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(56) Related Art

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(54) Title: THERAPIES FOR CHRONIC INFLAMMATORY DEMYELINATING POLYNEUROPATHY USING INTERFERON-β

(57) Abstract: The present invention provides methods for the treatment, and pharmaceuticals for the use in the treatment, of mammalian subjects having, or at risk of developing, chronic demyelinating neuropathies, e.g., CIDP. The methods involve the administration of IFN-β therapeutics.

THERAPIES FOR CHRONIC INFLAMMATORY DEMYELINATING POLYNEUROPATHY USING INTERFERON-β

Background of the invention

Chronic inflammatory demyelinating polyneuropathy (CIDP) is a neurological disorder characterized by slowly progressive weakness and sensory dysfunction of the legs and arms. This disease is caused by damage to the myelin sheath of the peripheral nerves. Swelling of nerve roots is also a characteristic of the disease. Although it can occur at any age and in both genders, CIDP is more common in young adults, and in men more so than women. Symptoms include tingling or numbness (beginning in the toes and fingers), weakness of the arms and legs, aching pain in the muscles, loss of deep tendon reflexes (areflexia), fatigue, and abnormal sensations.

CIDP is associated with certain other diseases. For example, it has been found that inflammatory demyelinating neuropathies, e.g., CIDP, are diagnosed in one third of human immunodeficiency virus (HIV)-seropositive patients referred for peripheral nerve diseases. CIDP was also found to occur in subjects afflicted with lupus, paraproteinemia, lymphoma or diabetes.

Untreated, CIDP is characterized by accumulating disability that requires physical and occupational therapy, orthotic devices, and long-term treatment. Close follow-up care with a physician knowledgeable in the field is necessary to adjust treatment.

Current methods of treatment for CIDP include administration of corticosteroids, such as prednisone, which may be prescribed alone or in combination with immunosuppressant drugs. Immunosuppressant drugs may also be given in the absence of a steroid. Plasmapheresis (plasma exchange) and intravenous immunoglobulin (IVIg) therapy are also relatively effective and currently being used. IVIg may be used even as a first-line therapy. Also, physiotherapy may improve muscle strength, function and mobility, and minimize the development of contractures.

The course of CIDP varies widely among individuals. Some may have a bout of CIDP followed by spontaneous recovery, while others may have many bouts with partial recovery in between relapses. The disease is a treatable cause of acquired neuropathy and

initiation of early treatment to prevent loss of nerve cells is recommended. However, some individuals are left with some residual numbness or weakness.

Thus, the current methods of treatment of CIDP consist of methods that are harmful (e.g., steroids or immunosuppressants); expensive (e.g., IVIg and plasmapheresis); or inconvenient (e.g., plasmapheresis). Accordingly, it would be highly desirable to have effective therapeutic methods for treating chronic demyelinating neuropathies, e.g., CIDP, which are less toxic, less expensive and more convenient than the current methods.

Summary of the invention

In one embodiment, the invention provides methods for treating a chronic demyelinating motor neuropathy in a mammal. The method may comprise administering to the mammal a therapeutically effective amount of an IFN-β therapeutic. The IFN-β therapeutic may be administered via a non-subcutaneous parenteral route, e.g., intramuscularly. In a preferred embodiment, the neuropathy is chronic inflammatory demyelinating neuropathy (CIDP).

The IFN-β therapeutic may comprise human IFN-β. For example, the IFN-β therapeutic may comprise a protein that is at least about 95% identical to full length mature human IFN-β having SEQ ID NO: 4. The IFN-β therapeutic may also comprise full length mature human IFN-β having SEQ ID NO: 4. The IFN-β therapeutic may also comprise full length mature human IFN-β having SEQ ID NO: 4 fused to a heterologous polypeptide, e.g., the constant domain of a human immunoglobulin molecule. The immunoglobulin molecule may be the heavy chain of an IgG1. The IFN-β therapeutic may comprise SEQ ID NO: 14. The IFN-β therapeutic may also comprise a pegylated IFN-β.

The IFN-β therapeutic, or composition comprising such, may comprise a stabilizing agent, such as a protein or amino acid. For example, the stabilizing agent may be arginine. The IFN-β therapeutic, or composition comprising such, may have a pH between about 4.0 and 7.2.

The IFN-β therapeutic may be administered once, twice or three times weekly. In certain embodiments, the IFN-β therapeutic is administered at about 6 or 12 million international units (MIU). The IFN-β therapeutic may be administered intramuscularly or

subcutaneously. In a preferred embodiment, the subject is a mammal, and preferably a human.

The invention also provides methods for treating a neuropathy, e.g., CIDP, comprising administering to a subject having the neuropathy a pharmaceutically effective amount of an IFN-β therapeutic and further administering to the subject an immunosuppressant or subjecting the subject to plasmapheresis. The method may comprise administering to the subject an immunosuppressant selected from the group consisting of a steroid, azothioprine, cyclosporin, cyclophosphamide, and mycophenolate.

Also within the scope of the invention are methods for treating a neuropathy, e.g., CIDP, comprising administering to a subject having a neuropathy a pharmaceutically effective amount of an IFN-β therapeutic in combination with a second treatment for the neuropathy, wherein administration of the IFN-β therapeutic is via a non-subcutaneous parenteral route. The administration of the IFN-β therapeutic can be via an intramuscular administration. The IFN-β therapeutic may be administered weekly, e.g., a weekly administration of about 6 MIU of an IFN-β therapeutic. When the neuropathy is CIDP, the second treatment may be selected from the group consisting of administration of a steroid; administration of IVIg; administration of an anti-inflammatory drug and plasmapheresis.

In another embodiment, the invention provides methods for treating a neuropathy, e.g., CIDP, comprising administering to a subject having the neuropathy a pharmaceutically effective amount of an IFN-β therapeutic in combination with a second treatment for the neuropathy, wherein administration of the IFN-β therapeutic is weekly. If the neuropathy is CIDP, the second CIDP treatment may be selected from the group consisting of administration of a steroid; administration of IVIg; administration of an anti-inflammatory drug and plasmapheresis.

In yet another embodiment, the invention provides methods for treating CIDP in a subject receiving a first CIDP treatment selected from the group consisting of administration of a steroid; administration of an anti-inflammatory drug; administration of IVIG and plasmapheresis, the improvement comprising administering to the subject, in addition to the first CIDP treatment, a dose of an IFN-β therapeutic in an amount effective to significantly reduce the dose or frequency of the first CIDP treatment, wherein administration of the IFN-β therapeutic is via a non-subcutaneous parenteral route, to provide effective relief from symptoms of CIDP. In another method for treating CIDP, a

subject receives a first CIDP treatment selected from the group consisting of administration of a steroid; administration of an anti-inflammatory drug; administration of IVIG and plasmapheresis, the improvement comprising administering to the subject, in addition to the first CIDP treatment, once a week a dose of an IFN-β therapeutic in an amount effective to significantly reduce the dose or frequency of the first CIDP treatment, to provide effective relief from symptoms of CIDP. A subject having CIDP may also be treated by receiving a first CIDP treatment selected from the group consisting of administration of a steroid; administration of an anti-inflammatory drug; and plasmapheresis, the improvement comprising administering to the subject, in addition to the first CIDP treatment, a dose of an IFN-β therapeutic in an amount effective to significantly reduce the dose or frequency of the first CIDP treatment, to provide effective relief from symptoms of CIDP.

