways. In March 2004, sorafenib was granted Fast Track status by the FDA for metastatic renal cell cancer. In

20   April 2005, sorafenib was accepted into the Pilot 1 Program, which is designed for therapies that have been granted FDA Fast Track status and that have the potential to provide significant benefit over existing standard therapy. There are also several large, international, multi-institution phase III clinical studies of sorafenib underway in patients with advanced stage primary cancers of the kidney and liver, as well as metastatic melanoma.

25

With regard to Sorafenib, Phase I clinical trials tested two dose levels: Dose Level 1: 200 mg of Sorafenib by mouth twice a day for a 3 week cycle or Dose Level 2: 400 mg of Sorafenib by mouth twice a day for a 3 week cycle.

30   The results of a planned interim analysis of an ongoing phase III trial in patients with advanced kidney cancer were recently presented (Escudier et al. J Natl Cancer Inst. 2008 100:1454-63; the contents of which are incorporated herein in their entirety). Among 769 analyzed patients, progression-free survival (PFS) was doubled to a median value of 24

weeks with sorafenib, compared to 12 weeks with placebo. The benefits from sorafenib were observed in all patient subgroups, regardless of age, duration of disease, or prior therapies. Disease control was achieved in 80% of patients who received sorafenib: 78% had stable disease (compared to 55% in the placebo arm) and 2% had partial response (compared to

5   none in the placebo arm). The 12-week progression-free rate was 79% for sorafenib vs. 50% for placebo. Furthermore, sorafenib was very well tolerated in 768 patients, and the most common side effects were hypertension, fatigue, diarrhea, and rash, including a rash on the hand and foot (hand and foot syndrome).

Phase II efficacy trials are studying sorafenib as a single agent in advanced lung, breast, and

10   other cancers. Phase I/II clinical trials are investigating sorafenib in combination with a range of standard chemotherapeutics and other anticancer agents.

ISIS 5132 is another raf inhibitor that has shown acceptable toxicity in phase I studies. Phase II studies are now underway in a variety of cancer types.

15

Other inhibitors that are currently in FDA Phase trial include the MEK inhibitors. CI-1040, for example, is an oral, selective small-molecule inhibitor of MEK 1-2. Animal and culture studies have shown activity of this agent in breast cancer cell lines. Phase I studies have found mild gastrointestinal and skin side effects. Unfortunately, a phase II study in 67

20   patients with 4 different tumor types (advanced colorectal, NSCLC, breast, and pancreatic cancer) found no responses, although CI-1040 treatment was well tolerated.

PD 0325901, a second generation MEK inhibitor, has recently entered clinical development and appears to have noticeably better pharmacologic properties compared to CI-1040, which

25   investigators hope may translate into better anti-cancer efficacy. It has shown some partial response in melanoma patients.

With regard to PD 0325901, Phase I and Phase II clinical trials tested multiple dose levels. Administered orally either once or twice a day; several dosing schedules evaluated; current

30   dosing schedule 5 days on-drug, 2-days off drug for 3 weeks in a 28-day cycle. Doses evaluated ranged from 1 mg once a day to 30 mg twice daily. Clinical trials were prematurely discontinued due to safety concerns, specifically ocular and neurological toxicity presented at 10 mg twice-a-day and higher doses.

It is to be understood that this invention is not limited to the particular molecules, compositions, methodologies or protocols described, as these may vary. It is also to be understood that the terminology used in the description is for the purpose of describing

5   particular embodiments only, and is not intended to limit the scope of the present invention, which is limited only by the appended claims.

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Administration: Neuregulins and polypeptides containing EGF-like domains encoded by neuregulin genes may be administered to patients or experimental animals with a

10 pharmaceutically-acceptable diluent, carrier, or excipient, in unit dosage form. Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer such compositions to patients or experimental animals. Any appropriate route of administration may be employed, for example, intravenous, parenteral, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular,

15 intraspinal, intracisternal, intraperitoneal, intranasal, aerosol, oral, or topical (e.g., by applying an adhesive patch carrying a formulation capable of crossing the dermis and entering the bloodstream) administration. Therapeutic formulations may be in the form of liquid solutions or suspensions; for oral administration, formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or

20 aerosols. Any of the above formulations may be in a sustained-release formulation.

Methods well known in the art for making formulations are found in, for example, "Remington's Pharmaceutical Sciences.", which is incorporated herein in its entirety. Formulations for parenteral administration may, for example, contain excipients, sterile

25 water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated napthalenes. Sustained-release, biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other useful parenteral delivery systems for administering molecules of the invention include ethylene-vinyl acetate copolymer particles,

30 osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel.

Thus, and as stated earlier, the present invention includes within its scope, and extends to, the recited methods of treatment and to the use of such compounds for the preparation of medicaments useful for such methods.

5

Demyelinating Diseases: Myelin sheaths cover many nerve fibers in the central and peripheral nervous system. The presence of intact myelin sheaths accelerates axonal transmission of neural impulses. Disorders that affect myelin interrupt nerve transmission and disease symptoms may reflect deficits in any part of the nervous system.

10

Myelin formed by oligodendroglia in the central nervous system (CNS) differs chemically and immunologically from that formed by Schwann cells peripherally. Thus, some myelin disorders (e.g., Guillain-Barré syndrome, chronic inflammatory demyelinating polyneuropathy, and other peripheral nerve polyneuropathies) tend to affect primarily the

15   peripheral nerves, whereas other myelin disorders affect primarily the CNS. The most commonly affected areas in the CNS are the brain, spinal cord, and optic nerves.

Demyelination is often secondary to an infectious, ischemic, metabolic, or hereditary disorder. In primary demyelinating disorders, while the cause or causes are unknown, an

20   autoimmune mechanism is suspected because the disorder sometimes follows a viral infection or viral vaccination.

Demyelination tends to be segmental or patchy, affecting multiple areas simultaneously or sequentially. Remyelination can occur, however, with repair, regeneration, and complete

25   recovery of neural function. Extensive myelin loss, however, is usually followed by axonal degeneration and often cell body degeneration.

Multiple sclerosis (MS) is characterized by disseminated patches of demyelination in the brain and spinal cord. Common symptoms include visual and oculomotor abnormalities,

30   paresthesias, weakness, spasticity, urinary dysfunction, and mild cognitive impairment. Typically, neurologic deficits are multiple, with remissions and exacerbations gradually producing disability. Diagnosis is by history of remissions and exacerbations plus clinical signs, test results, lesions seen on magnetic resonance imaging (MRI), or other criteria

(depending on symptoms) to objectively demonstrate ≥ 2 separate neurologic abnormalities. Treatment generally includes corticosteroids for acute exacerbations, immunomodulatory drugs to prevent exacerbations, and supportive measures.

5   In MS, localized areas of demyelination (plaques) occur, with destruction of oligodendroglia, perivascular inflammation, and chemical changes in lipid and protein constituents of myelin in and around the plaques. Axonal damage is possible, but cell bodies and axons tend to be relatively well preserved. Fibrous gliosis develops in plaques that are disseminated throughout the CNS, primarily in white matter, particularly in the lateral and posterior

10   columns (especially in the cervical regions), optic nerves, and periventricular areas. Tracts in the midbrain, pons, and cerebellum are also affected. Gray matter in the cerebrum and spinal cord can be affected, but to a much lesser degree.

