Title: COMPOSITIONS AND METHODS FOR TREATING MYELIN DEFICIENCY DISORDERS

Abstract: The present invention provides compositions, including pharmaceutical compositions, comprising transferrin and insulin-like growth factor-I (IGF-I); as well as containers, devices, and kits comprising the compositions. The compositions are useful for treating myelin deficiency disorders. The present invention further provides methods of treating myelin deficiency disorders, the methods generally involving administering to an individual in need thereof an effective amount of a subject composition.
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COMPOSITIONS AND METHODS FOR TREATING MYELIN DEFICIENCY DISORDERS

CROSS-REFERENCE
[0001] This application claims the benefit of U.S. Provisional Patent Application No. 60/573,166, filed May 21, 2004, which application is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH
[0002] The U.S. government may have certain rights in this invention, pursuant to grant no. HD06576 awarded by the National Institutes of Health NICHD.

FIELD OF THE INVENTION
[0003] The present invention is in the field of demyelinating disorders.

BACKGROUND OF THE INVENTION
[0004] Central nervous system (CNS) development is a multi-step spatial-temporal process in which a pool of uncommitted progenitors neural stem cells (NSCs) develops, giving rise to sequentially more restricted and specified phenotypes of NSC/progenitors for neurons during embryonic life, followed perinatally first by the generation of astrocytes and then a wave of oligodendrocytes (OL) between P1 and P18. Neural stem cells are identified by PSA-NCAM and nestin expression. Nestin, a class IV intermediate filament is expressed at the embryonic neurulation stage in neuroepithelial precursors. Subsequently, as nestin expression decreases, vimentin and GFAP are expressed in the astrocyte lineage, and neurofilament appears in immature neurons.

[0005] Neural precursors are committed to the OL fate when they express early OL markers such as transferrin, A2B5, and sulfatides. Later, these cells express myelin components that are also good OL markers, such as myelin basic protein (MBP) and proteolipid protein (PLP) among many others. The OL myelinating glial cells of the CNS interact with axons forming an insulating compact membrane layer (myelin), to facilitate proper electrochemical signaling of neurons. OL also maintain myelin throughout the life of the organism.

[0006] Myelin loss has devastating effects in patients afflicted by multiple sclerosis or Pelizaeus-Merzbacher disease. The failure of OL to mature and terminally differentiate translates into myelin deficiency (md). In in vitro cell culture, md OL progenitors are able to
express markers that are synthesized by more mature OL, even though the md OL progenitors did not express such markers in vivo.

[0007] Insulin-like growth factor one (IGF-1) is a polypeptide growth hormone homologous to insulin, well known to promote OL progenitor maturation. IGF-1 is widely distributed in the fetal and neonatal CNS, but is restricted in the adult. Similarly, IGF-1 receptor gene expression is heterogeneous within the CNS during early stages of development, but levels decline postnatally.

[0008] Despite the availability of treatments for demyelinating diseases such as MS, there is an ongoing need in the art for treatments for such disorders. The present invention addresses this need.

Literature


SUMMARY OF THE INVENTION

[0010] The present invention provides compositions, including pharmaceutical compositions, comprising transferrin and insulin-like growth factor-I (IGF-I); as well as containers, devices, and kits comprising the compositions. The compositions are useful for treating myelin deficiency disorders. The present invention further provides methods of treating myelin
deficiency disorders, the methods generally involving administering to an individual in need thereof an effective amount of a subject composition.

**Brief Description of the Drawings**

[0011] Figures 1A and 1B depict the number of cells expressing BrdU in the contralateral side vs. the ipsilateral side of wt (Figure 1A) and md (Figure 1B) rat brain treated with Tf + IGF-1.

[0012] Figure 2 provides an amino acid sequence of transferrin.

[0013] Figure 3 depicts cell-specific markers used to characterize the cell specification process of various cell lineages form neural stem cells to mature neurons, oligodendrocytes, and astrocytes.

**Definitions**

[0014] As used herein, the terms "treatment," "treating," and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse affect attributable to the disease. "Treatment," as used herein, covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) increasing survival time; (b) decreasing the risk of death due to the disease; (c) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (d) inhibiting the disease, i.e., arresting its development (e.g., reducing the rate of disease progression); and (e) relieving the disease, i.e., causing regression of the disease. The term "treatment" also includes improving the quality of life. In many embodiments, "treatment" includes recovery (whole or partial) of CNS function.

[0015] The terms "individual," "host," "subject," and "patient," used interchangeably herein, refer to a mammal, including primates, rodents (e.g., mice, rats), livestock, mammalian pets, horses, etc. In some embodiments, an individual is a human.

[0016] The term "dosing event" as used herein refers to administration of a therapeutic agent (e.g., a subject composition) to a patient in need thereof, which event may encompass one or more releases of agent from a drug dispensing device.

[0017] "Continuous delivery" as used herein (e.g., in the context of "continuous delivery of a substance to a tissue") is meant to refer to movement of drug to a delivery site, e.g., into a tissue in a fashion that provides for delivery of a desired amount of substance into the tissue.
over a selected period of time, where about the same quantity of drug is received by the patient each minute during the selected period of time.

[0018] "Controlled release" as used herein (e.g., in the context of "controlled drug release") is meant to encompass release of substance at a selected or otherwise controllable rate, interval, and/or amount, which is not substantially influenced by the environment of use. "Controlled release" thus encompasses, but is not necessarily limited to, substantially continuous delivery, and patterned delivery (e.g., intermittent delivery over a period of time that is interrupted by regular or irregular time intervals).

[0019] The term "controlled drug delivery device" is meant to encompass any device wherein the release (e.g., rate, timing of release) of a drug or other desired substance contained therein is controlled by or determined by the device itself and not substantially influenced by the environment of use, or releasing at a rate that is reproducible within the environment of use.

[0020] By "substantially continuous" as used in, for example, the context of "substantially continuous infusion" or "substantially continuous delivery" is meant to refer to delivery of drug in a manner that is substantially uninterrupted for a pre-selected period of drug delivery, where the quantity of drug received by the patient during any 8 hour interval in the pre-selected period never falls to zero. Furthermore, "substantially continuous" drug delivery can also encompass delivery of drug at a substantially constant, pre-selected rate or range of rates (e.g., amount of drug per unit time, or volume of drug formulation for a unit time) that is substantially uninterrupted for a pre-selected period of drug delivery.

[0021] Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0022] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.
Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a transferrin polypeptide" includes a plurality of such polypeptides and reference to "the IGF-I polypeptide" includes reference to one or more IGF-I polypeptides and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

**Detailed Description of the Invention**

The present invention provides compositions, including pharmaceutical compositions, comprising transferrin and insulin-like growth factor-I (IGF-I); as well as containers, devices, and kits comprising the compositions. The compositions are useful for treating myelin deficiency disorders. The present invention further provides methods of treating myelin deficiency disorders, the methods generally involving administering to an individual in need thereof an effective amount of a subject composition.

**Compositions**

The present invention provides compositions, including pharmaceutical compositions, comprising transferrin and IGF-I. The compositions are in many embodiments pharmaceutical compositions. A subject composition is useful for treating myelin deficiency disorders.

In some embodiments, a subject composition comprises an IGF-I polypeptide; a transferrin polypeptide; and pharmaceutically acceptable excipient(s). Pharmaceutical
compositions generally include therapeutically effective amounts of IGF-1 and transferrin, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The carrier and composition are in many embodiments sterile. The formulation should suit the mode of administration.


[0030] Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions (e.g., NaCl), alcohols, gum arabic, vegetable oils, benzyl alcohols, polyethylene glycols, gelatin, carbohydrates such as lactose, amylose or starch, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid esters, hydroxyethylcellulose, and polyvinyl pyrrolidone. The pharmaceutical preparations can be sterilized and if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic substances and the like which do not deleteriously react with the active compounds. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, polyvinyl pyrrolidone, sodium saccharine, cellulose, magnesium carbonate, etc.

[0031] The composition can be formulated in accordance with the routine procedures as a pharmaceutical composition adapted for intravenous administration to an individual. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be
dispensed with an infusion bottle containing sterile pharmaceutical grade water, saline or dextrose/water. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0032] The active agents can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0033] In some embodiments, the effective amounts of IGF-1 and transferrin in a subject composition are synergistic amounts. As used herein, a “synergistic combination” or a “synergistic amount” of IGF-1 and transferrin is a combined dosage that is more effective in the therapeutic or prophylactic treatment of a demyelinating disorder than the incremental improvement in treatment outcome that could be predicted or expected from a merely additive combination of (i) the therapeutic or prophylactic benefit of IGF-1 when administered at the same dosage as a monotherapy and (ii) the therapeutic or prophylactic benefit transferrin when administered at that same dosage as a monotherapy.