Definitions of the specific embodiments of the invention as claimed herein follow.

According to a first embodiment of the invention, there is provided a method of treating a chronic demyelinating motor neuropathy in a mammal, comprising administering to the mammal a therapeutically effective amount of an IFN-β therapeutic, wherein the IFN-β therapeutic is administered via a non-subcutaneous parenteral route.

According to a second embodiment of the invention, there is provided a method of treating CIDP, comprising administering to a subject having CIDP a pharmaceutically effective amount of an IFN-β therapeutic and further administering to the subject an immunosuppressant or subjecting the subject to plasmapheresis, wherein the IFN-β therapeutic is administered via a non-subcutaneous parenteral route.

According to a third embodiment of the invention, there is provided a method of treating CIDP, comprising administering to a subject having CIDP a pharmaceutically effective amount of an IFN-β therapeutic in combination with a second CIDP treatment, wherein administration of the IFN-β therapeutic is via a non-subcutaneous parenteral route.

According to a fourth embodiment of the invention, there is provided a method of treating CIDP, comprising administering to a subject having CIDP a pharmaceutically effective amount of an IFN-β therapeutic in combination with a second CIDP treatment, wherein administration of the IFN-β therapeutic is weekly, and wherein the IFN-β therapeutic is administered via a non-subcutaneous parenteral route.

According to a fifth embodiment of the invention, there is provided a method of treating CIDP in a subject receiving a first CIDP treatment selected from the group consisting of administration of a steroid; administration of an anti-inflammatory drug; administration of IVIG and plasmapheresis, the improvement comprising administering to the subject, in addition to the first CIDP treatment, a dose of an IFN-β therapeutic in an amount effective to significantly reduce the dose or frequency of the first CIDP treatment, wherein administration of the IFN-β therapeutic is via a non-subcutaneous parenteral route, to provide effective relief from symptoms of CIDP.

According to a sixth embodiment of the invention, there is provided a method of treating CIDP in a subject receiving a first CIDP treatment selected from the group consisting of administration of a steroid; administration of an anti-inflammatory drug; administration of IVIG and plasmapheresis, the improvement comprising administering to the subject, in addition to the first CIDP treatment, once a week a dose of an IFN-β therapeutic in an amount effective to significantly reduce the dose or frequency of the first CIDP treatment, wherein the IFN-β therapeutic is administered via a non-subcutaneous parenteral route, to provide effective relief from symptoms of CIDP.

According to a seventh embodiment of the invention, there is provided a method of treating CIDP in a subject receiving a first CIDP treatment selected from the group consisting of administration of a steroid; administration of an anti-inflammatory drug; and plasmapheresis, the improvement comprising administering to the subject, in addition to the first CIDP treatment, a dose of an IFN-β therapeutic in an amount effective to significantly reduce the dose or frequency of the first CIDP treatment, wherein the IFN-β therapeutic is administered via a non-subcutaneous parenteral route, to provide effective relief from symptoms of CIDP.

Brief description of the figures

Figs. 1A-C show the nucleotide (SEQ ID NO: 11) and amino acid (SEQ ID NO: 12) sequences of a fusion protein consisting of the VCAM signal sequence fused to the mature full length human IFN-β (SEQ ID NO: 3 and 4), in which the glycine at amino acid 162 of SEQ ID NO: 4 is replaced with a cysteine, fused to the hinge, CH2 and CH3 domains of human IgG1Fc (ZL5107).

Figs. 2A-C show the nucleotide (SEQ ID NO: 13) and amino acid (SEQ ID NO: 14) sequences of a fusion protein consisting of the VCAM signal sequence fused to the mature full length human IFN-β (SEQ ID NO: 3 and 4), in which the glycine at amino acid 162 of SEQ ID

NO: 4 is replaced with a cysteine; fused to the G4S linker which is fused to the hinge, CH2 and CH3 domains of human IgG1Fc (ZL6206).

Detailed description of the invention

The invention provides methods for treating chronic demyelinating neuropathies, e.g., CIPD, comprising administering a pharmaceutically effective amount of an IFN-β therapeutic.

[Text continues on page 5.

1. Definitions:

To more clearly and concisely point out the subject matter of the claimed invention, the following definitions are provided for specific terms used in the written description and the appended claims.

As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

"Chronic Inflammatory Demyelinating Polyneuropathy" is used interchangeably herein with "CIDP." The following conditions are identical or considered essentially identical to CIDP and are therefore encompassed in the term "CIDP" when used herein: "chronic relapsing polyneuropathy," "chronic idiopathic demyelinating polyneuropathy," "chronic inflammatory demyelinating polyradiculoneuropathy," and "chronic acquired demyelinating polyneuropathy" ("CADP"). CIDP has a chronic progressive, stepwise or relapsing course (see, e.g., Dyck et al. (1993) in Dyck, P.J., Thomas, PlK., Griffin, J.W., Low, P.A., Poduslo, J.F. (Eds), Peripheral Neuropathy, 3rd ed. Saunders, Philadelphia, pp. 1498-1517). The pathological hallmarks of CIDP are segmental demyelination and remyelination and mononuclear cellular infiltrates in the endoneurium (Dyck et al., supra). CIDP is sometimes referred to as the peripheral counterpart of multiple sclerosis (MS) (Toyka and Hartung (1996) Curr. Opin. Neurol. 9, 240-250). It is also sometimes referred to as the chronic form of Guillain-Barre syndrome. Criteria for diagnosis of CIDP include clinical, electrophysiological and cerebrospinal fluid (CSF) criteria, described, e.g., in the report from an adhoc subcommittee of the American Academy of Neurology AIDS taks force (1991) Neurology 41:617. Diagnostic tests include the nine hole peg test; the 10 meters walking test; the Rankin scale or modified form thereof; and an MRC sumscore or modified form thereof (Mathiowetz et al. (1985) Occupational Therapy J. of Res. 5:24; Thompson et al. (1996) J. Neurol. 243:280; Collen et al. (1990) Int. Disability Studies 12:6; van Swieten et al. (1988) Stroke 19:604 and Kleyweg et al. (1991) Muscle Nerve 14:1103). Neurological assessements may also include the Neurologic Disability Scale (NDS); a disability scale (0, healthy; 1, minor signs; 2, able to walk without assistance but unable to run; 3, able to walk 5 meters only with help; 4, chair/bed bound); the Hammersmith Motor Ability Test (HMAT) (Dyck P.J., In "Peripheral Neuropathy" (1993), supra, pages 686-697; Scott et al. (1982) Muscle Nerve 5:291); and testing of nerve conduction. Muscular assessements may involve motor function assessments, e.g., by measuring maximal

voluntary isometric contractions (MVIC), as is known in the art; and measurements of muscle strength. Yet other tests of disability include the Ambulation Index; the Functional Independence Measure; Guy's Neurological Disability Scale (GNDS); the Medical Research Council sumscore; the sensory sumscore; and the Hughes functional scale (Hauser et al. (1983) N. Engl. J. Med. 308:173; Hall et al. (1993) J. Head Trauma Rehabilitation 8:60; Sharrack et al. (1996) J. Neurol. 243:S32 and Merkies et al. (2002) Neurology 59:84). Electrophysiological criteria for CIDP were proposed by an Ad Hoc Subcommittee of the American Academy of Neurology (AAN) in 1991, and were recently revised (Nicolas et al. (2002) Muscle Nerve 25:26. Another test that is used for sensory-motor immune-mediated polyneuropathies, e.g., CIDP and Guillain-Barre syndrome (GBS) is the psychometric evaluation of the inflammatory neuropathy cause and treatment (INCAT) sensory sumscore (ISS) (Merkies et al. (2000) Neurology 54:943). Human leukocyte antigens Dw3, DRw3, A1, and B8 occur more frequently in patients with CIDP than in the healthy population (Zvartau-Hind et al. (2002) Chronic Inflammatory Demyelinating Polyradiculoneuropathy, at www.emedicine.com/neuro).