Heart Disease

15   Heart disease is a general term for a number of different diseases which affect the heart. It is the leading cause of death in many industrialized countries, including the United States. The following broad categories of heart disease are presented by way of introduction. Extrinsic cardiomyopathies are cardiomyopathies, wherein the primary pathology lies outside the myocardium. Most cardiomyopathies are extrinsic, because the most common cause of

20   cardiomyopathy is ischemia. Intrinsic cardiomyopathies derive from weakness in the heart muscle that is not due to an identifiable external cause. Cardiovascular disease, on the other hand, refers to any number of specific diseases that affect the heart itself and/or the blood vessel system, especially the veins and arteries leading to and from the heart. Research on disease dimorphism suggests that women who suffer with cardiovascular disease usually

25   suffer from forms that affect the blood vessels while men usually suffer from forms that affect the heart muscle itself. Known or associated causes of cardiovascular disease include diabetes mellitus, hypertension, hyperhomocysteinemia and hypercholesterolemia. Ischaemic heart disease is yet another category of disease of the heart itself, typified by reduced blood supply to the organ.

30   Hypertensive heart disease is a term used to refer to heart disease caused by high blood pressure, especially localized high blood pressure. Inflammatory heart disease involves inflammation of the heart muscle and/or the tissue surrounding it. Valvular heart disease is any disease process involving one or more valves of the heart. The valves in the right side of

the heart are the tricuspid valve and the pulmonic valve and the valves in the left side of the
heart are the mitral valve and the aortic valve.

Congestive heart failure, one of the leading causes of death in industrialized nations, results

5   from an increased workload on the heart and a progressive decrease in its pumping ability. It
can result from any structural or functional cardiac disorder that impairs the ability of the
heart to fill with or pump a sufficient amount of blood through the body. Initially, the
increased workload that results from high blood pressure or loss of contractile tissue induces
compensatory cardiomyocyte hypertrophy and thickening of the left ventricular wall, thereby

10   enhancing contractility and maintaining cardiac function. Over time, however, the left
ventricular chamber dilates, systolic pump function deteriorates, cardiomyocytes undergo
apoptotic cell death, and myocardial function progressively deteriorates.

Factors that underlie congestive heart failure include high blood pressure, ischemic heart

15   disease, exposure to cardiotoxic compounds such as the anthracycline antibiotics, and genetic
defects known to increase the risk of heart failure.

By "congestive heart failure" is meant impaired cardiac function that renders the heart unable
to maintain the normal blood output at rest or with exercise, or to maintain a normal cardiac

20   output in the setting of normal cardiac filling pressure. A left ventricular ejection fraction of
about 40% or less is indicative of congestive heart failure (by way of comparison, an ejection
fraction of about 60% percent is normal). Patients in congestive heart failure display well-
known clinical symptoms and signs, such as tachypnea, pleural effusions, fatigue at rest or
with exercise, contractile dysfunction, and edema. Congestive heart failure is readily

25   diagnosed by well known methods (see, e.g., "Consensus recommendations for the
management of chronic heart failure." Am. J. Cardiol., 83(2A):1A-38-A, 1999).

Relative severity and disease progression are assessed using well known methods, such as
physical examination, echocardiography, radionuclide imaging, invasive hemodynamic

30   monitoring, magnetic resonance angiography, and exercise treadmill testing coupled with
oxygen uptake studies.

By "ischemic heart disease" is meant any disorder resulting from an imbalance between the

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myocardial need for oxygen and the adequacy of the oxygen supply. Most cases of ischemic heart disease result from narrowing of the coronary arteries, as occurs in atherosclerosis or other vascular disorders.

5   By "myocardial infarction" is meant a process by which ischemic disease results in a region of the myocardium being replaced by scar tissue.

By "cardiotoxic" is meant a compound that decreases heart function by directing or indirectly impairing or killing cardiomyocytes.

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By "hypertension" is meant blood pressure that is considered by a medical professional (e.g., a physician or a nurse) to be higher than normal and to carry an increased risk for developing congestive heart failure.

15   By "treating" is meant that administration of a neuregulin or neuregulin-like polypeptide slows or inhibits the progression of congestive heart failure during the treatment, relative to the disease progression that would occur in the absence of treatment, in a statistically significant manner. Well known indicia such as left ventricular ejection fraction, exercise performance, and other clinical tests, as well as survival rates and hospitalization rates may

20   be used to assess disease progression. Whether or not a treatment slows or inhibits disease progression in a statistically significant manner may be determined by methods that are well known in the art (see, e.g., SOLVD Investigators, N. Engl. J. Med. 327:685-691, 1992 and Cohn et al., N. Engl. J Med. 339:1810-1816, 1998).

25   By "decreasing progression of myocardial thinning" is meant maintaining hypertrophy of ventricular cardiomyocytes such that the thickness of the ventricular wall is maintained or increased.

By "inhibits myocardial apoptosis" is meant that neuregulin treatment inhibits death of
30   cardiomyocytes by at least 10%, more preferably by at least 15%, still more preferably by at least 25%, even more preferably by at least 50%, yet more preferably by at least 75%, and most preferably by at least 90%, compared to untreated cardiomyocytes.

Stroke

Stroke or cerebrovascular accident (CVA) is a term used to refer to the rapidly developing loss of brain functions due to a disturbance in the blood vessels supplying blood to the brain. A stroke occurs when the blood supply to part of the brain is suddenly interrupted or when a

5   blood vessel in the brain bursts, spilling blood into the spaces surrounding brain cells. Brain cells die when they no longer receive oxygen and nutrients from the blood or there is sudden bleeding into or around the brain. The symptoms of a stroke include sudden numbness or weakness, especially on one side of the body; sudden confusion or trouble speaking or understanding speech; sudden trouble seeing in one or both eyes; sudden trouble with

10   walking, dizziness, or loss of balance or coordination; or sudden severe headache with no known cause. There are two forms of stroke: ischemic, which is due to blockage of a blood vessel supplying the brain (e.g., caused by thrombosis or embolism); and hemorrhagic, which results from bleeding into or around the brain.

15   **Index for Therapeutic Window**

For each disease application described herein, a target therapeutic window for GGF2 serum plasma levels is established. In accordance with the experimental results presented herein, when GGF2 is administered to a mammal afflicted with a neurological disorder associated with demyelination, GGF2 must be administered in a dosing regimen to achieve and maintain a

20   narrow target therapeutic window of plasma GGF2 concentrations. As taught herein, precise dosing of GGF2 is necessary in order to achieve serum plasma levels of GGF2 required for therapeutic efficacy with respect to inducing myelination in a subject in need thereof.

In an embodiment of the invention directed to a patient in need of remyelination a particular

25   embodiment, the target serum plasma level of GGF2 is about 0.01 nM.

In another embodiment of the invention directed to a patient in need of remyelination, GGF2 is administered at an amount of about 500 ng/kg of patient body weight.

30   The compositions of the present invention may be used in the treatment of a condition in a patient that includes establishing a therapeutically effective concentration of GGF2 in the patient in need thereof. The compositions may be used for building up a level and or maintaining a therapeutically effective concentration of GGF2 in the patient. Where desirable, the compositions of the present invention may be formulated to avoid large peaks

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in initial release of GGF2. The compositions of the present invention when administered to

a patient in need thereof provide for the treatment of the above-indicated diseases.

Preferably, the compositions are administered so as to achieve a therapeutically effective

blood plasma level of GGF2 that is maintained in the patient for a period of at least 6 hours,

5   preferably at least 8 hours, and more preferably at least about 10-12.

EXAMPLES

10   Materials and Methods

Antibodies

For immunofluorescence analysis, monoclonal antibody (SM194) to myelin basic protein

(MBP) (Sternberger monoclonals) was used at a 1:500 dilution. For Western blot analysis,

polyclonal antibodies to active erbB2 (p-Neu/Tyr 1248), erbB2 and erbB3 were all obtained

15   from Santa Cruz and used at a 1:1000 dilution. Monoclonal antibody to phosphorylated Akt

and polyclonal antibody to phosphorylated MAPK were purchased from Cell Signaling and

were used at dilutions of 1:1000 and 1:500, respectively. Polyclonal antibodies to Akt and

MAPK (Promega) were used at dilutions of 1:1000 and 1:5000, respectively.