[0034] Typically, the IGF-1 and transferrin polypeptides in a subject composition are purified. “Purified,” when used in reference to a given polypeptide, means that the substance is of 95% or greater (by weight) purity (e.g., 98%, 99%, or greater than 99% purity), e.g., that the polypeptide is substantially free of proteins, lipids, and carbohydrates with which it is naturally associated.

[0035] A subject composition comprises transferrin and IGF-1 in amounts that are effective for recruiting, and/or mobilizing, and/or increasing phenotype specification and/or proliferation of oligodendrocyte progenitors.

[0036] A subject composition comprises effective amounts of transferrin and IGF-1. In some embodiments, “effective amounts” of transferrin and IGF-1 are amounts that in combination are effective to induce or promote recruitment of stem/oligodendrocyte progenitors to a site of myelin deficiency or demyelination in the central nervous system (CNS) of a mammalian subject.

[0037] In some embodiments, “effective amounts” of transferrin and IGF-1 are amounts that in combination are effective to increase the number of myelin-producing cells (e.g., myelin-producing oligodendrocyte) by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%,
at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 2-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, or more, compared to the number of myelin-producing cells in an untreated subject. In particular, in some embodiments, “effective amounts” of transferrin and IGF-1 are amounts that in combination are effective to increase the number of myelin-producing cells at or near the site of myelin deficiency or demyelination in the CNS of the subject.

[0038] In some embodiments, “effective amounts” of transferrin and IGF-1 are amounts that in combination are effective to increase the number of myelin-producing cells (e.g., myelin-producing oligodendrocyte) at or near a site of CNS demyelination by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 2-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, or more, compared to the number of myelin-producing cells at the site of CNS demyelination in an untreated subject.

[0039] In some embodiments, “effective amounts” of transferrin and IGF-1 are amounts that in combination are effective to induce differentiation and proliferation of stem/oligodendrocyte progenitors, such that the number of myelin-producing oligodendrocytes is increased. “Effective amounts” of transferrin and IGF-1 are in some embodiments amounts that induce mobilization of oligodendrocyte progenitors to a site of CNS demyelination, and that induce differentiation of the progenitors to myelin-producing oligodendrocytes.

[0040] In some embodiments, “effective amounts” of transferrin and IGF-1 are amounts that in combination are effective to increase the degree or extent of myelination of a CNS axon by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 2-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, or more, compared to the degree or extent of myelination of a CNS axon in the absence of a combination of transferrin and IGF-1.

[0041] In some embodiments, “effective amounts” of transferrin and IGF-1 are amounts that in combination are effective to decrease the degree or extent of demyelination of a CNS axon by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%, compared the degree or extent of demyelination of a CNS axon in the absence of a combination of transferrin and IGF-1.
The molar ratio of IGF-I to transferrin in a subject composition ranges from about 0.01:1 to about 1:0.01, e.g., the molar ratio of IGF-I to transferrin in a subject composition ranges from about 0.01:1 to about 0.05:1, from about 0.05:1 to about 0.1:1, from about 0.1:1 to about 0.5:1, from about 0.5:1 to about 1:1, from about 1:1 to about 1:0.5, from about 1:0.5 to about 1:0.1, from about 1:0.1 to about 1:0.5, or from about 1:0.5 to about 1:0.01.

The term “unit dosage form,” as used herein, refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of compounds of the present invention calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier or vehicle. The specifications for the unit dosage forms of the present invention depend on the particular compound employed and the effect to be achieved, and the pharmacodynamics associated with each compound in the host.

The amount of IGF-I polypeptide in a given unit dosage form, e.g., the amount of IGF-I polypeptide per ml, per tablet, per capsule, etc., ranges from about 0.1 μg to about 100 mg, e.g., from about 0.1 μg to about 0.5 μg, from about 0.5 μg to about 1.0 μg, from about 1.0 μg to about 5.0 μg, from about 5.0 μg to about 25 μg, from about 25 μg to about 50 μg, from about 50 μg to about 100 μg, from about 100 μg to about 500 μg, from about 500 μg to about 1 mg, from about 1 mg to about 25 mg, from about 25 mg to about 50 mg, or from about 50 mg to about 100 mg.

The amount of transferrin polypeptide in a given unit dosage form, e.g., the amount of transferrin polypeptide per ml, per tablet, per capsule, etc., ranges from about 0.05 mg to about 500 mg, e.g., from about 0.05 mg to about 0.075 mg, from about 0.075 mg to about 0.1 mg, from about 0.1 mg to about 0.2 mg, from about 0.2 mg to about 0.3 mg, from about 0.3 mg to about 0.4 mg, from about 0.4 mg to about 0.5 mg, from about 0.5 mg to about 1.0 mg, from about 1.0 mg to about 5 mg, from about 5 mg to about 25 mg, from about 25 mg to about 50 mg, from about 50 mg to about 100 mg, from about 100 mg to about 250 mg, or from about 250 mg to about 500 mg.

A subject composition comprises, as active agents, IGF-I and transferrin. The active agents (e.g., IGF-I and transferrin) are in some embodiments formulated into preparations for injection by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.
For oral preparations, the active agents (e.g., IGF-I polypeptide; transferrin; etc.) are formulated alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose; with lubricants, such as talc or magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives, and flavoring agents.

Furthermore, the active agents can be made into suppositories by mixing with a variety of bases such as emulsifying bases or water-soluble bases. The suppository can include vehicles such as cocoa butter, carbowaxes and polyethylene glycols, which melt at body temperature, yet are solidified at room temperature.

Unit dosage forms for oral or rectal administration such as syrups, elixirs, and suspensions may be provided wherein each dosage unit, for example, teaspoonful, tablespoonful, tablet or suppository, contains a predetermined amount of the composition containing one or more active agents. Similarly, unit dosage forms for injection or intravenous administration may comprise the active agents in a composition as a solution in sterile water, normal saline or another pharmaceutically acceptable carrier.

IGF-I

IGF-I polypeptides that are suitable for use in a subject composition include any IGF-I polypeptide, or variant, fragment, derivative, or analog thereof that retains at least about 25% of the biological activity of a naturally-occurring IGF-I, e.g., that binds to an IGF-I receptor and functions as an agonist of the IGF-I receptor.

Amino acid sequences of IGF-I polypeptides from various species are known. For example, amino acid sequences of human IGF-I are found under GenBank Accession No. P05019; P01343; AAA52789; CAA40342; AAA96152; and IGHU1. Typically, the mature form, or a fragment, derivative, variant, or analog thereof, is used. The mature form is typically amino acids 49-118 of the precursor protein.

As one non-limiting example, a suitable IGF-I has the following amino acid sequence: NH₂PHELCAELV DALQFVCGDR GFYFNKPTGY GSŠŠRRAQP TGISVECCFR SCDLRLLEMY CAPLKPAKSA -COOH (SEQ ID NO:1). Also suitable for use are any of the IGF-I polypeptides disclosed under GenBank Accession Nos. CAA00835, CAA00281, and CAA01643.

The term “IGF-I” also includes the variants described in U.S. Patent Nos. 6,509,443; 6,506,874; and 6,403,764. For example, an IGF-I variant can include an IGF-I polypeptide (a)
in which an amino acid residue located at a single position selected from the group consisting of positions 4, 5, 7, 10, 14, 17, 23, 24, and 43 of a native-sequence human IGF-I (e.g., SEQ ID NO:1) is replaced with an alanine residue; or (b) in which both amino acid residues at positions 1 and 70 of a native-sequence human IGF-I (e.g., SEQ ID NO:1) are replaced with a serine residue and a valine residue, respectively; or (c) in which both amino acid residues at positions 1 and 70 of a native-sequence human IGF-I (e.g., SEQ ID NO:1) are replaced with a serine residue and a valine residue, respectively, and an amino acid residue located at a single position selected from the group consisting of positions 3, 4, 5, 7, 10, 14, 17, 23, 24, 25, and 43 of a native-sequence human IGF-I (e.g., SEQ ID NO:1) is replaced with an alanine residue.