"IFN-β-1a" refers to an IFN-β molecule having the amino acid sequence of the wild-type human IFN-β and is glycosylated.

"IFN-β-1b" refers to an IFN-β molecule having the amino acid sequence of the wild-type IFN-β, wherein the cysteine at position 17 is replaced with a serine; the methione at position 1 ("initiator methionine") is lacking and the molecule is not glycosylated.

"IFN-β variant" refers to a wild-type IFN-β protein having one or more modifications, e.g., amino acid deletions, additions, substitutions, a posttranslational modification or including one or more non-naturally occurring amino acid residues or linkages between them. Portions of IFN-βs are included in the term "IFN-β variant." A "biologically active IFN-β variant" is an IFN-β variant that has at least some activity in treating a neuropathy, e.g., CIDP. An IFN-β variant can be a naturally-occurring IFN-β having, e.g., an insertion, deletion or substitution of one or more amino acids relative to the wild-type IFN-β, i.e., a naturally occurring mutant or a polymorphic variant, or it can be a non-naturally occurring IFN-β.

"International units" or (IU) of IFN-β refers to the units as defined by the World Health Organization (WHO) International Standard for Interferon.

"Isolated" (used interchangeably with "substantially pure") when applied to polypeptides means a polypeptide which, by virtue of its origin or manipulation: (i) is present in a host cell as the expression product of a portion of an expression vector; (ii) is linked to a protein or other chemical moiety other than that to which it is linked in nature; or (iii) does not occur in nature, for example, a protein that is chemically manipulated by appending, or adding at least one hydrophobic moiety to the protein so that the protein is in a form not found in nature. By "isolated" it is further meant a protein that is: (i) synthesized chemically; or (ii) expressed in a host cell and purified away from associated and contaminating proteins. The term generally means a polypeptide that has been separated from other proteins and nucleic acids with which it naturally occurs. Preferably, the polypeptide is also separated from substances such as antibodies or gel matrices (polyacrylamide) which are used to purify it. "Isolated" (used interchangeably with "substantially pure")- when applied to nucleic acids, refers to an RNA or DNA polynucleotide, portion of genomic polynucleotide, cDNA or synthetic polynucleotide which, by virtue of its origin or manipulation: (i) is not associated with all of a polynucleotide with which it is associated in nature (e.g., is present in a host cell as an expression vector or a portion thereof); or (ii) is linked to a nucleic acid or other chemical moiety other than that to which it is linked in nature; or (iii) does not occur in nature. By "isolated" it is further meant a polynucleotide sequence that is: (i) amplified *in vitro* by, for example, polymerase chain reaction (PCR); (ii) synthesized chemically; (iii) produced recombinantly by cloning; or (iv) purified, as by cleavage and gel separation.

"Multifocal motor neuropathy" or "MMN" is a chronic immune mediated demyelinating neuropathy, that is characterized by a stepwise progression of asymmetric muscle weakness and amyotrophy localised in the anatomical distribution areas of peripheral nerves (Pestronk et al. (1988) Ann. Neurol. 24:73 and Kornberg et al. (1995) Ann. Neurol. (suppl. 1) S43). The electrohysiological hallmark of multifocal motor neuropathy is persistent conduction block. Clinically, this disease is also described as an asymmetric pure motor variant of CIDP with multifocal motor conduction blocks. During the evolution of multifocal motor neuropathy, the multifocal character may gradually evolve in an essentially symetrical pattern, clinically resembling the motor form of CIDP. Pathological studies have linked these two diseases (Krendel et al. (1996) Ann. Neurol. 40:948 and Oh et al. (1995) Neurology 45:1828). Most patients with multifocal motor

neuropathy have high titer antibodies against the ganglioside GM1 (Pestronk et al., supra and Kornberg et al., supra).

A nucleic acid is "operably linked" to another nucleic acid when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader (e.g., signal sequence or signal peptide) is operably linked to DNA encoding a polypeptide if the DNA is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; and a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous and, in the case of, e.g., a secretory leader, contiguous and in reading phase. Linking can be accomplished by ligation, e.g., at convenient restriction sites. If such sites do not exist, synthetic oligonucleotide adaptors or linkers can be used in accordance with conventional practice.

"Percent identity" or "percent similarity" refers to the sequence similarity between two polypeptides, molecules, or between two nucleic acids. When a position in both of the two compared sequences is occupied by the same base or amino acid monomer subunit, then the respective molecules are identical at that position. The percentage identity between two sequences is a function of the number of matching or identical positions shared by the two sequences divided by the number of positions compared × 100. For instance, if 6 of 10 of the positions in two sequences are matched or are identical, then the two sequences are 60% homologous. By way of example, the DNA sequences CTGACT and CAGGTT share 50% homology (3 of the 6 total positions are matched). Generally, a comparison is made when two sequences are aligned to give maximum identity. Such alignment can be provided using, for instance, the method of Karlin and Altschul described in more detail below. When referring to a nucleic acid, "percent homology" and "percent identity" are used interchangeably, whereas when referring to a polypeptide, "percent homology" refers to the degree of similarity, where amino acids representing conserved substitutions of other amino acids are considered identical to these other amino acids. A "conservative substitution" of a residue in a reference sequence is a replacement with an amino acid that is physically or functionally similar to the corresponding reference residue, e.g., that have a similar size, shape, electric charge, chemical properties, including the ability to form covalent or hydrogen bonds, or the like. Particularly preferred conservative

substitutions are those fulfilling the criteria defined for an "accepted point mutation" in Dayhoff et al., 5: Atlas of Protein Sequence and Structure, 5: Suppl. 3, chapter 22: 354-352, Nat. Biomed. Res. Foundation, Washington, D.C. (1978). The percent homology or identity of two amino acids sequences or two nucleic acid sequences can be determined using the alignment algorithm of Karlin and Altschul (Proc. Nat. Acad. Sci., USA 87: 2264 (1990) as modified in Karlin and Altschul (Proc. Nat. Acad. Sci., USA 90: 5873 (1993). Such an algorithm is incorporated into the NBLAST or XBLAST programs of Altschul et al., J. Mol. Biol. 215: 403 (1990). BLAST searches are performed with the NBLAST program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to a nucleic acid of the invention. BLAST protein searches are performed with the XBLAST program, score = 50, wordlength = 3, to obtain amino acid sequences homologous to a reference polypeptide. To obtain gapped alignments for comparisons, gapped BLAST is used as described in Altschul et al., Nucleic Acids Res., 25: 3389 (1997). When using BLAST and Gapped BLAST, the default parameters of the respective programs (XBLAST and NBLAST) are used. See http://www/ncbi.nlm.nih.gov.

Quality of life can be measured by the EuroQoL visual analogue scale and the EuroQoL questionnaire sum score; the Medical Outcome Study 36-item short-form health status scale (SF-36); and a Visual Analogue Scale (VAS) (EuroQoL Group (1990) Health Policy 16: 199 and Merkies et al. (2002) Neurology 59:84).