20   *Type-II and Type-III Neuregulin-1*

Recombinant human glial growth factor-II (rhGGF-II, Type-II Nrg1) was obtained from

Acorda Therapeutics, Inc. Recombinant human sensory and motor neuron derived factor

(rhSMDF, Type-III Nrg1) was purchased from R&D Systems. In the present study, rhGGF-

II and rhSMDF are referred simply as GGF (or GGF2) and SMDF, respectively. The GGF

25   was the N-terminus 419 amino acid residues containing the EGF domain and the Ig-like

domain. Accordingly, GGF is a soluble protein lacking a transmembrane and cytoplasmic

domains.

Primary rat Schwann cell culture

30   Schwann cells were prepared from sciatic nerves of newborn rats (1-2 day old) as described

previously (Brockes et al., Brain Res. 1979;165:105-118). For routine culture, Schwann cells

were grown in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum

(FBS) supplemented with EGF-domain neuregulin-1 (R&D Systems) (10 ng/ml) and

Forskolin (2 µM). Cells between passage 2-4 were used in all experiments described in the text.

Dorsal root ganglion (DRG) neuron-Schwann cell co-culture

5 Dissociated DRG were prepared from embryonic day14.5 rat embryos as described previously (Eldridge et al., J Cell Biol. 1987;105(2):1023-34) and plated onto collagen (type-1 rat tail collagen,)-coated 12 mm glass coverslips at a density of 1.25 DRG/coverslip. Five to six hours later, the cultures were flooded with neurobasal medium (Cellgro,) supplemented with B27 (GIBCO), 20% glucose, NGF (50 ng/ml) and 5-fluorodeoxyuridine

10 (FUdR, 10 µM) and maintained in the medium for additional 2-3 days in order to remove proliferating non-neuronal cells. Cultures were then switched to fresh medium without FUdR and maintained until the DRG axons reached the periphery of the coverslips. After the axonal networks were established, Schwann cells were plated onto the neurons at a density of 100,000 cells/coverslip. Four to five days later, cultures were switched to myelinating

15 medium: Minimal Essential Medium (MEM) supplemented with 10% heat-inactivated FBS, 20% glucose, NGF (50 ng/ml) and ascorbic acid (50 µg/ml). Ten to eleven days later, myelination was assessed by immunostaining for MBP.

Superior cervical ganglion (SCG) neuron-Schwann cell co-culture

20 Dissociated SCG were prepared from postnatal day 1-2 rats as described previously and plated onto collagen-coated 12 mm glass coverslips at a density of 0.8 SCG/coverslip. Next day, the cultures were flooded with neurobasal medium supplemented with B27 (GIBCO), 20% glucose, NGF (50 ng/ml) and 5-fluorodeoxyuridine (FUdR, 10 µM) and maintained in the medium for an additional 2-3 days in order to remove proliferating non-neuronal cells.

25 The cultures were switched back to fresh medium without FudR and maintained until the axons extended out to the periphery of the coverslips. Schwann cells were plated onto the neurons and maintained in neurobasal medium with supplements until the Schwann cells populate the axons (about 7-10 days). Myelination was initiated by placing the cultures in myelinating medium as described for DRG-Schwann cell co-culture. Forty days later,

30 myelination was assessed by MBP immunostaining.

Immunoprecipitation and Western blot analysis

. To prepare cell lysates, 90-95% confluent rat Schwann cells on 60 mm plates or co-cultures
were washed twice in phosphate-buffered saline (PBS) and then lysed in 300 μl ice-cold lysis
buffer (50 mM Tris HCl pH7.4, 1% NP-40, 0.25% Sodium Deoxycholate, 150 mM NaCl, 1
mM EGTA, 10 μg/ml Leupeptin, 2 μg/ml Aprotinin, 1 mM PMSF and 0.5 mM sodium

5   orthovanadate). Lysates were cleared by centrifugation for 15 min at 14,000 rpm in the cold
and the protein concentration of the supernatants was determined according to manufacturer
specifications (Bio-Rad: Hercules, CA). For Western blot analysis, 50-70 μg of Schwann
cell lysates were size-fractionated on 10% SDS-polyacrylamide gels and transferred onto
PVDF membranes. After blocking in 5% milk, the membranes were incubated with

10 appropriate primary antibodies prepared in blocking solution. After incubating with
horseradish peroxidase conjugated secondary antibodies, the protein bands were visualized
by enhanced chemiluminescence. For immunoprecipitation, 500 μg of Schwann cell lysates
were incubated with 0.6 μg of primary antibody for 3 hours at 4°C, then incubated with 50 μl
Sepharose A beads for 1 hour. Beads were washed 5 times in the lysis buffer and proteins

15 bound to beads were fractionated on SDS-polyacrylamide gels and subjected to Western blot
analysis.

Immunofluorescence staining for MBP
DRG-Schwann cell or SCG-Schwann cell cultures were rinsed in phosphate buffered saline

20 (PBS) then fixed in 4% paraformaldehyde for 20 minutes. After washing with PBS, samples
were permeabilized in ice-cold methanol for 25 minutes then incubated in blocking solution
(5% normal goat-serum + 0.3% Triton X) for 1 hour at room temperature. This was followed
by incubation with primary antibody prepared in blocking solution overnight. After washing
with PBS, samples were incubated with Alexa-488 conjugated goat-anti-mouse secondary

25 antibody for 45 minutes. Nuclei of cells were visualized by staining with DAPI.

Real-time quantitative PCR
Statistical analysis
One way ANOVA was performed using SAS programming software with 95% significance

30 level.

Results
The inhibitory function of GGF2 on myelination is mediated by the MAPK activation

An earlier study has shown that Nrg1 type II (GGF2), when added to Schwann cell-neuron co-cultures, inhibits myelination. It has also been reported that activation of Ras/Raf/MAPK pathway inhibits myelin-associated gene expression in Schwann cells, whereas activation of the PI3-kinase pathway promotes myelination, leading to a notion that the myelination state of

5   Schwann cell is determined by the balance between PI3-kinase and Ras/Raf/MAPK pathways (Ogata et al. J Neurosci 2004;24:6724-32). The present inventors predicted that if GGF2 acts via MAPK activation to inhibit myelination, inhibition of GGF2-induced MAPK activation would reverse the inhibitory effect on myelination. To assess the possibility that the inhibitory effect of GGF2 on myelination could be due to its ability to induce a robust MAPK activation in

10   Schwann cells, the present inventors used a well-established *in vitro* myelinating culture system in which Schwann cells are co-cultured with dorsal root ganglion (DRG) neurons and induced to myelinate the associated axons by addition of ascorbic acid to the culture media. First, to determine the effect of GGF2 on MAPK activation in the co-cultures, primary Schwann cells were plated onto DRG neurons and allowed to propagate the axons. Once the cultures stopped

15   proliferating, the co-cultures were stimulated with GGF2 at 0.6 nM. Twenty minutes later, cell lysates were prepared and MAPK activation was determined by Western blot analysis. In control co-cultures, there was a low level of active MAPK. As shown in Figure 1, treatment with GGF2 further increased the level of MAPK activation. To determine whether the GGF2-induced MAPK activation could be blocked by treatment with U0126, a pharmacologic inhibitor of

20   MAPK kinase, co-cultures were pre-treated with increasing concentrations (0.5, 1, 3 and 10 µM) of U0126 for 30 minutes prior to GGF2 stimulation and these concentrations were maintained in the culture medium. Control cultures were treated with the inhibitor in the absence of GGF2 treatment. In both control and experimental cultures, U0126-mediated MAPK inhibition was concentration-dependent, as indicated by the progressive decrease in the levels of phospho-

25   MAPK. In cultures treated with GGF2 and U0126 at a concentration of 1 µM, the level of activation was reduced to the basal level, while at 10 µM U0126, MAPK activation in the co-culture was completely abolished. The U0126 had no effect on GGF2-induced PI3kinase activation.