The term "IGF-I" includes modifications of any known IGF-I, e.g., where suitable modifications are readily identified by those skilled in the art, e.g., by identifying: (1) conservation of amino acid sequence among species; (2) presence of "conservative" amino acid substitutions among species (i.e., amino acids with similar shape, charge or other salient characteristics); (3) receptor shielding of tyrosine residues from radiiodination (Maly and Luthi, J. Biol. Chem. 263:7068-7072 (1988)); (4) predominance of hydrophilic residues, suggesting the location of a receptor-binding domain on the surface of the polypeptide, a presumptive requirement for receptor interaction; and (5) consideration of hydrophobic and polar regions of three-dimensional models (e.g., Blundell et al., Fed. Proc. 42:2592-2597 (1983)) and identifying therefrom regions which are possible binding sites, e.g., IGF-I receptor binding sites.

The term "IGF-I" also includes fragments of IGF-I, especially fragments retaining at least about 25% of the biological activity of a naturally-occurring IGF-I polypeptide. Fragments include fragments of from about 25 contiguous amino acids to about 65 contiguous amino acids of a mature IGF-I polypeptide, e.g., fragments of from about 25 to about 30, from about 30 to about 40, from about 40 to about 50, or from about 50 to about 65 contiguous amino acids of a mature IGF-I polypeptide. Exemplary, non-limiting IGF-I fragments include: IGF-I (55-70) Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala Lys Ser Ala (SEQ ID NO:2); an IGF-I polypeptide comprising the sequence GPETL CGAEL VDALQ FVC GD RG FYF NKPTG YGSSS (SEQ ID NO:3); IGF-I (21-40) drgfyfnkpt g ygssrrap qlspkklfg cipr sasrv (SEQ ID NO:4); and the like.

Suitable IGF-I polypeptides include cyclic peptides, which are cyclized fragments of IGF-I. Typically, a cyclized IGF-I fragment is from about 5 amino acids to about 40 amino acid residues, e.g., from about 5 amino acids to about 25 amino acids in length, or from about 25 amino acids to about 40 amino acids in length. Such peptides are generally modeled after
the looped domains of the IGF molecules. Such loops may be a consequence of natural
disulfide bond formation, while others are a consequence of the folding of the protein as it
achieves a minimal energy conformation or a receptor-induced conformation to permit binding.
Cyclization can be effected by joining the amino and carboxyl ends of a linear peptide, either
directly to form an amide (lactam) bond, or by disulfide bond formation employing terminal
cysteine groups. Any internal cysteine groups present are typically selectively blocked before
cyclization and may be unblocked afterward using well-established procedures. Alternatively,
internal cysteines may be replaced by an amino acid which would be expected to have a
minimal influence on peptide conformation, e.g. alanine, which is frequently used in scanning
mutagenesis studies. Suitable cyclized IGF-I polypeptides, and methods of making same, are
described in U.S. Patent No. 6,723,699.

[0057] The sequence of any known IGF-I polypeptide may be altered in various ways known
in the art to generate targeted changes in sequence. A variant polypeptide will usually be
substantially similar to the sequences provided herein, i.e. will differ by at least one amino
acid, and may differ by at least two but not more than about ten amino acids. The sequence
changes may be substitutions, insertions or deletions. Conservative amino acid substitutions
typically include substitutions within the following groups: (glycine, alanine); (valine,
iso-leucine, leucine); (aspartic acid, glutamic acid); (asparagine, glutamine); (serine, threonine);
(lysine, arginine); or (phenylalanine, tyrosine).

[0058] The IGF-I formulation may comprise an N-blocked species, wherein the N-terminal
amino acid is acylated with an acyl group, such as a formyl group, an acetyl group, a malonyl
group, and the like. Also suitable for use is a consensus IGF-I.

[0059] IGF-I polypeptides can be produced by any known method. DNA sequences encoding
IGF-I may be synthesized using standard methods. In many embodiments, IGF-I polypeptides
are the products of expression of manufactured DNA sequences transformed or transfected into
bacterial hosts, e.g., E. coli, or in eukaryotic host cells (e.g., yeast; mammalian cells, such as
CHO cells; and the like). In these embodiments, the IGF-I is “recombinant IGF-I.” Where the
host cell used to produce the IGF-I is a bacterial host cell, the IGF-I is modified to comprise an
N-terminal methionine.

[0060] It is to be understood that an IGF-I polypeptide suitable for use herein may comprise
one or more modified amino acid residues, e.g., glycosylations, chemical modifications, and
the like.
Transferrin

[0061] Transferrin is fully discussed in Welch, S., Transferrin: The Iron Carrier, CRC Press, Boca Raton (1992) and the references cited therein, which are hereby incorporated by reference. Transferrin is a glycoprotein which is needed for the transportation of iron in the blood of more complex organisms. In humans, transferrin is a plasma glycoprotein with a molecular weight of approximately 80,000 daltons and is able to bind reversibly to two atoms of ferric iron in the presence of bicarbonate. Related proteins have been found in all mammals, birds, reptiles, amphibians, bony fish, and cartilaginous fish.

[0062] The tertiary structure of transferrin has been elucidated. Bailey, S., et al., "Molecular Structure of Serum Transferrin at 3.3 Angstrom Resolution," Biochemistry, 27:5804 (1988), which is hereby incorporated by reference. The polypeptide chain is folded into two globular lobes joined by a short intervening region. The N-lobe, comprising the N-terminal half of the protein, contains the first 330 amino acid residues. The C-lobe, comprising the C-terminal half of the protein, contains the last 330 amino acids. Each lobe is an ellipsoid and contains one iron binding site. The two lobes are connected by a short three turn helix of 15-20 amino acids.

[0063] Human transferrin is a glycoprotein with two N-linked oligosaccharide chains attached to asparagine residues 413 and 611 in both the C-lobe. The structure of these sugar chains can vary greatly.

[0064] The amino acid sequences of human transferrin polypeptides are known. See, e.g., MacGillivray et al. Proc Natl Acad Sci U S A. 1982 April; 79 (8): 2504–2508. Human transferrin amino acid sequences are also disclosed under GenBank Accession Nos. AAH59367; AAB22049; P02787; and AAP45055. One non-limiting example of a suitable transferrin is a polypeptide comprising the amino acid sequence depicted in Figure 2 and set forth in SEQ ID NO:5.

[0065] The sequence of any known transferrin polypeptide may be altered in various ways known in the art to generate targeted changes in sequence. A variant polypeptide will usually be substantially similar to the sequences provided herein, i.e. will differ by at least one amino acid, and may differ by at least two but not more than about ten amino acids. The sequence changes may be substitutions, insertions or deletions. Conservative amino acid substitutions typically include substitutions within the following groups: (glycine, alanine); (valine, isoleucine, leucine); (aspartic acid, glutamic acid); (asparagine, glutamine); (serine, threonine); (lysine, arginine); or (phenylalanine, tyrosine).

[0066] The term “transferrin polypeptide” also includes active fragments of transferrin. Active fragments include fragments containing from about 50 to about 100, from about 100 to about
200, from about 200 to about 300, from about 300 to about 400, from about 400 to about 500, or from about 500 to about 600 contiguous amino acids of any naturally-occurring or synthetic transferrin polypeptide.

**Modifications**

[0067] Modifications of interest that may or may not alter the primary amino acid sequence include chemical derivatization of polypeptides, e.g., acetylation, carboxylation, glycosylation, PEGylation, etc.; changes in amino acid sequence that introduce or remove a glycosylation site; changes in amino acid sequence that make the protein susceptible to PEGylation; and the like. Also included are modifications of glycosylation, e.g., those made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing or in further processing steps; e.g., by exposing the polypeptide to enzymes that affect glycosylation, such as mammalian glycosylating or deglycosylating enzymes. Also embraced are sequences that have phosphorylated amino acid residues, e.g., phosphotyrosine, phosphoserine, or phosphothreonine. Also suitable are depsipeptide analogs; and retro-inverse peptide analogs of an IGF-I and/or a transferrin polypeptide.

[0068] In some embodiments, an IGF-I and/or a transferrin polypeptide is a fusion protein comprising an IGF-I polypeptide or a transferrin polypeptide and a heterologous polypeptide (e.g., a fusion partner). Suitable fusion partners include peptides and polypeptides that confer enhanced stability in vivo (e.g., enhanced serum half-life); provide ease of purification, e.g., (His)$_n$, e.g., 6His, and the like; provide for secretion of the fusion protein from a cell; provide an epitope tag, e.g., glutathione-S-transferase, hemagglutinin (HA; e.g., CYPYDVPDYA; SEQ ID NO:6), FLAG (e.g., DYKDDDDK; SEQ ID NO:7), c-myc (e.g., CEQKLISEEDL; SEQ ID NO:8), and the like; provide a detectable signal, e.g., an enzyme that generates a detectable product (e.g., β-galactosidase, luciferase), or a protein that is itself detectable, e.g., a green fluorescent protein, etc.; provides for multimerization, e.g., a multimerization domain such as an Fc portion of an immunoglobulin; and the like.