An IFN-β therapeutic is said to have "therapeutic efficacy," and an amount of the IFN-β therapeutic is said to be "therapeutically effective," if administration of that amount of the IFN-β therapeutic alone in combination therapy is sufficient to cause a clinically significant improvement in at least one symptom of a disease relative to the absence of IFN-β treatment. In a preferred embodiment, administration of a therapeutically effective amount of IFN-β therapeutic in a subject having CIDP results in an improvement of at least one symptom of CIDP, e.g., muscular or neural impairments.

"Wild-type IFN-β" refers to an IFN-β, whether native or recombinant, having the normally occurring amino acid sequence of native IFN-β. The nucleotide and amino acid sequence of native human IFN-β are set forth in SEQ ID NO: 1 and 2, respectively, which are the sequences shown, e.g., in GenBank Accession Nos. M28622 (and E00029) and AAA36040, respectively.

The term "comprise" and variants of the term such as "comprises" or "comprising" are used herein to denote the inclusion of a stated integer or stated integers but not to exclude any other integer or any other integers, unless in the context or usage an exclusive interpretation of the term is required.

Any reference to publications cited in this specification is not an admission that the disclosures constitute common general knowledge in Australia.

2. IFN-β therapeutics

IFN-β therapeutics that can be used according to the invention include wild-type IFN-βs and biologically active variants thereof, e.g., naturally-occurring and non-naturally-occurring variants. The nucleotide and amino acid sequences of wild-type naturally-occurring human IFN-β are set forth in SEQ ID NOs: 1 and 2, respectively, which are identical to GenBank Accession Nos. M28622 and AAA36040, respectively. These IFNs are also described, e.g., in Seghal (1985) J. Interferon Res. 5:521. The full length human IFN-β protein is 187 amino acids long and the coding sequence of SEQ ID NO: 1 corresponds to nucleotides 76-639. The signal sequence corresponds to amino acids 1 to 21. The amino acid sequence of the mature form of this IFN-β corresponds to amino acids 22-187 (nucleotides 139-639 of SEQ ID NO: 1). The mature human IFN-β protein and nucleotide sequence encoding such are set forth as SEQ ID NOs: 4 and 3, respectively.

IFN-β produced in mammalian cells is glycosylated. Naturally-occurring wild-type IFN-β is glycosylated at residue 80 (Asn 80) of the mature polypeptide of SEQ ID NO: 4 or residue 101 (Asn 101) of the immature polypeptide of SEQ ID NO: 2.

IFN-β therapeutics also include non-human IFN-βs, e.g., from a vertebrate, such as a mammal, e.g., a non-human primate, bovine, ovine, porcine, equine, feline, canine, rat and mouse; or an avian or amphibian. IFN-β sequences from these species can be obtained from GenBank and/or publications, or can be determined from nucleic acids isolated by low stringency hybridization with an IFN-β gene from another species.

Variants of wild-type IFN-β proteins include proteins having an amino acid sequence that is at least about 70%, 80%, 90%, 95%, 98% or 99% identical or homologous to a wild-type IFN-β, e.g., human IFN-β having SEQ ID NO: 2 or 4. Variants may have one or more amino acid substitutions, deletions or additions. For example, biologically active fragments of wild-type IFN-β proteins can be used. Such fragments may have 1, 2, 3, 5, 10 or up to 20 amino acids deleted, added or substituted at the C- or N-terminus of the protein. Variants may also have 1, 2, 3, 5, 10 or up to 20 amino acid substitutions, deletions or additions. Some variants may have less than about 50, 40, 30, 25, 20, 15, 10, 7, or 5 amino acid substitutions, deletions or additions. Substitutions can be with naturally occurring amino acids or with analogs thereof, e.g., D-stereoisomeric amino acids.

Also within the scope of the invention are IFN-β variants encoded by nucleic acids that hybridize under stringent conditions to a nucleic acid encoding a naturally-occurring IFN-β, e.g., represented by SEQ ID NOs: 1 or 3, or the complement thereof. Appropriate stringency conditions which promote DNA hybridization, for example, 6.0 x sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0 x SSC at 50°C, are known to those skilled in the art or can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6; Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, N.Y; S. Agrawal (ed.) Methods in Molecular Biology, volume 20; and Tijssen (1993) Laboratory Techniques in biochemistry and molecular biology-hybridization with nucleic acid probes, e.g., part I chapter 2 "Overview of principles of hybridization and the strategy of nucleic acid probe assays", Elsevier, New York. For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0 x SSC at 50°C to a high stringency of about 0.2 x SSC at 50°C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22°C, to high stringency conditions at about 65°C. Both temperature and salt concentration may be varied, or temperature or salt concentration may be held constant while the other variable is changed. Exemplary hybridization conditions include hybridization in 6.0 x sodium chloride/sodium citrate (SSC) at about 50°C, followed by a wash in 0.2 x SSC at room temperature. The temperature of the wash step may be increased to about 45°C, 50°C, 55°C, 60°C, or 65°C to increase the stringency of the hybridization. Hybridization may also be carried out in 5xSSC, 4xSSC, 3xSSC, 2xSSC, 1xSSC or 0.2xSSC. The hybridization can be conducted for at least about 1 hour, 2 hours, 5 hours, 12 hours or 24 hours. The hybridization may also include another agent affecting the stringency, e.g., formamide. For example, stringent hybridization may be conducted in the presence of 50% formamide, which increases the stringency of hybridization at a defined temperature. The wash step may be conducted in the presence of a detergent, e.g., SDS, such as 0.1 or 0.2% SDS. The hybridization may be followed by a wash consisting of a single wash step or at least two wash steps, which may be at the same or a different salinity and temperature. For example, hybridization can be followed by two wash steps at 65°C each for about 20 minutes in 2xSSC, 0.1% SDS, followed by two wash steps at 65°C each for about 20 minutes in 0.2xSSC, 0.1%SDS. Exemplary stringent hybridization conditions include overnight hybridization at 65% in a solution comprising or consisting of 50% formamide, 10xDenhardt (0.2% Ficoll, .2% Polyvinylpyrrolidone, 0.2%

bovine serum albumin) and 200 μg/ml of denatured carrier DNA, e.g., sheared salmon sperm DNA, followed by two wash steps at 65°C each for about 20 minutes in 2xSSC, 0.1% SDS, and two wash steps at 65°C each for about 20 minutes in 0.2xSSC, 0.1%SDS. Hybridization may consist of hybridizing two nucleic acids in solution, or a nucleic acid in solution to a nucleic acid attached to a solid support, e.g., a filter. In certain situations, e.g., when one nucleic acid is on a solid support, hybridization may be preceded by a prehybridization step, which may be carried out for at least about 1 hour, 3 hours or 10 hours, and may be in the same solution and at the same temperature as the hybridization solution (without the probe). In a preferred embodiment, a nucleic acid encoding an IFN-β variant will hybridize to one of SEQ ID NOs: 1 or 3 or complement thereof under moderately conditions, for example at, and including a wash at, about 2.0 x SSC and about 40 °C. In a particularly preferred embodiment, a nucleic acid encoding an IFN-β variant will hybridize to one of SEQ ID NOs: 1 or 3 or complement thereof under high stringency conditions, e.g., at, and including a wash at 0.2 SSC and about 65 °C.