30   In order to evaluate the effect of MAPK inhibition on myelination, co-cultures were treated with GGF2 in the presence or the absence of U0126 at the time of initiating myelination and the same conditions were maintained under the described myelinating condition. Control cultures were left untreated under the described myelinating condition. Ten to eleven days later, cultures were fixed and immunostained for myelin basic protein (MBP) to visualize myelin segments. In

cultures treated with GGF2, there was a marked decrease in the number of myelin segments as shown previously, revealing the inhibitory effect of GGF2 on myelination. In cultures co-treated with U0126, however, there was a dose-dependent increase in myelination, indicating that blocking MAPK activation reversed the inhibitory effect of GGF2.

GGF2 promotes myelination at low concentrations: Although the level of MAPK activation steadily increased in Schwann cells treated with increasing concentrations of GGF2, the present inventors observed that at low concentrations below 0.01 μM, while the level of Akt activation increased significantly above the basal level, there was no detectable level of MAPK activation. If the myelination state of a Schwann cell is determined by the balance between the Akt and MAPK activation, the present inventors sought to evaluate if the increase in Akt activation in the absence of MAPK activity at these concentrations is correlated with a positive effect on myelination. To investigate this potentiality, co-cultures were treated with GGF2 at concentrations ranging from 0.0005 and 0.03 nM at the time of initiating myelination. Cultures were later fixed and immunostained for MBP. As predicted based on the instant findings, there was an increase in the level of myelination in cultures treated with low doses of GGF2, ranging from 0.0005 to 0.01 nM, compared to the untreated control cultures. When quantitated, the result demonstrated that there was a dose-dependent increase in the number of myelin segments (Figure 2): a 1.9-, 2.7-, and 3.5-fold increase in myelination relative to the control level, at 0.0005, 0.001 and 0.01 nM GGF2, respectively. At 0.03 nM, there was a drastic decrease in the level of myelination to a level close to, or slightly below the control cultures. Subsequent increases in the amount of GGF2 resulted in further decreases in myelination. Myelination responsive to GGF2 was completely inhibited at 0.6 nM GGF2. This concentration corresponded to the appearance of active MAPK in the co-cultures as shown in Figure 1. These results suggest that GGF2 plays dual roles during myelination: one that promotes and the other that inhibits myelination, and the two opposing functions are determined by the dosage of GGF2 presented to the Schwann cells.

The opposing functions of GGF2 are mediated by Mek/Erk activation: To investigate further the opposing functions of GGF2, additional experiments were performed. Previous studies have implicated Ras/Raf/Erk and PI-3 kinase, respectively, as negative and positive regulators of myelination, suggesting that a balance between the two is correlated with the myelination state of the Schwann cells. To delineate further the activation states of the

29

pathways induced by GGF2, co-cultures were treated with the soluble GGF2 protein at 1 nM. The present inventors determined that at this concentration, GGF2 effectively inhibited myelination. Cell lysates were prepared 30 minutes following the GGF2 treatment and the presence of the phosphorylated proteins was determined by Western blot analysis (Figure

5   3A). At 1 nM (Figure 3A, boxed lanes) GGF2 increased Akt activation above the basal level. An increase in Erk activation was also observed in GGF2-treated cultures at this concentration. Concentrations of GGF2 as low as 0.6 nM were shown to be sufficient for Erk activation in GGF2 treated cultures.

10   To corroborate the above results and investigate further the correlative link between Erk activation and the inhibitory effect of GGF on myelination, additional experiments were performed. Accordingly, co-cultures were treated with GGF2 along with increasing concentrations of U0126, the above-described specific inhibitor of Mek1/Erk pathway. Western blot analysis presented in Figure 3B shows that U0126 inhibited GGF2-induced Erk

15   activation in a dosage-dependent manner while it had no effect on Akt activation. The low level of endogenous Erk activity normally observed in the co-culture system was also decreased with the drug treatment.

The present inventors further assessed the effect of Mek1/Erk inhibition on myelination. As

20   shown in Figures 3C and 3D, addition of GGF2 at high concentration almost completely inhibited myelination in the co-cultures. In cultures co-treated U0126, however, the inhibitory effect of GGF2 was reversed, as indicated by the dosage-dependent increase in the level of myelination (Figures 3C and 3D). This result provides direct evidence that the inhibitory effect of GGF2 on myelination is mediated by the Erk activation. Interestingly,

25   U0126 treatment in co-cultures in the absence of GGF2 also resulted in an increase in the level of myelination (Figure 3E), which indicates that the endogenous Mek1/Erk activity functions as an intrinsic negative regulator of myelination.

Western blot analysis on lysates prepared from the co-cultures also revealed that GGF2

30   treatment increased expression of c-Jun protein, a negative regulator of Schwann cell differentiation and myelination. Subsequent inhibition of the GGF2-induced Mek1/Erk activity down-regulated c-Jun levels, which, in turn, was accompanied by an increase in myelin protein expression. Unlike the effect on c-Jun, U0126 treatment resulted in an

increase in Krox20 expression in the co-cultures. This is in agreement with a recent report
suggesting a cross antagonistic relationship between c-Jun and Krox20 in regulating
myelination (Parkinson et al, 2008, Journal of Cell Biology 181:625-637).

5   GGF2 promotes Schwann cell myelination: To corroborate and extend results presented
      herein and evaluate further the opposing functions of GGF2, the present inventors assessed
      the concentration-dependent effect of GGF2 on Ras/Raf/Erk and PI3-kinase activation in
      Schwann cells. Cells were treated with various concentrations of GGF2 ranging from 0.0003
      to 10 nM and the level of Erk and Akt activation was determined by Western blot analysis.
10   Images and the relative increase in the activation levels are presented in Figures 4A and 4B.
      The level of active Akt increased steadily beginning at the lowest dose tested, whereas Erk
      activation required higher concentrations of GGF2. The differential activation of the two
      pathways at low concentrations, as a result, generated a narrow window of doses (0.003 to
      0.01 nM, boxed in Figure 4B) in which Akt was the predominant pathway activated in
15   response to GGF2. Next, the effect of various doses of GGF2 on myelination was
      determined in the co-culture system. At the low concentration window, GGF2 elicited a
      dosage-dependent promyelinating effect: 1.5-, 2.3-, 2.2- and 2.8-fold increase in myelination
      compared to the control cultures at 0.0005, 0.001, 0.003 and 0.01 nM of GGF2, respectively
      (Figure 4C). As the concentration increased further, GGF2 began to inhibit myelination,
20   coinciding with the appearance of Erk activation. The promyelinating effect of GGF2 was
      also demonstrated in CRD-Nrg1$^{+/-}$ co-cultures in which low doses of GGF2 rescued the
      myelination defect on the mutant axons (Figure 4D).