[0069] As a non-limiting example, a polypeptide (e.g., an IGF-1 polypeptide, a transferrin polypeptide) is modified with one or more polyethylene glycol moieties, i.e., PEGylated. The PEG molecule is conjugated to one or more amino acid side chains of the polypeptide. In some embodiments, a polypeptide (e.g., an IGF-1 polypeptide, a transferrin polypeptide) contains a PEG moiety on only one amino acid. In other embodiments, a polypeptide (e.g., an IGF-1 polypeptide, a transferrin polypeptide) contains a PEG moiety on two or more amino acids, e.g., the polypeptide (e.g., an IGF-1 polypeptide, a transferrin polypeptide) contains a PEG moiety attached to two, three, four, five, six, seven, eight, nine, or ten different amino
acid residues. A polypeptide (e.g., an IGF-1 polypeptide, a transferrin polypeptide) may be coupled directly to PEG (i.e., without a linking group) through an amino group, a sulfhydryl group, a hydroxyl group, or a carboxyl group. Methods of PEGylating proteins are well known in the art. See, for example, Zaplisky and Lee, Polyethylene Glycol Chemistry: Biotechnical and Biomedical Applications, J. M. Harris, ed., Plenum Press, NY, Chapter 21 (1992).

Polypeptides administered in methods of the invention may be chemically modified in such a way as to increase the transport of the polypeptide across the blood-brain barrier, e.g., by modifications of the polypeptide that increase lipophilicity, alter glycosylation, or increase net positive charge. For example, in some embodiments, the transferrin and/or the IGF-I of a subject composition is conjugated with L-glutamic acid. In other embodiments, the transferrin and/or the IGF-I of a subject composition is conjugated to an oligomer, as described in U.S. Patent No. 6,703,381. In other embodiments, the transferrin and IGF-I are adsorbed onto polysorbate-80-coated nanoparticles (e.g., polybutylcyanoacrylate nanoparticles).

Since the ability of peptides to penetrate the blood-brain barrier is related to their lipophilicity or their net ionic charge, suitable modifications of these peptides (e.g., by substituting pentafluorophenylalanine for phenylalanine, or by conjugation to cationized albumin) to increase their transportability (Kastin et al, Pharmac Biochem. Behav. 11:713-716 (1979); Rapoport et al., Science 207:84-86 (1980); Pardridge et al., Biochem. Biophys. Res. Commun. 146:307-313 (1987); Riekkinen et al., Peptides 8:261-265 (1987)) may be important for their bioavailability following administration outside the blood-brain barrier, and these modifications are within the scope of the invention. In addition, since bioavailability of peptides may be limited by their susceptibility to degradation by proteases and peptidases (Littlewood, et al., Neurochem Int. 12:383-389 (1988)), modifications of these peptides (e.g., replacement of L-amino acids with D-amino acids) to increase their metabolic stability (Coy et al., 1976) may also be important for their therapeutic efficacy, and these modified peptides are also within the scope of the invention.

A retro-isomer of a peptide is defined by a reversal of the direction of the peptide bond while maintaining the side-chain topochemistry. In retro-inverso peptides, D-amino acids are substituted for L-amino acids to retain the overall conformation for biological response and receptor binding similar to the native peptides (Hayward et al., Peptides 1974: Proc. 13th Eur. Peptide Symp., ed. Y. Wolman, pp. 287-297; Goodman et al., Acc. Chem.Res. 12:1-7 (1979)). It has been shown that the retro-inverso peptides introduced well defined conformational constraints and showed limited biodegradation by endopeptidases.
CONTAINERS, DEVICES, AND KITS

[0073] The present invention provides a container comprising a subject composition; and devices comprising the container(s). The invention further provides a kit comprising a formulation comprising a unit dosage form of a subject composition in a container, and a label that provides instructions for use of the kit.

[0074] A subject device (a drug delivery device) for delivering a subject composition to an individual in need thereof comprises a container that holds a subject composition (usually a fluid composition). In some embodiments, a subject device further comprises a catheter in fluid communication with the container (e.g., a reservoir), where the reservoir has an inlet and an outlet, and where the catheter is coupled to the outlet. In some embodiments, the device is implantable. In some embodiments, the catheter has a proximal end coupled to and in fluid communication with the outlet the reservoir; and a distal end from which the composition enters the body of the individual being treated. In many embodiments, the implantation site is distant from the delivery site. For example, the implantation site may be subcutaneous or intravenous; and the delivery site may be in the brain.

[0075] The device will in some embodiments include a fluid flow control means (e.g., a valve, a pump) for controlling the flow of a subject composition from the reservoir to the catheter, where the fluid control means may be situated at the outlet of the reservoir. In some embodiments, the fluid flow control means comprises a pump, e.g., a programmable flow rate pump, operably connected to the reservoir, which pump controls the rate of flow of the subject fluid composition from the reservoir through the catheter. The pump allows the operator of the device to change fluid delivery parameters, such as flow rate, infusion period, ramp time, and bolus volume. Any of a variety of pumps known in the art can be used, including, e.g., an electromechanical pump, an electroosmotic pump, a hydrolytic pump, a piezoelectric pump, an elastomeric pump, a vapor pressure pump, and an electrolytic pump. 

[0076] Suitable containers include those adapted for administration by subcutaneous injection, including a syringe (for use with a needle), an injector pen, and the like. In some embodiments, a subject agonist is administered with a pen injector (e.g., a medication delivery pen), a number of which are known in the art. Exemplary devices which can be adapted for use in the present methods are any of a variety of pen injectors from Becton Dickinson, e.g., BD™ Pen, BD™ Pen II, BD™ Auto-Injector; a pen injector from Innoject, Inc.; any of the medication delivery pen devices discussed in U.S. Patent Nos. 5,728,074, 6,096,010, 6,146,361, 6,248,095, 6,277,099, and 6,221,053; and the like. The medication delivery pen
can be disposable, or reusable and refillable. Also suitable for use is an Intraject® needle-free injection system (Aradigm Corp.).

[0077] Suitable containers also include those suitable for use with an implantable device. For example, a container is in some embodiments a reservoir for use with an implantable device. Also suitable for use are containers suitable for use with an injection device, e.g., a needle and syringe, e.g., suitable for stereotaxic intraspinal or intrathecal injection.

[0078] In some embodiments, the present invention provides a device for delivery of a subject composition, where the device includes a container that includes a subject composition, operably connected to a catheter. In some embodiments, the container is a reservoir, e.g., an Ommaya reservoir.

[0079] The present invention provides kits for use in carrying out a subject method. A subject kit generally includes a device for administering an active agent(s) to an individual in need thereof, where the device includes a container comprising a unit dosage form of a subject composition. In some embodiments, the device will further include an additional container that comprises a second therapeutic agent, e.g., a steroid (e.g., dexamethasone, methylprednisolone, etc.); an interferon (IFN-β1a, IFN-β1b); etc. In many embodiments, a subject kit will further include instructions for practicing the subject methods or means for obtaining the same (e.g., a website URL directing the user to a webpage which provides the instructions), where these instructions are typically printed on a substrate, which substrate may be one or more of: a package insert, the packaging, reagent containers and the like.

TREATMENT METHODS

[0080] The present invention provides methods of treating myelin deficiency disorders. The methods generally involve administering to an individual in need thereof an effective amount of a subject composition. The present invention features methods for recruiting/mobilizing and/or increasing proliferation of oligodendrocyte progenitors, as well as methods for recruiting/mobilizing and/or increasing proliferation of the progeny of such cells, by administration of a composition comprising effective amounts of transferrin and IGF-1. The mobilized oligodendrocyte progenitors become committed to the oligodendrocyte lineage, mature into myelin-producing oligodendrocytes, and increase the degree or level of myelination of axons.

[0081] Myelin deficiency disorders and demyelinating disorders that can be treated with a subject method include Multiple Sclerosis (MS), optic neuritis, transverse neuritis and Guillain-Barre Syndrome (GBS), Pelizaeus-Merzbacher disease, leukodystrophies, extrapontine demyelination, intrapontine demyelination (also known as osmotic demyelination
syndrome), and the like. Demyelination resulting from head injury or spinal cord trauma can also be treated with a subject method.