Exemplary modifications are conservative modifications, which have a minimal effect on the secondary and tertiary structure of the protein. Exemplary conservative substitutions include those described by Dayhoff in the Atlas of Protein Sequence and Structure 5 (1978), and by Argos in EMBO J., 8, 779-785 (1989). For example, amino acids belonging to one of the following groups represent conservative changes: ala, pro, gly, gln, asn, ser, thr; cys, ser, tyr, thr;
val, ile, leu, met, ala, phe; lys, arg, his; and phe, tyr, trp, his.

Other modifications include the substitution of one amino acid for another amino acid that may not necessarily represent a conservative substitution. For example substitutions that essentially do not affect the three dimensional structure of IFN-β can be made. The three dimensional structure of non-glycosylated human IFN-β is described, e.g., in Radhakrishnan et al. (1996) Structure 4: 1453 and the three dimensional structure of glycosylated IFN-β is described, e.g., in Karpusas et al. (1997) PNAS 94:11813). Essentially, IFN-β comprises five helices: helix A, which consists of about amino acids 2-22 of SEQ ID NO: 4; helix B, which consists of about amino acids 51-71 of SEQ ID NO: 4; helix C, which consists of about amino acids 80-107 of SEQ ID NO: 4; helix D, which consists of about amino acids 118-136 of SEQ ID NO: 4 and helix E, which consists of about amino acids 139-162 of SEQ ID NO: 4 (Karpusas et al., *supra*). Helices A, B, C and

E form a left-handed, type 2 four-helix bundle. There is a long overhand loop, the AB loop, that connects helices A and B and three shorter loops (named BC, CD and DE) that connects the rest of the helices (Karpusa et al., *supra*). Previous studies have shown that the N-terminal, C-terminal and the glycosylated C helix regions of the IFN-beta molecule do not lie within the receptor binding site (see, WO 00/23472 and USSN 09/832,659). Accordingly, mutations in these regions would not significantly adversely affect the biological activity of the IFN molecule. It has also been previously shown that mutations in helix C (amino acids 81, 82, 85, 86 and 89 of mature human IFN-β) results in a molecule having higher antiviral activity relative to the wild-type IFN-β (see, WO 00/23472 and USSN 09/832,659). Similarly, it has been shown that mutants in the helix A (amino acids 2, 4, 5, 8 and 11 of mature human IFN-β) and CD loop (amino acids 110, 11, 113, 116 and 119) have a higher binding activity to the receptor and higher antiviral and anti-proliferative activities relative to the naturally occurring wild-type human IFN-β (see, WO 00/23472 and USSN 09/832,659).

Other preferred modifications or substitutions eliminate sites for intermolecular crosslinking or incorrect disulfide bond formation. For example, IFN-β is known to have three cys residues, at wild-type positions 17, 31 and 141 of SEQ ID NO: 4. One IFN variant is an IFN in which the cys (C) at position 17 has been substituted with ser (S), as described, e.g., in U.S. Pat. No. 4,588,585. Other IFN-β variants include IFN-β variants having, e.g., one or more of ser (S) substituted for cys (C) at position 17 and val (V) at position 101 substituted with phe (F), trp (W), tyr (Y), or his (H), preferably phe (F), when numbered in accordance with wild type IFN-β, having, e.g., SEQ ID NO: 4, such as described, e.g., in U.S. Patent 6,127,332. Other preferred variants include polypeptides having the sequence of a wild-type IFN-β, e.g., having SEQ ID NO: 4, wherein the val (V) at position 101, when numbered in accordance with wild type IFN-β, is substituted with phe (F), tyr (Y), trp (W), his (H), or phe (F), also as described, e.g., in U.S. Patent 6,127,332.

Other IFN-β variants are mature IFN-β molecules lacking the initiator methionine, e.g., methionine 1 of SEQ ID NO: 4. Exemplary IFN-β variants lack an iniatiator methionine and have at least one amino acid substitution, e.g., at position 17 of the mature form, as disclosed in U.S. patent No. 4,588,585.

IFN-β molecules can also be modified by replacing one or more amino acids with one or more derivatized amino acids, which are natural or nonnatural amino acid in which

the normally occurring side chain or end group is modified by chemical reaction. Such modifications include, for example, gamma-carboxylation, beta-carboxylation, pegylation, sulfation, sulfonation, phosphorylation, amidization, esterification, N-acetylation, carbobenzylation, tosylation, and other modifications known in the art.

Other modifications include the use of amino acid analogs or derivatized amino acids wherein a side chain is lengthened or shortened while still providing a carboxyl, amino or other reactive precursor functional group for cyclization, as well as amino acid analogs having variant side chains with appropriate functional groups. For instance, the subject compound can include an amino acid analog such as, for example, cyanoalanine, canavanine, djenkolic acid, norleucine, 3-phosphoserine, homoserine, dihydroxy-phenylalanine, 5-hydroxytryptophan, 1-methylhistidine, 3-methylhistidine, diaminopimelic acid, ornithine, or diaminobutyric acid. Other naturally occurring amino acid metabolites or precursors having side chains which are suitable herein will be recognized by those skilled in the art and are included in the scope of the present invention.

Other IFN-β variants include reversed or retro peptide sequences. A "reversed" or "retro" peptide sequence refers to that part of an overall sequence of covalently-bonded amino acid residues (or analogs or mimetics thereof) wherein the normal carboxyl-to amino direction of peptide bond formation in the amino acid backbone has been reversed such that, reading in the conventional left-to-right direction, the amino portion of the peptide bond precedes (rather than follows) the carbonyl portion. See, generally, Goodman, M. and Chorev, M. Accounts of Chem. Res. 1979, 12, 423. The reversed orientation peptides described herein include (a) those wherein one or more amino-terminal residues are converted to a reversed ("rev") orientation (thus yielding a second "carboxyl terminus" at the left-most portion of the molecule), and (b) those wherein one or more carboxyl-terminal residues are converted to a reversed ("rev") orientation (yielding a second "amino terminus" at the right-most portion of the molecule). A peptide (amide) bond cannot be formed at the interface between a normal orientation residue and a reverse orientation residue. Therefore, certain reversed polypeptides of the invention can be formed by utilizing an appropriate amino acid mimetic moiety to link the two adjacent portions of the sequences utilizing a reversed peptide (reversed amide) bond. In case (a) above, a central residue of a diketo compound may conveniently be utilized to link structures with two amide bonds to achieve a peptidomimetic structure. In case (b) above, a central residue of a diamino compound will likewise be useful to link structures with two amide bonds to form a peptidomimetic

structure. The reversed direction of bonding in such polypeptides will generally, in addition, require inversion of the enantiomeric configuration of the reversed amino acid residues in order to maintain a spatial orientation of side chains that is similar to that of the non-reversed peptide. The configuration of amino acids in the reversed portion of the peptides is preferably (D), and the configuration of the non-reversed portion is preferably (L). Opposite or mixed configurations are acceptable when appropriate to optimize a binding activity. Modifications of polypeptides are further described, e.g., in U.S. Patent No. 6,399,075.