      Soluble Nrg1 can both promote and inhibit myelination: binary choice determined by the
25   concentration: In the peripheral nervous system (PNS), GGF2 has been regarded as an Nrg1
      isoform associated with the Schwann cell injury response. Ectopic *in vivo* expression of
      GGF-β3 in myelinating Schwann cells stimulates cell proliferation and induces demyelination
      (Huijbregts et al. J Neurosci 2003;23:7269-80). Moreover, addition of high concentrations
      of GGF2 (e.g., those exceeding 0.25 nM GGF2) to Schwann cell-DRG neuron co-cultures
30   has been shown to inhibit myelination (Zanazzi et al. J Cell Biol 2001;152:1289-99). Thus,
      an unexpected result of the present study was the discovery that at low concentration, GGF2
      exhibits myelination promoting effects. The promyelinating effect was, however, limited to a

low concentration range and an increase in GGF2 concentration from this range results in
inhibition of myelination as described previously. This is an intriguing finding as it
demonstrates that soluble GGF2 can elicit two contrasting biological functions under the
same cellular context solely based on the amount presented to the cell. It also suggests that

5   threshold levels of GGF2 determine the promyelinating and inhibitory function during
myelination. As demonstrated for the first time in the present study, this can be explained by
concentration-dependent differential activation of the receptor downstream signaling
effectors. More specifically, the present data show that the promyelinating function of GGF2
is observed at concentrations that preferentially activate Akt, while the transition into the

10   inhibitory role at higher concentrations coincides with the appearance of Erk activation
despite the continuous increase in the level active Akt. This result also supports the previous
notion that the balance between PI3-kinase and Ras/Raf/Erk activation is crucial in
determining the state of myelination in Schwann cells (Ogata et al. J Neurosci 2004;24:6724-
32). The present findings, however, provide direct evidence that activation of the

15   Ras/Raf/Erk pathway functions as a negative regulator of myelination.

The inhibitory function Nrg1 on myelination is mediated through Erk/Mek1 activation: The
inhibitory role of Ras/Raf/Erk pathway on myelination has been suggested previously by
studies wherein expression of constitutively active Mek1 in Schwann cells blocks forskolin-

20   induced myelin gene expression, whereas dominant-negative Ras blocks myelin gene down-
regulation induced by Nrg1. Its direct effect on myelination, however, has not been
elucidated prior to the instant results. As demonstrated herein, GGF2, when used above a
threshold concentration, inhibits myelination in the co-cultures. The present inventors show
herein that inhibition of Mek1/Erk1 activation restored myelination in GGF2-treated co-

25   cultures, demonstrating that the inhibitory role of GGF2 was mediated through its
Ras/Raf/Erk1 activation. The mechanism by which Mek1/Erk activation inhibits myelination
is unclear. A possible mechanism includes suppression of myelin gene expression as
described previously. Supportive of this suggestion, the present data revealed that an
increase in myelination in U0126 treated cultures was accompanied by an increase in P0

30   expression. It is also possible that the Mek1/Erk pathway might modulate expression of
transcription factors involved in myelination or Schwann cell differentiation. Recently it has
been shown that ectopic expression of c-Jun in Schwann cells inhibits myelination, thus

suggesting that c-Jun functions as a negative regulator of the myelin program. The present findings are consistent with this conclusion since the present inventors determined that GGF2 treatment that inhibits myelination is accompanied by c-Jun induction and furthermore, inhibition of the Nrg1-induced Mek1/Erk1 activity blocks c-Jun expression. This result

5   suggests that the inhibitory function of Mek1/Erk1 on myelination is in part mediated through induction of c-Jun.

Another interesting finding of the present study is the presence of an intrinsic Mek1/Erk-dependent signal in the co-cultures that serves as a negative regulator of myelination. This

10   was shown in an experiment in which treatment of normal myelinating co-cultures with U0126 promoted myelination. The nature of the signal that contributes to the Mek1/Erk1 activity during myelination is presently unknown, although it is likely to be axonal in origin, independent of the axonal CRD-Nrg1. Possible candidates are type I and II Ig-Nrg1 that are expressed by the PNS neurons and later released from the axonal membrane by proteolytic

15   cleavage. Another possible Mek1/Erks activator is FGF-2, which is expressed in PNS neurons and the receptor for which is expressed on Schwann cells. Treatment with FGF-2 down-regulates myelin gene expression and inhibits myelination in vitro. Loss of FGF-2 expression results in an increase in the number of myelinated axons during sciatic nerve regeneration. Peripheral neurons also express PDGF and IGF, with the corresponding

20   receptor tyrosine kinases expressed on the associated Schwann cells. It will be of a great interest to assess the regulatory role of these growth factors during myelination of the PNS.

Therapeutic use of GGF2: Experimental transplantation has provided overwhelming proof for the potential of repairing damaged nerves by transplantation of myelinating glial cells.

25   Schwann cells are good candidates for such therapy as they are easily expanded in culture and offer the possibility of autologous transplantation to promote remyelination and restoration of nerve conduction at the demyelinated lesions not only in the PNS but also in the CNS. Remyelination by Schwann cells on adult regenerating axons, however, is often incomplete resulting in formation of thinner myelin sheath and shorter internode compared to

30   normal nerves. Therefore, the present demonstration of the promyelinating function of GGF2 and the ability to avoid inadvertent hindrance of myelination due to GGF2 dose levels is significant as it provides a therapeutic strategy for the treatment of demyelinating diseases as well as for rebuilding myelin following nerve injury.

Other Embodiments: All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference.

5

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure come

10   within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth, and follows in the scope of the appended claims.

In the claims:

1.   A method for avoiding inhibition of Schwann cell myelination following administration of glial growth factor 2 (GGF2) in a subject, said method comprising:

        providing a subject in need of neuron myelination;

5   providing GGF2 or EGFL domain of GGF2 in a pharmaceutically acceptable carrier; and

        administering the GGF2 or EGFL domain of GGF2 to the subject in an amount sufficient to elicit a plasma level of between about 0.0005 nM and about 0.01 nM GGF2 or EGFL domain of GGF2.

10

2.   The method of claim 1, wherein the GGF2 or the EGFL domain of GGF2 is administered intravenously, intrathecally, or topically.

3.   The method of claim 1, wherein the plasma level is about 0.01 nM GGF2 or EGFL

15   domain of GGF2.

4.   The method of claim 1, wherein the amount of GGF2 administered is about 500 ng of GGF2 or EGFL domain of GGF2 per kg of body weight.

20   5.   A method for promoting myelination in a patient afflicted with a disease or disorder associated with reduced levels of myelination, the method comprising:

        selecting a patient afflicted with a disease or disorder associated with reduced levels of myelination; and,

        administering glial growth factor 2 (GGF2) or EGFL domain of GGF2 to the patient

25   at an amount that achieves a plasma level of between about 0.0005 nM and about 0.01 nM GGF2 or EGFL domain of GGF2.

6.   The method of claim 5, wherein 500 ng of GGF2 or EGFL domain of GGF2 per kg is administered to the patient to achieve a GGF2 or EGFL domain of GGF2 serum plasma level

30   of 0.01 nM.

7.   The method of any one of the preceding claims substantially as herein described.

8.   A method of treatment of a patient afflicted with a disease or disorder associated with reduced levels of myelination comprising administering GGF2 or an EGFL domain of GGF2 to the patient in an amount sufficient to elicit a plasma level of between about 0.0005 nM and about 0.01 nM GGF2 or EGFL domain of GGF2, in combination with a Mek1/Erk pathway inhibitor, wherein the Mek1/Erk pathway inhibitor is chosen from: Arctigenin, PD 98059, SB202190, SB203580, SP600125, U0126, tipifarnib (Zarnestra®), sorafenib, ISIS 5132, CI-1040, and PD 0325901.

9.   The method according to claim 8, wherein the administration is intravenous, intrathecal, or topical.

10.   The method according to claim 8, wherein the disease or disorder associated with reduced levels of myelination is selected from Guillain-Barre Syndrome, chronic inflammatory demyelinating polyneuropathy, peripheral demyelination due to traumatic injury, multiple sclerosis, optic neuritis, central demyelination due to traumatic injury, transverse myelitis, progressive multifocal leukoencephalopathy, Devic's disease (neuromyelitis optica), acute disseminated encephalomyelitis, adrenoleukodystrophy and adrenoleukoneuropathy.