[0082] The subject methods generally involve administering to an individual in need thereof an effective amount of a composition comprising transferrin and IGF-I. In some embodiments, an "effective amount" of a subject composition comprising transferrin and IGF-I is an amount that is effective to ameliorate a symptom of a demyelinating disorder by at least about 10%, at least about 20%, a at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%, or more, compared to the degree or level of the symptom(s) in an untreated subject.

[0083] Symptoms of demyelinating disorders are known in the art. For example, symptoms of MS include difficulty walking; numbness ("pins and needles") in the extremities; pain on moving the eyes; dim or blurred vision, double vision, or loss of central vision; tremors (shakiness); clumsiness; slurred speech; poor memory; reduced cognitive abilities; painful muscle spasms; etc.

[0084] The subject methods generally involve administering to an individual in need thereof an effective amount of a composition comprising transferrin and IGF-I. In some embodiments, an "effective amount" of a subject composition comprising transferrin and IGF-I is an amount that is effective to increase locomotor function by at least about 10%, at least about 20%, a at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%, or more, compared to an untreated subject.

[0085] In some embodiments, an "effective amount" of a subject composition is an amount that is effective to reduce the extent or degree of demyelination by at least about 10%, at least about 20%, a at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%, or more, compared to the extent or degree of demyelination an untreated subject.

[0086] A subject composition can be administered twice daily, daily, every other day, once a week, twice a week, three times a week, every other week, three times per month, or once monthly, or substantially continuously or continuously. In some embodiments, a subject composition is administered in a single dose directly into the brain parenchyma.

[0087] A subject composition is administered for a period of about 1 day to about 7 days, or about 1 week to about 2 weeks, or about 2 weeks to about 3 weeks, or about 3 weeks to about 4 weeks, or about 1 month to about 2 months, or about 3 months to about 4 months, or about 4 months to about 6 months, or about 6 months to about 8 months, or about 8 months to about 12 months, or at least one year, and may be administered over longer periods of time.
A subject composition can be administered according to the appearance of symptoms, e.g., a subject composition is in some embodiments administered within a period of time of from about 5 minutes to about 24 hours following the appearance of symptoms of a demyelinating disorder, e.g., a subject composition is administered within from about 5 minutes to about 15 minutes, from about 15 minutes to about 30 minutes, from about 30 minutes to about 1 hour, from about 1 hour to about 4 hours, from about 4 hours to about 8 hours, from about 8 hours to about 12 hours, from about 12 hours to about 18 hours, or from about 18 hours to about 24 hours, of the appearance of a symptom of, or triggering of, a demyelinating disorder.

Combination therapies

In some embodiments, a subject method for treating a demyelinating disorder involves administering a subject composition, and at least a second therapeutic agent that treats a demyelinating disorder. Suitable second therapeutic agents include, but are not limited to, IFN-β1a (e.g., Avonex® IFN-β1a; Rebif® IFN-β1a; etc.); IFN-β1b (e.g., Betaseron® IFN-β1b); random copolymer of Ala, Lys, Glu, and Tyr (e.g., Copaxone® glatiramer acetate); a corticosteroid (e.g., methylprednisolone (e.g., Medrol®, Solu-Medrol®, etc.); prednisone (Deltacon®); prednisolone (Delta-Cortel®); dexamethasone (Decadron®); etc.); antineoplastic agents (e.g., methotrexate; Novatron® mitoxantrone; etc.); purines, e.g., guanosine and guanosine analogs and derivatives; and the like.

In some embodiments, a subject method includes administration of a symptom management agent. Symptom management agents that are suitable for use in a subject treatment method include agents that are effective for increasing or maintaining bladder control (e.g., DDAVP® desmopressin; Detrol® tolterodine tartrate; Debenzylin® phenoxybenzamine; Ditropan XL® oxybutynin chloride; Hytrin® terazosin hydrochloride; Pro-Banthine® propantheline; Tegretol® carbamazepine; etc.); agents that are effective for treating depression, such as tricyclic anti-depressants, selective serotonin re-uptake inhibitors, etc. (e.g., Aventyl® nortriptyline; Elavil® amitriptyline; Prozac® fluoxetine; Tofranil® imipramine; etc.); agents that are effective for treating fatigue (e.g., Symmetrel® amantadine; Cylert® pemoline; Provigil® modafinil; and the like); agents effective for pain reduction (e.g., Dilantin® phenytoin; Naprosyn; Neurontin® gabapentin; Tegretol® carbamazepine; etc.); agents that treat spasticity (e.g., Clonidine® catapres-TTS; Dantrium® dantrolene; Lioresal® baclofen; Valium® diazepam; Zanaflex® tizanidine; etc.); agents that reduce tremors (e.g., Klonopin® clonazepam; etc.); agents that treat vertigo and dizziness (e.g., Antivert® meclizine; Dimenhydrinate; Transderm Scop® scopolamine; etc.); and the like.
In some embodiments, a subject method includes administration of a side-effect management agent.

A subject composition and the second agent can be administered substantially simultaneously, or within about 30 minutes, about 1 hour, about 2 hours, about 4 hours, about 8 hours, about 16 hours, about 24 hours, about 36 hours, about 72 hours, about 4 days, about 7 days, or about 2 weeks of one another.

In some embodiments, the additional agent(s) is administered during the entire course of treatment with a subject composition, and the beginning and end of the treatment periods coincide. In other embodiments, the additional agent(s) is administered for a period of time that is overlapping with that of treatment with a subject composition, e.g., treatment with the additional therapeutic agent(s) begins before the treatment with a subject composition begins and ends before the treatment with a subject composition ends; treatment with the additional therapeutic agent(s) begins after the treatment with a subject composition begins and ends after the treatment with a subject composition ends; treatment with the additional therapeutic agent(s) begins after the treatment with a subject composition begins and ends before the treatment with a subject composition ends; or treatment with the additional therapeutic agent(s) begins before the treatment with a subject composition begins and ends after the treatment with a subject composition ends.

Dosages of second therapeutic agents for treating a demyelinating disorder such as MS are well known in the art. For example, Avonex® is typically administered by intramuscular injection once per week at a dose of 30 µg per injection; Betaseron® is typically administered by subcutaneous injection every other day at a dose of 250 µg per injection; Copaxone® is typically administered by subcutaneous injection 3 times per week at a dose of 44 µg per injection; Novantrone® is typically administered once every three months by intravenous infusion at a dose of 12 µg/m². Dosages for other therapeutic agents are well known in the art.

Determining efficacy of treatment

Whether a subject treatment regimen is effective to treat a demyelinating disorder can be readily determined by standard methods that are well known in the art. For example, efficacy in treating MS is determined, e.g., by magnetic resonance imaging (e.g., to detect gaps in the myelin sheath on the spinal cord or brain; to evaluate the degree or extent of demyelination); evaluation of locomotor movement (e.g., gait, etc.); lumbar puncture (e.g., to obtain a sample of cerebrospinal fluid (CSF) for evaluating the number of autoreactive T lymphocytes in the CSF, for evaluating the presence of autoantibodies in the CSF, etc); evoked potential testing (e.g., to measure electrical signals in the brain in response to stimulation,
which allows an evaluation of the degree or extent of demyelination; and neuropsychological tests (e.g., to evaluate cognitive abilities, etc.).

**Dosages, Formulations, and Routes of Administration**

[0006] An active agent (e.g., IGF-I polypeptide; transferrin; etc.) also referred to herein as a “drug” or a “therapeutic agent” or an “active agent”) is administered to individuals in a formulation with a pharmaceutically acceptable excipient(s). A wide variety of pharmaceutically acceptable excipients are known in the art and need not be discussed in detail herein. Pharmaceutically acceptable excipients have been amply described in a variety of publications, including, for example, A. Gennaro (2000) “Remington: The Science and Practice of Pharmacy,” 20th edition, Lippincott, Williams, & Wilkins; Pharmaceutical Dosage Forms and Drug Delivery Systems (1999) H.C. Ansel et al., eds., 7th ed., Lippincott, Williams, & Wilkins; and Handbook of Pharmaceutical Excipients (2000) A.H. Kibbe et al., eds., 3rd ed. Amer. Pharmaceutical Assoc.

[0007] The pharmaceutically acceptable excipients, such as vehicles, adjuvants, carriers or diluents, are readily available to the public. Moreover, pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are readily available to the public.