IFN-β therapeutics also include IFN-β proteins and variants thereof (e.g., a mature protein) fused to one or more heterologous polypeptides. A heterologous polyeptide may be added, e.g., for the purpose of prolonging the half-life of the IFN-β protein or improving its production. Exemplary heterologous polypeptides include immunoglobulin (Ig) molecules or portions thereof, e.g., the constant domain of a light or heavy chain of an Ig molecule. In one embodiment, an IFN-β protein or variant thereof is fused or otherwise linked to all or part of the hinge and constant regions of an immunoglobulin light chain, heavy chain, or both. Thus, this invention features a molecule which includes: (1) an IFN-β protein moiety (i.e., an IFN-β or variant thereof), (2) a second peptide, e.g., one which increases solubility or *in vivo* life time of the IFN-β moiety, e.g., a member of the immunoglobulin super family or fragment or portion thereof, e.g., a portion or a fragment of IgG, e.g., the human IgG1 heavy chain constant region, e.g., CH2, CH3, and hinge regions. Specifically, an "IFN-β/Ig fusion" is a protein comprising a biologically active IFN-β moiety linked to the N-terminus of an immunoglobulin chain. A species of IFN-β/Ig fusion is an "IFN-β /Fc fusion" which is a protein comprising an IFN-β moiety linked to at least a portion of the constant domain of an immunoglobulin. A preferred Fc fusion comprises an IFN-β moiety linked to a fragment of an antibody containing the C terminal domain of the heavy immunoglobulin chains.

A fusion protein may comprise an IFN-β polypeptide or variant thereof to which heterologous polypeptides are linked to both its N- and its C-termini. A heterologous polypeptide can also be internal to the IFN-beta polypeptide of variant thereof.

In one embodiment, a fusion protein has the generic formula X-Y-Z, wherein X is a polypeptide having an amino acid sequence of IFN-β, or portion or variant thereof; Y is an optional linker moiety; and Z is a polypeptide comprising at least a portion of a polypeptide

other than the interferon beta of moiety X. In other embodiments, the fusion protein has the formula Z-Y-X, in which the non-IFN-β polypeptide is fused to the N-terminal portion of the linker which is fused to the N-terminal portion of the IFN-β polypeptide or portion or variant thereof. Moiety Z can be a portion of a polypeptide that contains immunoglobulin-like domains. Examples of such other polypeptides include CD1, CD2, CD4, and members of class I and class II major histocompatability antigens. See U.S. 5,565,335 (Capon et al.) for examples of such polypeptides.

Moiety Z can include, for instance, a plurality of histidine residues or, preferably, the Fc region of an immunoglobulin, "Fc" defined herein as a fragment of an antibody containing the C terminal domain of a heavy immunoglobulin chain.

Moiety Y can be any linker that permits the IFN-β moiety to retain its biological activity. Moiety Y can be one amino acid long or at least two amino acids long. Y can also be from about 2 to about 5 amino acids; from about 3 to about 10 amino acid long or 10 or more amino acids. In a preferred embodiment, Y consists of or comprises GlyGlyGlyGlySer (SEQ ID NO: 6), which is encoded, e.g., by the nucleotide sequence GGCGGTGGTGGCAGC (SEQ ID NO: 5). Y can also consist of or comprise an enterokinase recognition site, e.g., AspAspAspAspLys (SEQ ID NO: 8), which is encoded by, e.g., GACGATGATGACAAG (SEQ ID NO: 7). In another embodiment, Y consists of or comprises SerSerGlyAspAspAspAspLys (SEQ ID NO: 10), which is encoded, e.g., by AGCTCCGGAGACGATGATGACAAG (SEQ ID NO: 9).

Moreover, the coupling between the IFN-β moiety (X) and the second, non-IFN-β moiety Z (e.g., an Fc region of an immunoglobulin) can also be effected by any chemical reaction that will bind the two molecules together so long as the X and Z moieties essentially retain their respective activities. This chemical linkage can include many chemical mechanisms such as covalent binding, affinity binding, intercalation, coordinate binding and complexation. Representative coupling agents (i.e., linkers "Y" in the generic formula) to develop covalent binding between the IFN-β moiety and Z moiety can include organic compounds such as thioesters, carbodiimides, succinimide esters, diisocyanates such as tolylene-2,6-diisocyanate, gluteraldehydes, diazobenzenes and hexamethylene diamines such as bis-(p-diazonium-benzoyl)-ethylenediamine, bifunctional derivatives of imidoesters such as dimethyl adipimidate, and bis-active fluorine compounds such as 1,5-difluoro-2,4-dinitrobenzene. This listing is not intended to be exhaustive of the various classes of chemical coupling agents known in the art. Many of these are commercially

available such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide hydrochloride (EDC); 4-succinimidyloxycarbonyl-alpha-methyl-alpha-(2-pyridyl-dithio)-toluene (SMPT: Pierce Chem. Co., Cat. # 21558G).

A preferred IFN-β /Ig fusion protein consists of or comprises SEQ ID NO: 12, which contains the full length mature form of human IFN-β, i.e., SEQ ID NO: 4, fused to human IgG1Fc (ZL5107) (see WO 00/23472 and USSN 09/832,659) (see Fig. 1). The corresponding nucleotide sequence is set forth in SEQ ID NO: 11. The DNA encoding human IFN-β ends at nucleotide triplet 568-570 (AAC encoding an arginine) and DNA encoding a human IgG1 constant region starts at the triplet (GAC encoding an aspartic acid) beginning with nucleotide number 574 of SEQ ID NO: 11.

Another preferred IFN-β/Ig fusion protein is set forth in SEQ ID NO: 14 and encoded by SEQ ID NO: 13 (see WO 00/23472 and USSN 09/832,659) (see Fig. 2). This latter fusion protein consists of human IFN-β linked to the G4S linker that is itself linked to human IgG1Fc (ZL6206). The G4S linker (encoded by nucleotides 571 to 585 of SEQ ID NO: 7) consists of the amino acid sequence GGGGS (SEQ ID NO: 9). Methods for producing these proteins are described in WO 00/23472 and USSN 09/832,659.

In a preferred embodiment, the IFN-β polypeptide is fused via its C-terminus to at least a portion of the Fc region of an immunoglobulin. The IFN-β forms the amino-terminal portion, and the Fc region forms the carboxy terminal portion. In these fusion proteins, the Fc region is preferably limited to the constant domain hinge region and the CH2 and CH3 domains. The Fc region in these fusions can also be limited to a portion of the hinge region, the portion being capable of forming intermolecular disulfide bridges, and the CH2 and CH3 domains, or functional equivalents thereof. These constant regions may be derived from any mammalian source (preferably human) and may be derived from any appropriate class and/or isotype, including IgA, IgD, IgM, IgE and IgG1, IgG2, IgG3 and IgG4.

Recombinant nucleic acid molecules which encode the Ig fusions may be obtained by any method known in the art (Maniatis et al., 1982, Molecular Cloning; A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.) or obtained from publicly available clones. Methods for the preparation of genes which encode the heavy or light chain constant regions of immunoglobulins are taught, for example, by Robinson, R. et al., PCT Application, Publication No. WO87/02671. The cDNA sequence encoding the

interferon molecule or fragment may be directly joined to the cDNA encoding the heavy Ig contant regions or may be joined via a linker sequence. In further embodiments of the invention, a recombinant vector system may be created to accommodate sequences encoding interferon beta in the correct reading frame with a synthetic hinge region. Additionally, it may be desirable to include, as part of the recombinant vector system, nucleic acids corresponding to the 3' flanking region of an immunoglobulin gene including RNA cleavage/polyadenylation sites and downstream sequences. Furthermore, it may be desirable to engineer a signal sequence upstream of the immunoglobulin fusion protein-encoding sequences to facilitate the secretion of the fused molecule from a cell transformed with the recombinant vector.