11.   The method according to claim 10, wherein the disease or disorder associated with reduced levels of myelination is multiple sclerosis.

12.   The method according to claim 10, wherein the disease or disorder associated with reduced levels of myelination is peripheral demyelination due to traumatic injury.

13.   The method according to claim 10, wherein the disease or disorder associated with reduced levels of myelination is central demyelination due to traumatic injury.

14.   The method according to claim 10, wherein the disease or disorder associated with reduced levels of myelination is optic neuritis.

15.   The method according to claim 8, wherein amount of the GGF2 or the EGFL domain of GGF2 administered is about 500 ng per kg of body weight.

16.   The method according to claim 15, wherein the plasma level is about 0.01 nM GGF2 5   or EGFL domain of GGF2.
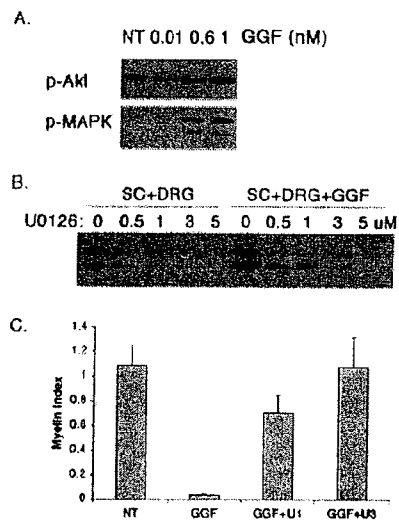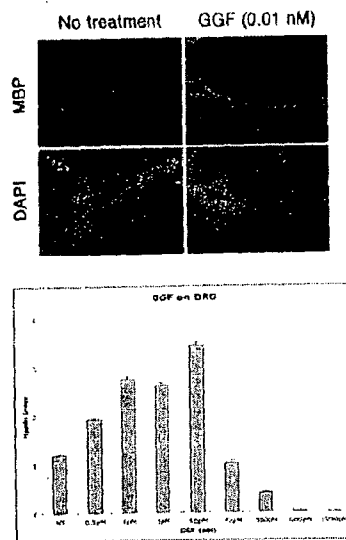
Figure 1

A.

NT 0.01 0.6 1 GGF (nM)

p-Akt

p-MAPK

B.

SC+DRG   SC+DRG+GGF

U0126: 0 0.5 1 3 5 0 0.5 1 3 5 uM
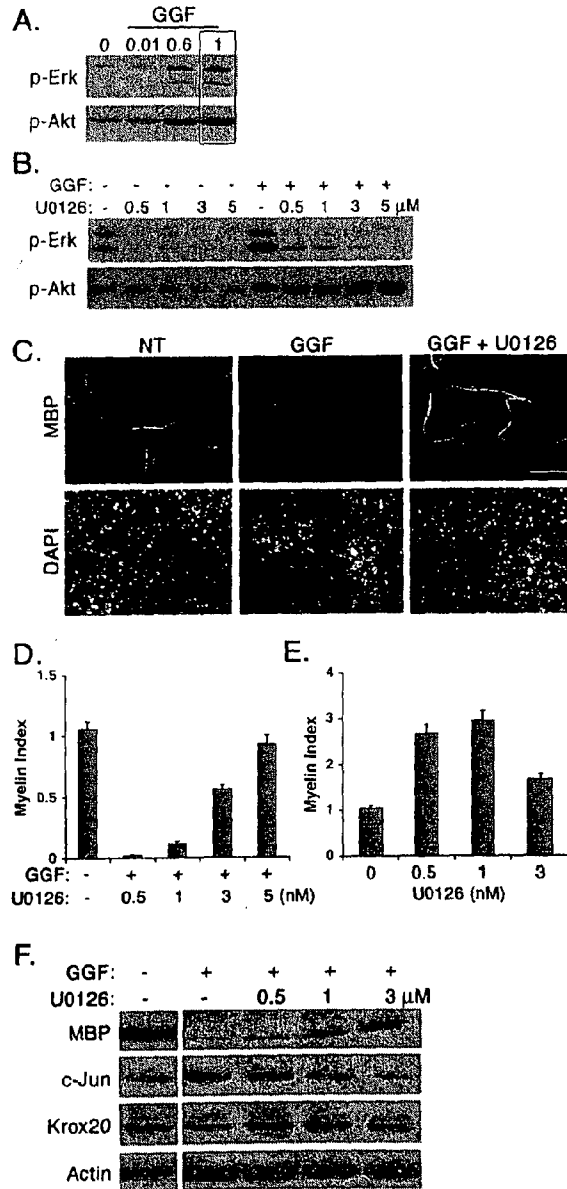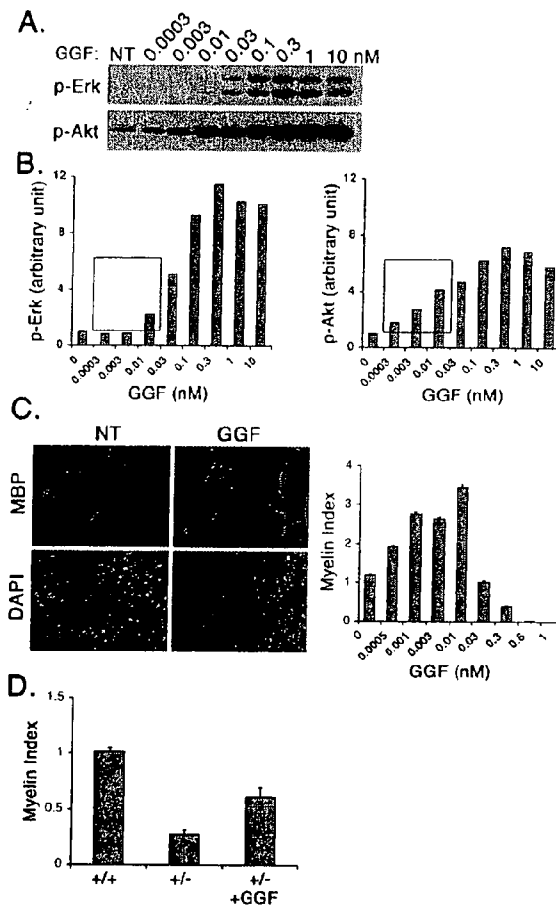
C.