[0008] In the subject methods, an active agent (e.g., IGF-I polypeptide; transferrin; etc.) may be administered to the host using any convenient means capable of resulting in the desired therapeutic effect. Thus, the agents can be incorporated into a variety of formulations for therapeutic administration. More particularly, an active agent (e.g., IGF-I polypeptide; transferrin; etc.) can be formulated into pharmaceutical compositions by combination with appropriate, pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants and aerosols.

[0009] As such, administration of an active agent(s) can be achieved in various ways, including oral, buccal, rectal, parenteral, intraperitoneal, intradermal, intrathecal, intraspinal, intracisternal, intracapsular, subcutaneous, intravenous, intramuscular, transdermal, intratracheal, etc., administration. In some embodiments, e.g., where two different agents are administered, two different routes of administration are used. Where the active agent is to be provided parenterally, such as by intravenous, subcutaneous, ophthalmic, intraperitoneal, intramuscular, buccal, rectal, vaginal, intraorbital, intracerebral, intracranial, intraspinal, intraventricular, intrathecal, intracisternal, intracapsular, intranasal or by aerosol
administration, the agent typically comprises part of an aqueous or physiologically compatible fluid suspension or solution.

[00100] A liquid is in some embodiments the dosage form that is used for intravenous, intrathecal, intraspinal, intraventricular, or intramedullary administration of an active agent for use in a subject method. For preparing liquids, solvents can be used, as exemplified by purified water, physiological saline, alcohols such as ethanol, propylene glycol, glycerin and polyethylene glycol, and triacetin. The thus prepared liquids may be used as dilutions with a lactated Ringer's solution, a maintaining solution, a postoperative recovery fluid, a solution for supplying water to compensate for dehydration, physiological saline for use in dripping. The preparations may further be admixed with adjuvants such as antiseptics, moistening agents, emulsifiers, dispersing agents and stabilizers. Suspensions are another exemplary dosage form to be administered.

[00101] In some embodiments, active agent (e.g., IGF-I polypeptide; transferrin; etc.) is administered intrathecally, including, e.g., administration into a cerebral ventricle, administration into the lumbar area, and administration into the cisterna magna; or by an intraspinal route. For specific delivery within the CNS intrathecal delivery can be used with, for example, an Ommaya reservoir. U.S. Pat. No. 5,455,044 provides for use of a dispersion system for CNS delivery or see U.S. Pat. No. 5,558,852 for a discussion of CNS delivery.

[00102] As used herein, the term "intrathecal administration" includes delivering an active agent directly into the cerebrospinal fluid of a subject, by techniques including lateral cerebroventricular injection through a burrhole or cisternal or lumbar puncture or the like (e.g., as described in Lazorthes et al. Advances in Drug Delivery Systems and Applications in Neurosurgery, 143-192 and Omaya et al., Cancer Drug Delivery, 1: 169-179, the contents of which are incorporated herein by reference). The term "lumbar region" includes the area between the third and fourth lumbar (lower back) vertebrae. The term "cisterna magna" includes the area where the skull ends and the spinal cord begins at the back of the head. The term "cerebral ventricle" includes the cavities in the brain that are continuous with the central canal of the spinal cord: Administration of an active agent to any of the above mentioned sites can be achieved by direct injection of the active agent or by the use of infusion pumps. For injection, the active agent can be formulated in liquid solutions, e.g., in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the active agent may be formulated in solid form and re-dissolved or suspended immediately prior to use. Lyophilized forms are also included. The injection can be, for example, in the form of a bolus injection or continuous infusion (e.g., using infusion pumps) of the active agent.
Subcutaneous administration of active agent (e.g., IGF-I polypeptide; transferrin; etc.) can be accomplished using standard methods and devices, e.g., needle and syringe, a subcutaneous injection port delivery system, and the like. See, e.g., U.S. Patent Nos. 3,547,119; 4,755,173; 4,531,937; 4,311,137; and 6,017,328. A combination of a subcutaneous injection port and a device for administration of an active agent to a patient through the port is referred to herein as “a subcutaneous injection port delivery system.” In some embodiments, subcutaneous administration is achieved by a combination of devices, e.g., bolus delivery by needle and syringe, followed by delivery using a continuous delivery system.

In some embodiments, active agent (e.g., IGF-I polypeptide; transferrin; etc.) is delivered by a continuous delivery system. The terms “continuous delivery system,” “controlled delivery system,” and “controlled drug delivery device,” are used interchangeably to refer to controlled drug delivery devices, and encompass pumps in combination with catheters, injection devices, and the like, a wide variety of which are known in the art.

Mechanical or electromechanical infusion pumps can also be suitable for use with the present invention. Examples of such devices include those described in, for example, U.S. Pat. Nos. 4,692,147; 4,360,019; 4,487,603; 4,360,019; 4,725,852; 5,820,589; 5,643,207; 6,198,966; and the like. In general, the present methods of drug delivery can be accomplished using any of a variety of refillable, pump systems. Pumps provide consistent, controlled release over time. Typically, the agent is in a liquid formulation in a drug-impermeable reservoir, and is delivered in a continuous fashion to the individual.

In one embodiment, the drug delivery system is an at least partially implantable device. The implantable device can be implanted at any suitable implantation site using methods and devices well known in the art. An implantation site is a site within the body of a subject at which a drug delivery device is introduced and positioned. Implantation sites include, but are not necessarily limited to a subdermal, subcutaneous, intramuscular, or other suitable site within a subject’s body. Subcutaneous implantation sites are generally preferred because of convenience in implantation and removal of the drug delivery device.

Drug release devices suitable for use in the invention may be based on any of a variety of modes of operation. For example, the drug release device can be based upon a diffusive system, a convective system, or an erodible system (e.g., an erosion-based system). For example, the drug release device can be an electrochemical pump, osmotic pump, an electroosmotic pump, a vapor pressure pump, or osmotic bursting matrix, e.g., where the drug is incorporated into a polymer and the polymer provides for release of drug formulation concomitant with degradation of a drug-impregnated polymeric material (e.g., a biodegradable,
drug-impregnated polymeric material). In other embodiments, the drug release device is based upon an electrodiffusion system, an electrolytic pump, an effervescent pump, a piezoelectric pump, a hydrolytic system, etc.

[00108] Drug release devices based upon a mechanical or electromechanical infusion pump can also be suitable for use with the present invention. Examples of such devices include those described in, for example, U.S. Pat. Nos. 4,692,147; 4,360,019; 4,487,603; 4,360,019; 4,725,852, and the like. In general, the present methods of drug delivery can be accomplished using any of a variety of refillable, non-exchangeable pump systems. Pumps and other convective systems are generally preferred due to their generally more consistent, controlled release over time. Osmotic pumps are particularly preferred due to their combined advantages of more consistent controlled release and relatively small size (see, e.g., PCT published application no. WO 97/27840 and U.S. Pat. Nos. 5,985,305 and 5,728,396). Exemplary osmotically-driven devices suitable for use in the invention include, but are not necessarily limited to, those described in U.S. Pat. Nos. 3,760,984; 3,845,770; 3,916,899; 3,923,426; 3,987,790; 3,995,631; 3,916,899; 4,016,880; 4,036,228; 4,111,203; 4,203,440; 4,203,442; 4,210,139; 4,327,725; 4,627,850; 4,865,845; 5,057,318; 5,059,423; 5,112,614; 5,137,727; 5,234,692; 5,234,693; 5,728,396; and the like.

[00109] In some embodiments, the drug delivery device is an implantable device. The drug delivery device can be implanted at any suitable implantation site using methods and devices well known in the art. As noted infra, an implantation site is a site within the body of a subject at which a drug delivery device is introduced and positioned. Implantation sites include, but are not necessarily limited to a subdermal, subcutaneous, intramuscular, intraspinal, or other suitable site within a subject’s body.

[00110] In some embodiments, a therapeutic agent is delivered using an implantable drug delivery system, e.g., a system that is programmable to provide for administration of a therapeutic agent. Exemplary programmable, implantable systems include implantable infusion pumps. Exemplary implantable infusion pumps, or devices useful in connection with such pumps, are described in, for example, U.S. Pat. Nos. 4,350,155; 5,443,450; 5,814,019; 5,976,109; 6,017,328; 6,171,276; 6,241,704; 6,464,687; 6,475,180; and 6,512,954. A further exemplary device that can be adapted for the present invention is the SynchroMed® infusion pump (Medronic).

[00111] In pharmaceutical dosage forms, active agent (e.g., IGF-I polypeptide; transferrin; etc.) may be administered in the form of their pharmacologically acceptable salts, or they may also be used alone or in appropriate association, as well as in combination, with other
pharmacologically active compounds. The following methods and excipients are merely exemplary and are in no way limiting.