The present invention provides for dimeric fusion molecules as well as monomeric or multimeric molecules comprising fusion proteins. Such multimers may be generated by using those Fc regions, or portions thereof, of Ig molecules which are usually multivalent such as IgM pentamers or IgA dimers. It is understood that a J chain polypeptide may be needed to form and stabilize IgM pentamers and IgA dimers. Alternatively, multimers of IFN-β fusion proteins may be formed using a protein with an affinity for the Fc region of Ig molecules, such as Protein A. For instance, a plurality of IFN-β/ immunoglobulin fusion proteins may be bound to Protein A-agarose beads.

These polyvalent forms are useful since they possess multiple interferon beta receptor binding sites. For example, a bivalent soluble IFN-β may consist of two tandem repeats of amino acids 1 to 166 of SEQ ID NO: 4 (or those encoded by nucleic acids numbered 1 to 498 of SEQ. ID. NO: 3) (moiety X in the generic formula) separated by a linker region (moiety Y), the repeats bound to at least a portion of an immunoglobulin constant domain (moiety Z). Alternate polyvalent forms may also be constructed, for example, by chemically coupling IFN-β/Ig fusions to any clinically acceptable carrier molecule, a polymer selected from the group consisting of Ficoll, polyethylene glycol or dextran using conventional coupling techniques. Alternatively, IFN-β may be chemically coupled to biotin, and the biotin-interferon beta Fc conjugate then allowed to bind to avidin, resulting in tetravalent avidin/biotin/interferon beta molecules. IFN-β/Ig fusions may also be covalently coupled to dinitrophenol (DNP) or trinitrophenol (TNP) and the resulting conjugate precipitated with anti-DNP or anti-TNP-IgM, to form decameric conjugates with a valency of 10 for interferon beta receptor binding sites

Derivatives of proteins of the invention also include various structural forms of the primary protein which retain biological activity. Due to the presence of ionizable amino and carboxyl groups, for example, IFN-β proteins and variants thereof may be in the form of acidic or basic salts, or may be in neutral form. Individual amino acid residues may also be modified by oxidation or reduction. Further, the primary amino acid structure (including the N- and/or C-terminal ends) or the glycan of the IFN-β may be modified ("derivatized") by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, polyalkylene glycol polymers such as polyethylene glycol, lipids, phosphate, acetyl groups and the like, or by creating amino acid sequence mutants.

Other derivatives of interferon beta/ Ig include covalent or aggregative conjugates of interferon beta or its fragments with other proteins or polypeptides, such as by synthesis in recombinant culture as additional N-termini, or C-termini. For example, the conjugated peptide may be a signal (or leader) polypeptide sequence at the N-terminal region of the protein which co-translationally or post-translationally directs transfer of the protein from its site of synthesis to its site of function inside or outside of the cell membrane or wall (e.g., the yeast *alpha* -factor leader). For example, the signal peptide can be that of IFN-β, i.e., amino acids 1-21 of SEQ ID NO: 2, corresponding to nucleotides 76-138 of SEQ ID NO: 1. The signal peptide can also be that of VCAM, i.e., amino acids 1-24 of SEQ ID NO: 12, which is encoded by nucleotides 1-72 of SEQ ID NO: 11.

A heterologous polypeptide (e.g., peptide) or other molecule may also be used as a label or for helping in the purification of the IFN-β therapeutic. Such peptides are well known in the art. For example, the polynucleotide of the present invention may be fused in frame to a marker sequence, also referred to herein as "Tag sequence" encoding a "Tag peptide," which allows for marking and/or purification of the polypeptide of the present invention. In a preferred embodiment, the marker sequence is a hexahistidine tag, e.g., supplied by a PQE-9 vector. Numerous other Tag peptides are available commercially. Other frequently used Tags include myc-epitopes (e.g., see Ellison et al. (1991) *J Biol Chem* 266:21150-21157), which includes a 10-residue sequence from c-myc, the pFLAG system (International Biotechnologies, Inc.), the pEZZ-protein A system (Pharmacia, NJ), and a 16 amino acid portion of the *Haemophilus influenza* hemagglutinin protein. Furthermore, any polypeptide can be used as a Tag so long as a reagent, e.g., an antibody

interacting specifically with the Tag polypeptide is available or can be prepared or identified.

In one embodiment, an IFN-β protein or variant thereof is fused at the N- or C-terminus with one of the following peptides: HisHisHis HisHisHis (SEQ ID NO: 16), which may be encoded by the nucleotide sequence CATCATCATCATCATCAT (SEQ ID NO: 15); SerGlyGlyHisHisHisHisHisHis (SEQ ID NO: 18), which may be encoded by the nucleotide sequence TCCGGGGGCCATCATCATCATCATCAT (SEQ ID NO: 15) and SerGlyGlyHisHisHisHisHisHisSerSerGlyAspAspAspAspLys (SEQ ID NO: 20), which may be encoded by the nucleotide sequence TCCGGGGGCCATCATCATCATCATCATAGCTCCGGAGACGATGATGACAAG (SEQ ID NO: 19).

The amino acid sequence of interferon beta can also be linked to the peptide AspTyrLysAspAspAspAspLys (DYKDDDDK) (SEQ ID NO: 21) (Hopp et al., Bio/Technology 6:1204,1988). The latter sequence is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. This sequence is also specifically cleaved by bovine mucosal enterokinase at the residue immediately following the Asp-Lys pairing.

In another embodiment, an IFN-β therapeutic comprises an IFN-β protein or variant thereof fused to an albumin protein, variant or portion thereof. Such a fusion protein can be created as described in, e.g., WO 01/77137.

IFN-β therapeutics may also include a molecule that is not a polypeptide. For example, an IFN-β protein or variant thereof can be linked covalently or not covalently to a polymer, e.g., a biodegradable polymer. For example, an IFN-β protein or variant thereof can be pegylated, e.g., linked to polyethylene glycol (PEG), as described in WO 00/23114.

Within the broad scope of the present invention, a single polymer molecule may be employed for conjugation with an IFN-β, although it is also contemplated that more than one polymer molecule can be attached as well. It will be recognized that the conjugating polymer may utilize any groups, moieties, or other conjugated species, as appropriate to the end use application. By way of example, it may be useful in some applications to covalently bond to the polymer a functional moiety imparting UV-degradation resistance, or antioxidation, or other properties or characteristics to the polymer. As a further example, it may be advantageous in some applications to functionalize the polymer to render it

reactive or cross-linkable in character, to enhance various properties or characterisics of the overall conjugated material. Accordingly, the polymer may contain any functionality, repeating groups, linkages, or other constitutent structures which do not preclude the efficacy of the conjugated IFN-β composition for its intended purpose.

The IFN-β is conjugated most preferably via a terminal reactive group on the polymer although conjugations can also be branched from the non-terminal reactive groups. The polymer with the reactive group(s) is designated herein as "activated polymer." The reactive group selectively reacts with free amino or other reactive groups on the protein. The activated polymer(s) are reacted so that attachment may occur at any available IFN-β amino group such as the alpha amino groups or the epsilon-amino groups of lysines. Free carboxylic groups, suitably activated carbonyl groups, hydroxyl, guanidyl, oxidized carbohydrate moieties and mercapto groups of the IFN-β (if available) can also be used as attachment sites.