Figure 2

Figure 3

Figure 4

Fig. 5A

Nucleotide Sequence & Deduced Acid Sequence of GGF2HBS5

```
GGAATTCCTT TTTTTTTT TTTTTTCTT NNTTTTTTT TGCCCTTATA CCTCTTCGCC        60

TTTCTGTGGT TCCATCCACT TCTTCCCCCT CCTCCTCCCA TAAACAACTC TCCTACCCCT       120

GCACCCCCAA TAAATAAATA AAAGGAGGAG GGCAAGGGGG GAGGAGGAGG AGTGGTGCTG       180

CGAGGGGAAG GAAAAGGGAG GCAGCGCGAG AAGAGCCGGG CAGAGTCCGA ACCGACAGCC       240

AGAAGCCCGC ACGCACCTCG CACC ATG AGA TGG CGA CGC GCC CCG CGC CGC CGC     291
                          Met Arg Trp Arg Arg Ala Pro Arg Arg Arg

TCC GGG CGT CCC GGC CCC CGG GCC CAG CGC CCC GGC TCC GCC GCC CGC        339
Ser Gly Arg Pro Gly Pro Arg Ala Gln Arg Pro Gly Ser Ala Ala Arg

TCG TCG CCG CCG CTG CCG CTG CCA CTA CTG CTG CTG CTG CTG GGG ACC       387
Ser Ser Pro Pro Leu Pro Leu Pro Leu Leu Leu Leu Leu Leu Gly Thr
                                 Val Cys Leu Leu Thr Val
                                              GGF-II 09

GCG GCC CTG GCG CCG GGG GCG GCG GCC AAC GAG GCG GCT CCC GCG          435
Ala Ala Leu Ala Pro Gly Ala Ala Ala Gly Asn Glu Ala Ala Pro Ala
Ala Ala Leu Pro Pro

GGG GCC TCG TGC TAC TCG TCC CCG CCC AGC GTG GGA TCG GTG CAG         483
Gly Ala Ser Val Cys Tyr Ser Ser Pro Pro Ser Val Gly Ser Val Gln
    Ala Ser        Pro Val Ser Pro Val Ser
                                        GGF-II 08

GAG CTA GCT CAG CGC GCC GCG GTG GTG ATC GAG GGA AAG GTG CAC CCG      531
Glu Leu Ala Gln Arg Ala Ala Val Val Ile Glu Gly Lys Val His Pro
Glu Leu Val Gln Arg Trp Phe Val Ile Glu Gly Lys
                     GGF-II 04
```

Fig. 5B

Nucleotide Sequence & Deduced Acid Sequence of GGF2HBS5

```
CAG CGG CAG CAG GCA CTC GAC AGG AAG GCG GCG GCG GCG GCG      579
Gln Arg Gln Gln Ala Leu Asp Arg Lys Ala Ala Ala Ala Ala

GGC GAG GCA GGG GCG TGG GGC GGC GAT CGC GAG CCG CCA GCC GCG GGC   627
Gly Glu Ala Gly Ala Trp Gly Gly Asp Arg Glu Pro Pro Ala Ala Gly

CCA CGG GCG GGG CCC CCG GCC GAG GAG CTG CCG CTC GCC GCC AAC       675
Pro Arg Ala Gly Pro Pro Ala Glu Glu Leu Pro Leu Ala Ala Asn

GGG ACC GTG CCC TCT TGG CCC ACC GCC GTG CCC GTG AGC GCC GGC GAG   723
Gly Thr Val Pro Ser Trp Pro Thr Ala Val Pro Val Ser Ala Gly Glu

CCC GGG GAG GCG CCC TAT CTG GTG AAG GTG CAC CAG GTG TGG GCG       771
Pro Gly Glu Ala Pro Tyr Leu Val Lys Val His Gln Val Trp Ala
                        Leu     Lys Val His Glu Val Trp Ala
                                GGF-II 01 & GGF-II 11

GTG AAA GCC GGG GGC TTG GGC AAG GAC TCG CTC CTG CTC ACC GTG CGC CTG   819
Val Lys Ala Gly Gly Leu Gly Lys Asp Ser Leu Leu Leu Thr Val Arg Leu
Ala Lys                         Asp Leu Leu Leu Xaa Val     Leu
                                            GGF-II 10

GGG ACC TGG GGC CAC CCC GCC TTC CCC TCC TGC GGG AGG CTC AAG GAG       867
Gly Thr Trp Gly His Pro Ala Phe Pro Ser Cys Gly Arg Leu Lys Glu
Gly Ala Trp     Pro Pro Ala Phe Pro Val Xaa     Tyr Arg Leu Glu
                    GGF-II 03

GAC AGC AGG TAC TTC ATC TTC ATG GAG CCC GAC GCC AAC AGC ACC AGC       915
Asp Ser Arg Tyr Phe Ile Phe Met Glu Pro Asp Ala Asn Ser Thr Ser
Asp Ser Arg Tyr Ile Phe     Met Glu Pro Gla Ala Xaa Ser Ser Gly
                                GGF-II 02
```

Fig. 5C

Nucleotide Sequence & Deduced Acid Sequence of GGF2HBS5

```
CGC GCG CCG GCC GCC TTC CGA GCC TCT TTC CCC CCT CTG GAG ACG GGC      963
Arg Ala Pro Ala Ala Phe Arg Ala Ser Phe Pro Pro Leu Glu Thr Gly

CGG AAC CTC AAG AAG GAG GTC AGC CGG CTG TGC AAG CGG TGC GCC         1011
Arg Asn Leu Lys Lys Glu Val Ser Arg Leu Cys Lys Arg Cys Ala

TTG CCT CAA TTG AAA GAG ATG AAG AGC CAG GAA TCG GCT GCA GGT         1059
Leu Pro Gln Leu Lys Glu Met Lys Ser Gln Glu Ser Ala Ala Gly

TCC AAA CTA GTC CTT CGG TGT GAA ACC AGT TCT GAA TAC TCC TCT CTC     1107
Ser Lys Leu Val Leu Arg Cys Glu Thr Ser Ser Glu Tyr Ser Ser Leu
          Leu Val Leu Arg
          GGF-II 06

AGA TTC AAG TGG TTC AAG AAT GGG AAT GAA TTG AAT CGA AAA AAC AAA     1155
Arg Phe Lys Trp Phe Lys Asn Gly Asn Glu Leu Asn Arg Lys Asn Lys

CCA CAA AAT ATC AAG ATA CAA CAA AAA AAG GGG CCA TCA GAA CTT CGC     1203
Pro Gln Asn Ile Lys Ile Gln Gln Lys Lys Gly Pro Ser Glu Leu Arg

ATT AAC AAA GCA TCA CTG GCT GAT TCT GGA GAG TAT ATG TGC AAA GTG     1251
Ile Asn Lys Ala Ser Leu Ala Asp Ser Gly Glu Tyr Met Cys Lys Val
                                        Tyr Met Xaa Lyx
                                        GGF-II 12

ATC AGC AAA TTA GGA AAT GAC AGT TCT GCC AAT ATC ACC ATC GTG         1299
Ile Ser Lys Leu Gly Asn Asp Ser Ser Ala Asn Ile Thr Ile Val

GAA TCA AAC GCT ACA TCT ACA TCC ACC ACA GGG AGC CAT CTT GTA         1347
Glu Ser Asn Ala Thr Ser Thr Ser Thr Gly Ser His Leu Val
```

Fig. 5D

Nucleotide Sequence & Deduced Acid Sequence of GGF2HBS5

```
AAA TGT GCG GAG AAG GAG AAA ACT TTC TGT GTG AAT GGA GGG GAG TGC                    1395
Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu Cys

TTC ATG GTG AAA GAC CTT TCA AAC CCC TCG AGA TAC TTG TGC AAG TGC                    1443
Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu Cys Lys Cys

CCA AAT GAG TTT ACT GGT GAT CGC TGC CAA AAC TAC GTA ATG GCC AGC                    1491
Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr Val Met Ala Ser

TTC TAC AGT ACG TCC ACT CCC TTT CTG TCT CCT GAA                                    1530
Phe Tyr Ser Thr Ser Thr Pro Phe Leu Ser Pro Glu

TAGGAGCATG CTCAGTTGGT GCTGCTTTCT TGTTGCTGCA TCTCCCCTCA GATTCCACCT                  1590

AGAGCTAGAT GTGTCTTACC AGATCTAATA TTGACTGCCT CTGCCTGTCG CATGAGAACA                  1650

TTAACAAAAG CAATTGTATT ACTTCCTCTG TTCGCGACTA GTTGGCTCTG AGATACTAAT                  1710

AGGTGTGTGA GGCTCCGGAT GTTTCTGGAA TTGATATTGA ATGATGTGAT ACAAATTGAT                  1770

AGTCAATATC AAGCAGTGAA ATATGATAAT AAAGGCATTT CAAAGTCTCA CTTTTATTGA                  1830

TAAAATAAAA ATCATTCTAC TGAACAGTCC ATCTTCTTTA TACAATGACC ACATCCTGAA                  1890

AAGGGTGTTG CTAAGCTGTA ACCGATATGC ACTTGAAATG ATGGTAAGTT AATTTTGATT                  1950

CAGAATGTGT TATTTGTCAC AAATAAACAT AATAAAAGGA AAAAAAAAA AAA                          2003
```