[00112] An active agent (e.g., IGF-I polypeptide; transferrin; etc.) can be formulated into preparations for injection by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

[00113] For oral preparations, an active agent (e.g., IGF-I polypeptide; transferrin; etc.) is formulated alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose; with lubricants, such as talc or magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives, and flavoring agents.

[00114] Furthermore, an active agent can be made into suppositories by mixing with a variety of bases such as emulsifying bases or water-soluble bases. An active agent can be administered rectally via a suppository. The suppository can include vehicles such as cocoa butter, carbowaxes and polyethylene glycols, which melt at body temperature, yet are solidified at room temperature.

[00115] Unit dosage forms for oral or rectal administration such as syrups, elixirs, and suspensions may be provided wherein each dosage unit, for example, teaspoonful, tablespoonful, tablet or suppository, contains a predetermined amount of the composition containing one or more active agents. Similarly, unit dosage forms for injection or intravenous administration may comprise the agent(s) in a composition as a solution in sterile water, normal saline or another pharmaceutically acceptable carrier.

SUBJECTS SUITABLE FOR TREATMENT

[00116] Subjects suitable for treatment with a subject method include individuals who have been diagnosed as having a demyelinating disorder or a myelin deficiency disorder, e.g., Multiple Sclerosis (MS), optic neuritis, transverse neuritis and Guillain-Barre Syndrome (GBS), or Pelizaeus-Merzbacher disease. Also suitable are individuals who have suffered traumatic head and/or spinal cord injury; and individuals who have suffered osmotic striatal extra- or intra-pontine demyelination.
Subjects suitable for treatment with a subject method include individuals having benign MS, relapsing/remitting MS, secondary/progressive MS, primary/progressive MS, or progressive/relapsing MS.

Subjects suitable for treatment with a subject method include individuals who have been treated with a therapeutic agent for the treatment of a demyelinating disorder, and who are refractory to such treatment (e.g., treatment failure patients). Subjects suitable for treatment with a subject method include individuals who have been treated with a therapeutic agent for the treatment of a demyelinating disorder, and who can for any reason no longer tolerate treatment with the agent. Subjects suitable for treatment with a subject method include individuals who have been treated with a therapeutic agent for the treatment of a demyelinating disorder, who were in remission, but who subsequently relapsed.

**EXAMPLES**

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); and the like.

Example 1: Treatment with transferrin and IGF-1 promotes recruitment of stem/oligodendrocyte progenitors in the normal and myelin-deficient postnatal brain.

**Materials and Methods**

*Animals and Growth factors administration*

The myelin deficient rat mutants (+md/Y), the littermate controls (++/Y), and the wild type (Wt) Wistar rats were bred at UCLA in a restricted access, temperature-controlled vivarium on a 12:12 h cycle, and free access to food and water. At postnatal day 4 (P4) male rat pups, either wild type or born to md (myelin deficient) carrier mothers, were separated into
two groups: the first group did not receive any factors (control group) and the second group received a stereotaxic injection of IGF-1 (100 ng/pup) and Tf (15 μg/pup) combined in a total volume of 2 μl of Hank’s solution without calcium nor magnesium. Intraparenchymal injections of the trophic factors were performed as previously described (Espinosa et al. (1999) Neurochem Res, 24: 235-248). The md and Wt animals were perfused 15, 5, or 2 days post-injection (3 weeks of age) and brains were cryoprotected. Tissues were embedded in OCT compound to prepare and 20μm frozen sections as described (Espinosa et al, (1999) supra; Espinosa et al. (2002) Rat.Dev. Neurosci. 24:227-241).

Evaluation of growth factors’ treatment

[00121] Double immunofluorescence for PSA-NCAM from the Hybridoma Bank (Pharmacia 556309), nestin and Tf Nordic Immunology, 2693) detection was performed as previously described by Espinosa et al, (1999) supra. An IgG anti-mouse FITC (Sigma, F-9006) and IgG anti- rabbit Texas red (Jackson Immuno Research Laboratories Inc. Cat. # 112 075 075) were used to visualize these markers. To establish that nestin positive cells did not give rise to astrocytes or neurons, we used glial fibrillary acidic protein antibody (GFAP) (Sigma G-9269) as an astrocyte marker, and the neuronal marker, neurofilament 200 (Sigma N-4142). The Zeiss LSM 510 Meta, confocal microscope and Adobe Photoshop software, were respectively used to acquire and record the images depicting the effects of the trophic factors.

BrdU incorporation assay

[00122] To assess cell proliferation, animals were injected with 10 uM BrdU, (1ml/100g body weight BrdU Kit II, 1299 964, Roche Molecular Biochemicals), and left 24 hours. Next day, animals were given a second injection and 2h after the second injection they were perfused as previously described (Espinosa et al., (2002) supra), brains harvested and left in 30% sucrose for cryoprotection. The day of the immunocytochemistry, sections were immersed in 70% ethanol in 50mM glycine buffer pH2.0 at -20°C for 20 min, and rehydrated in PBS. After DNA denaturation with 2N HCl at 37°C for 1h, sections were washed and incubated with 1% Triton X-100 at 37°C for 20 min and then at room temperature for 10 min. Samples were blocked with 10% goat serum in 0.1% Triton X-100 in PBS. The sections were then incubated with anti-BrdU antibody alone or mixed with other antibodies for cell type identification such as the early marker for OL transferrin (Tf) and nestin to recognize OL progenitors and stem cells respectively. The secondary antibody to visualize BrdU was provided in the BrdU kit II.
**Results**

**Nestin and Transferrin Expression in md and Wt injected with Tf+IGF-1**

*At the level of the third ventricle*

[00123] To determine if cells of the subventricular zone (SVZ) in normal and md rats responded to a single injection of Tf+ IGF-1 administered to the brain in corpus callosum at P4. The expression of nestin was examined in these brains at 2 and 13 days post-injection. Coronal sections of md and unaffected littermates treated with the combined factors (Tf+IGF-1) or without were examined.

[00124] At seven days of age the md brain was widely positive for nestin fibers. At the level of the 3rd ventricle animals treated with the combination of IGF-1+ Tf expressed nestin as a network revealing an array of nestin cell processes extending from the ventricular wall into the subventricular region and the parenchyma. Tf immunoreactivity was found in md non-treated animals in the ventricular zone (VZ) and the choroids plexus and also in the SVZ, where many nestin positive cells extended processes from the ventricular wall into the SVZ. Adjacent to the SVZ and already in the parenchymal tissue some nestin+/Tf+ and Nestin negative/Tf+ cells were also present. However, neither of the two types of populations Nestin+/Tf+ nor Tf+ nestin negative, seemed to migrate beyond this region, because they were not seen anywhere else. Unaffected littermates and wild type animals without factors injection were negative for nestin at the level of the third ventricle. The combined factors had different effects on unaffected and md pups. On unaffected and wild type animals, nestin was upregulated and the expression was found mainly in the paraventricular thalamic anterior nucleus (PVA) and the triangular septal nucleus (TS). In the md rat with the combined factors the wall of the ventricle was negative for nestin while the SVZ and the parenchyma showed nestin labeled cell processes but not cell bodies. There were also a few Tf+ cells with or without nestin expression in the parenchyma.

[00125] Thirteen days after injection of the combined factors, the effects on nestin expression were very modest when compared to younger animals. The unaffected brain did not have any nestin positive fibers but Nestin positive/Tf positive small cells were in the SVZ and neighboring parenchyma. The non treated md rat did not have Tf or nestin expression at this age, while the double injection resulted only in a modest and restricted region of the SVZ with a weak nestin expression. The untreated unaffected brain had nestin only in some cells of the choroids plexus and a little in the dorsal portion of the third ventricle wall.
At the level of the ipsilateral ventricular region

[00126] Unaffected animals at 7 and 15 days of age without the factors treatment were negative in the lateral ventricle regions. In contrast, md animals at 7 days had nestin positive radial cell processes and some cells that co-expressed Tf and nestin in the SVZ. However, these cell had enlarged body and some seemed to have vacuoles. Interestingly, the injection of Tf+IGF-1 combined, resulting in an increase of both markers in the SVZ and much more accentuated in the apical portion of the lateral right ventricle. The expression of both markers was seen in the cell bodies and only a few fibers were nestin positive/Tf negative in the same region. Another important finding was that very large Tf positive/nestin negative cells of enlarged cell bodies were located along the wall of the lateral ventricle. However, these enlarged cells were not seen in the SVZ or the parenchyma. The untreated md brain did not show any significant expression of either marker, only very few short and isolated nestin positive sprout-like fibers. The mutant 13 days after the injection no longer had nestin in the ventricular wall and only a thin stream of nestin positive/Tf positive small bipolar cells was observed. We also analyzed the contralateral VZ where the effects of the treatment (Tf+IGF-1) were milder compared to the ipsilateral side.