Fig. 6

EGFL1

```
AGC CAT CTT GTC AAG TGT GCA GAG AAG GAG AAA ACT TTC TGT GTG AAT    48
Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn

GGA GGC GAG TGC TTC ATG GTG AAA GAC CTT TCA AAT CCC TCA AGA TAC    96
Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr

TTG TGC AAG TGC CCA AAT GAG TTT ACT GGT GAT CGC TGC CAA AAC TAC   144
Leu Cys Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr

GTA ATG GCC AGC TTC TAC AGT ACG TCC ACT CCC TTT CTG TCT CTG CCT   192
Val Met Ala Ser Phe Tyr Ser Thr Ser Thr Pro Phe Leu Ser Leu Pro

GAA TAG                                                           198
Glu
```

Fig. 7

EGFL2

```
AGC CAT CTT GTC AAG TGT GCA GAG AAG GAG AAA ACT TTC TGT GTG AAT    48
Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn

GGA GGC GAG TGC TTC ATG GTG AAA CTT GAC TCA AAT CCC TCA AGA TAC    96
Gly Gly Glu Cys Phe Met Val Lys Leu Asp Ser Asn Pro Ser Arg Tyr

TTG TGC AAG TGC CAA CCT GGA TTC ACT GGA GCG AGA TGT ACT GAG AAT   144
Leu Cys Lys Cys Gln Pro Gly Phe Thr Gly Ala Arg Cys Thr Glu Asn

GTG CCC ATG AAA GTC CAA ACC CAA GAA AAA GCG GAG GAG CTC TAC TAA   192
Val Pro Met Lys Val Gln Thr Gln Glu Lys Ala Glu Glu Leu Tyr Tyr
```

Fig. 8

EGFL3

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AGC CAT CTT GTC AAG TGT GCA GAG AAG GAG AAA ACT TTC TGT GTG AAT
Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn    48

GGA GGC TGC GAG TTC ATG GTG AAA GAC CTT TCA AAT CCC TCA AGA TAC
Gly Gly Cys Glu Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr    96

TTG TGC AAG TGC CCA AAT GAG TTT ACT GGT GAT CGC TGC CAA AAC TAC
Leu Cys Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr   144

GTA ATG GCC AGC TTC TAC AAA GCG GAG GAG CTC TAC TAA
Val Met Ala Ser Phe Tyr Lys Ala Glu Glu Leu Tyr             183
```

Fig. 9

EGFL4

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AGC CAT CTT GTC AAG TGT GCA GAG AAG GAG AAA ACT TTC TGT GTG AAT      48
Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn

GGA GGC GAG TGC TTC ATG GTG AAA GAC CTT TCA AAT CCC TCA AGA TAC      96
Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr

TTG TGC AAG TGC CCA AAT GAG TTT ACT GGT GAT CGC TGC CAA AAC TAC     144
Leu Cys Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr

GTA ATG GCC AGC TTC TAC AAG CAT CTT GGG ATT GAA TTT ATG GAG AAA     192
Val Met Ala Ser Phe Tyr Lys His Leu Gly Ile Glu Phe Met Glu Lys

GCG GAG GAG CTC TAC TAA                                             210
Ala Glu Glu Leu Tyr Tyr
```

Fig. 10

EGFL5

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AGC CAT CTT GTC AAG TGT GCA GAG AAG GAG AAA ACT TTC TGT GTG AAT    48
Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn

GGA GGC GAG TGC TTC ATG GTG AAA GAC CTT TCA AAT CCC TCA AGA TAC    96
Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr

TTG TGC AAG TGC CAA CCT GGA TTC ACT GGA GCG AGA TGT ACT GAG AAT   144
Leu Cys Lys Cys Gln Pro Gly Phe Thr Gly Ala Arg Cys Thr Glu Asn

GTG CCC ATG AAA GTC CAA ACC CAA GAA AAG TGC CCA AAT GAG TTT ACT   192
Val Pro Met Lys Val Gln Thr Gln Glu Lys Cys Pro Asn Glu Phe Thr

GGT GAT CGC TGC CAA AAC TAC GTA ATG TAC GCC AGC TTC TAC AGT ACG TCC   240
Gly Asp Arg Cys Gln Asn Tyr Val Met Tyr Ala Ser Phe Tyr Ser Thr Ser

ACT CCC TTT CTG TCT CTG CCT GAA TAG                                267
Thr Pro Phe Leu Ser Leu Pro Glu
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SUBSTITUTE SHEET (RULE 26)

Fig. 11

EGFL6

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AGC CAT CTT GTC AAG TGT GCA GAG AAG GAG AAA ACT TTC TGT GTG AAT    48
Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn

GGA GGC GAG TGC TTC ATG GTG AAA CTT TCA GAC CCC TCA AGA TAC        96
Gly Gly Glu Cys Phe Met Val Lys Leu Ser Asp Pro Ser Arg Tyr

TTG TGC AAG TGC CAA CCT GGA TTC ACT GGA GCG AGA TGT ACT GAG AAT   144
Leu Cys Lys Cys Gln Pro Gly Phe Thr Gly Ala Arg Cys Thr Glu Asn

GTG CCC ATG AAA GTC CAA ACC CAA GAA AAG TGC CCA AAT GAG TTT ACT   192
Val Pro Met Lys Val Gln Thr Gln Glu Lys Cys Pro Asn Glu Phe Thr

GGT GAT CGC TGC CAA AAC TAC GTA ATG GCC AGC TTC TAC AAA GCG GAG   240
Gly Asp Arg Cys Gln Asn Tyr Val Met Ala Ser Phe Tyr Lys Ala Glu

GAG CTC TAC TAA                                                   252
Glu Leu Tyr
```

RNA								
Size	2224 bp							
TRANSCRIPTS	number of transcripts	9	type	messenger				
text	other alternative forms							
identification	major	type	bp	product				
				kDa	AA	expressed in	author	
HRG-gamma, NRG1 type 1	-	splicing	1651	-	211	-	Holmes	
					-			
GGF2, NRG1 type 2	-	splicing	1986	-	422	nervous system, skeletal muscle	Marchionni, Holmes	
	soluble form							
SMDF, NRG1 type3	-	splicing	1860	31,6	296	in the nervous system spinal cord, motoneuron, fetal lung, liver, kidney	Holmes, Michailov	
	a C-terminal EGF-like domain and a unique N-terminal sequence which lacks an Ig-like domain, cysteine-rich domain (CRD)							
HRG-alpha, NRG1 type1	yes	splicing	2224	70.2	640	breast, ovary, testis, prostate, heart, muscle	Holmes, Michailov	
					-			
HRG-beta 1, NRG1 type 1	-	splicing	2199	-	645	neuronal	Holmes	
					-			
HRG-beta 2, NRG1 type 1	-	splicing	2490	-	637	-	Holmes	
					-			
HRG-beta 3, NRG1 type 1	-	splicing	1715	-	241	brain spinal cord	Marchionni, Holmes	
	may be a nuclear isoform							
HRG-NDF43, NRG1 type 1	-	splicing	1793	-	462	-	Wen, Holmes	
					-			
GGF, NRG1 type 2	-	splicing	1199	-	241	nervous system, skeletal muscle	Marchionni, Holmes	
	immunoglobulin-like and EGF-like domain and a kringle-like sequence							

Figure 12