Expression of Nestin and Transferrin in the Ependymal canal

[00127] The ependymal canal and the apical portion of the 3rd ventricle 8.20 mm intraneural and minus 0.8 from Bregma were also compared, where the walls of the third ventricle are vertical and the ependymal canal extends as deep as the optic chiasm. Intense nestin labeling was found in the ependymal canal of untreated P7 pups born to md carrier mothers. Thin hair-like labeled cell processes were also present in the adjacent parenchyma. In unaffected untreated littermates nestin expression was confined to the ependymal canal and it was absent from the adjacent parenchyma. In md pups two days after Tf+IGF-1 treatment, nestin expression remained localized to the ependymal canal that had thickened and the hair-like processes had displaced farther into the parenchyma. After treatment the nestin labeled ependymal canal had also thickened and hair-like staining had appeared in unaffected pups. The nestin expression pattern resembled the md untreated brain. Neither the ependymal canal nor the adjacent parenchyma expressed Tf, whether animals were unaffected or mutants nor in response to the injection of Tf+IGF-1. Older untreated animals P18/19 days no longer expressed nestin in these regions. Moreover, the effect of the double injection of Tf and IGF-1 was not found in md brains two weeks after injection, suggesting that there is a developmental window for nestin expression. No Tf immunoreactivity was observed in the canal or in the adjacent parenchyma.
Expression of Nestin and GFAP

The question of whether the treatment with Tf+IGF-1 had also given rise to astroglial cells in the SVZ was examined. Double immunocytochemistry was performed with these markers. Examination of coronal sections from 7-day-old untreated md animals revealed the presence of round GFAP expressing cells in the SVZ. These cells were among nestin labeled cells and their processes that were randomly oriented. Two days after Tf+IGF-1 treatment the SVZ of md pups showed an almost total absence of GFAP staining. Nestin immunoastaining revealed radial cell processes that were all perpendicular to the ventricular wall and extended into the parenchyma. The unaffected littermates without treatment showed a reduced GFAP staining when compared to the untreated md mutant. Moreover, no nestin positive cells or fibers were present. However, Wt animals responded to the treatment by displaying numerous radial like processes extending perpendicularly from the subventricular region into the brain parenchyma, these fibers were GFAP negative. Thus, the two factors induced nestin expression in regions of neurogenesis yet not in GFAP expressing cells neither in the unaffected nor in the md animal. Neither unaffected nor mutants displayed colocalization of GFAP with nestin.

Expression of Nestin and Neurofilaments

The possibility of finding co-expression of NF-200 and nestin, which would indicate that the factors would have induced neuronal differentiation, was examined. As in the case of GFAP, there was no colocalization of NF with nestin. Interestingly, the md animal whether treated or not treated, did not show NF expressing cells in the SVZ. In contrast the Wt animal treated with the two factors displayed a few scattered small (less than 1%) NF positive cells that appeared to travel along the nestin cell processes.

BrdU incorporation

Whether treatment with Tf+IGF-1 influences cell proliferation in the mutant and unaffected brain was examined. The lateral and contralateral hemispheres were examined; it was found that unaffected animals responded to IGF-1 + Tf treatment with moderate BrdU incorporation where the maximum effect induced twice as many BrdU-bearing cells when compared to the Wt non-treated brain. Cell counts per area were performed on the coronal sections. The areas selected were those in the white matter notably the corpus callosum (CC) and the dorsal portion of the internal capsule region that exhibited enriched BrdU staining. The regions where cells were counted are shown on a diagram of the coronal rat brain. The cell counts are shown in Figure 1. It was found that both the ipsilateral and contralateral hemispheres displayed a gradient of BrdU positive cells along the CC. Interestingly, in the ipsilateral hemisphere BrdU incorporation increased from the mid line peaking around the
injection site. The points closest to the midline in both hemispheres had the lowest and equivalent number of BrdU positive cells. However, the ipsilateral CC had twice as many BRDU positive cells when compared to the corresponding regions of the CC in the contralateral side and equivalent to the non treated animal. The combined factors had a greater impact on cell proliferation in the md brain when compared with their wild type counterparts. The pattern of BrdU incorporation was also accentuated in the CC where the highest number of BrdU positive cells was in regions neighboring to the injection site and fewer BrdU positive cells were found towards the midline and the lateral portions of the CC (Figure 1A, insert). In the case of the contralateral hemisphere, there was a less intense response, the highest number of BrdU+ cells was in the point closest to the midline and therefore closer to the injection side. Thereafter the number of cells BRDU+ remained low and constant (Fig. 1B solid bars).

Fig. 1A and 1B depict the number of cells expressing BrdU in the contralateral side vs. the ipsilateral side of wt (Figure 1A) and md (Figure 1B) rat brain treated with Tf + IGF-1. Figures 1A and 1B show the results of examination of BRDU incorporation on coronal brain sections two days after injection. Bars represent the number of BRDU+ cells per area in different regions of the CC. Both the ipsilateral and contralateral hemispheres displayed a BRDU gradient of positive cells along the corpus callosum (CC). In the ipsilateral hemisphere BRDU incorporation increased from the mid line peaking around the injection site. The points closest to the midline in both hemispheres had the lowest number of BRDU+ cells and they were equivalent to each other. The ipsilateral CC had twice as many BRDU+ cells when compared to the corresponding regions of the CC in the contralateral side. Numbers represent mean values of counts in three different fields within the same area.

Phenotype of BrdU expressing cells

To assess the phenotype of cells labeled with BrdU, colocalization of BrdU staining with Tf, NF or GFAP markers for OL, neurons and astrocytes was examined. It was found that in treated md brains two days post-injection many cells were BrdU labeled in the dorsal portion of the lateral ventricle extending towards the ventral portion of the CC. Yet only a few BRDU positive cells co-expressed Tf. Surprisingly, three days later (5 days post injection) the BRDU labeled cells had migrated into the parenchyma within the CC and CaPu and they all co-expressed Tf on a one to one bases.

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and
scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.
CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
   a) insulin-like growth factor-1 or an active fragment thereof;
   b) transferrin or an active fragment thereof; and
   c) a pharmaceutically acceptable excipient.

2. The composition of claim 1, wherein the composition is formulated for subcutaneous injection.

3. The composition of claim 1, wherein the composition is formulated for intramuscular injection.

4. The composition of claim 1, wherein the composition is formulated for intravenous injection.

5. The composition of claim 1, comprising an amount of IGF-1 in a range of from about 0.1 μg to about 100 μg.

6. The composition of claim 1, comprising an amount of transferrin in a range of from about 0.1 mg to about 100 mg.

7. The composition of claim 1, formulated for oral administration.

8. A device for delivery of the composition of claim 1 to an individual in need thereof, the device comprising a container comprising the composition of claim 1.

9. The device of claim 8, further comprising a catheter in fluid communication with the container.

10. The device of claim 9, wherein the container is a reservoir.
11. The device of claim 10, further comprising a fluid control means operably connected to the reservoir.

12. A method of increasing the number of myelin-producing oligodendrocytes at or near a site of demyelination in the central nervous system, the method comprising administering an effective amount of the composition of claim 1.

13. A method of reducing the extent or degree of demyelination in the central nervous system of an individual suffering from a demyelinating disorder, the method comprising administering to the individual an effective amount of the composition of claim 1.

14. A method of treating a demyelinating disorder in an individual, the method comprising administering to an individual in need thereof an effective amount of the composition of claim 1.

15. The method of claim 14, wherein the demyelinating disorder is multiple sclerosis.

16. The method of claim 14, further comprising administering at least one additional therapeutic agent for treating a demyelinating disorder.

17. The method of claim 16, wherein the at least one additional therapeutic agent is an interferon-beta.

18. The method of claim 17, wherein the interferon-beta is selected from IFN-β1a and IFN-β1b.

19. The method of claim 16, wherein the at least one additional therapeutic agent is a corticosteroid.

20. The method of claim 19, wherein the corticosteroid is selected from methylprednisolone, prednisone, prednisolone, and dexamethasone.
21. The method of claim 16, wherein the at least one additional therapeutic agent is a random copolymer of alanine, lysine, glutamic acid, and tyrosine.
FIG. 1B
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