Although the polymer may be attached anywhere on the IFN-β molecule or variant thereof or other amino acid linked directly or indirectly to the IFN-β molecule, the most preferred site for polymer coupling is the N-terminus of the IFN-β molecule. Secondary site(s) are at or near the C-terminus and through sugar moieties. Thus, the invention contemplates as its most preferred embodiments: (i) N-terminally coupled polymer conjugates of IFN-β or variant thereof; (ii) C-terminally coupled polymer conjugates of IFN-β or variant thereof ; (iii) sugar-coupled conjugates of polymer conjugates; (iv) as well as N-, C- and sugar-coupled polymer conjugates of IFN-β proteins or variants thereof.

Generally from about 1.0 to about 10 moles of activated polymer per mole of protein, depending on protein concentration, is employed. The final amount is a balance between maximizing the extent of the reaction while minimizing non-specific modifications of the product and, at the same time, defining chemistries that will maintain optimum activity, while at the same time optimizing, if possible, the half-life of the protein. Preferably, at least about 50% of the biological activity of the protein is retained, and most preferably 100% is retained.

The reactions may take place by any suitable method used for reacting biologically active materials with inert polymers, preferably at about pH 5-7 if the reactive groups are on the alpha amino group at the N-terminus. Generally the process involves preparing an

activated polymer (that may have at least one terminal hydroxyl group) and thereafter reacting the protein with the activated polymer to produce the soluble protein suitable for formulation. The above modification reaction can be performed by several methods, which may involve one or more steps.

As mentioned above, the most preferred embodiments of the invention utilize the N-terminal end of IFN-β as the linkage to the polymer. Suitable methods are available to selectively obtain an N-terminally modified IFN-β. One method is exemplified by a reductive alkylation method which exploits differential reactivity of different types of primary amino groups (the epsilon amino groups on the lysine versus the amino groups on the N-terminal methionine) available for derivatization on IFN-β. Under the appropriate selection conditions, substantially selective derivatization of IFN-β at its N-terminus with a carbonyl group containing polymer can be achieved. The reaction is performed at a pH which allows one to take advantage of the pKa differences between the epsilon-amino groups of the lysine residues and that of the alpha-amino group of the N-terminal residue of IFN-β. This type of chemistry is well known to persons with ordinary skill in the art.

For example, a reaction scheme can be used in which this selectivity is maintained by performing reactions at low pH (generally 5-6) under conditions where a PEG-aldehyde polymer is reacted with IFN-β in the presence of sodium cyanoborohydride. After purification of the PEG-IFN-β and analysis with SDS-PAGE, MALDI mass spectrometry and peptide sequencing/mapping, this resulted in an IFN-β whose N-terminus is specifically targeted by the PEG moiety.

The crystal structure of IFN-β indicates that the N- and C-termini are located close to each other (see Karpusas et al., 1997, Proc. Natl. Acad. Sci. 94: 11813-11818). Thus, modifications of the C- terminal end of IFN-β should also have minimal effect on activity. While there is no simple chemical strategy for targeting a polyalkylene glycol polymer such as PEG to the C-terminus, it would be straightforward to genetically engineer a site that can be used to target the polymer moiety. For example, incorporation of a Cys at a site that is at or near the C-terminus would allow specific modification using a maleimide, vinylsulfone or haloacetate- activated polyalkylene glycol (e.g., PEG). These derivatives can be used specifically for modification of the engineered cysteines due to the high selectively of these reagents for Cys. Other strategies such as incorporation of a histidine tag which can be targeted (Fancy et al., (1996) Chem. & Biol. 3: 551) or an additional glycosylation site, represent other alternatives for modifying the C-terminus of IFN-β.

The glycan on the IFN-β is also in a position that would allow further modification without altering activity. Methods for targeting sugars as sites for chemical modification are also well known and therefore it is likely that a polyalkylene glycol polymer can be added directly and specifically to sugars on IFN-β that have been activated through oxidation. For example, a polyethyleneglycol-hydrazide can be generated which forms relatively stable hydrazone linkages by condensation with aldehydes and ketones. This property has been used for modification of proteins through oxidized oligosaccharide linkages. See Andresz, H. et al., (1978), Makromol. Chem. 179: 301. In particular, treatment of PEG-carboxymethyl hydrazide with nitrite produces PEG-carboxymethyl azide which is an electrophilically active group reactive toward amino groups. This reaction can be used to prepare polyalkylene glycol-modified proteins as well. See, U.S. Patents 4,101,380 and 4,179,337.

Thiol linker-mediated chemistry can further facilitate cross-linking of proteins. This can be performed, e.g., by generating reactive aldehydes on carbohydrate moieties with sodium periodate, forming cystamine conjugates through the aldehydes and inducing cross-linking via the thiol groups on the cystamines (see Pepinsky, B. et al., (1991), J. Biol. Chem., 266: 18244-18249 and Chen, L.L. et al., (1991) J. Biol. Chem., 266: 18237-18243). Accordingly, this type of chemistry is expected to be appropriate for modification with polyalkylene glycol polymers where a linker is incorporated into the sugar and the polyalkylene glycol polymer is attached to the linker. While aminothiol or hydrazine-containing linkers will allow for addition of a single polymer group, the structure of the linker can be varied so that multiple polymers are added and/or that the spatial orientation of the polymer with respect to the IFN-β is changed.

Exemplary polymers include water soluble polymer such as a polyalkylene glycol polymer. A non-limiting list of such polymers include other polyalkylene oxide homopolymers such as polypropylene glycols, polyoxyethylenated polyols, copolymers thereof and block copolymers thereof. Other examples of suitable water-soluble and non-peptidic polymer backbones include poly(oxyethylated polyol), poly(olefinic alcohol), poly(vinylpyrrolidone), poly(hydroxypropylmethacrylamide), poly(α-hydroxy acid), poly(vinyl alcohol), polyphosphazene, polyoxazoline, poly(N-acryloylmorpholine) and copolymers, terpolymers, and mixtures thereof. In one embodiment, the polymer backbone is poly(ethylene glycol) or monomethoxy polyethylene glycol (mPEG) having an average molecular weight from about 200 Da to about 400,000 Da. It should be understood that

other related polymers are also suitable for use in the practice of this invention and that the use of the term PEG or poly(ethylene glycol) is intended to be inclusive and not exclusive in this respect. The term PEG includes poly(ethylene glycol) in any of its forms, including alkoxy PEG, difunctional PEG, multi-armed PEG, forked PEG, branched PEG, pendent PEG, or PEG with degradable linkages therein.

In one embodiment, polyalkylene glycol residues of C1-C4 alkyl polyalkylene glycols, preferably polyethylene glycol (PEG), or poly(oxy)alkylene glycol residues of such glycols are incorporated in the polymer systems of interest. Thus, the polymer to which the protein is attached can be a homopolymer of polyethylene glycol (PEG) or is a polyoxyethylated polyol, provided in all cases that the polymer is soluble in water at room temperature. Non-limiting examples of such polymers include polyalkylene oxide homopolymers such as PEG or polypropylene glycols, polyoxyethylenated glycols, copolymers thereof and block copolymers thereof, provided that the water solubility of the block copolymer is maintained. Examples of polyoxyethylated polyols include, for example, polyoxyethylated glycerol, polyoxyethylated sorbitol, polyoxyethylated glucose, or the like. The glycerol backbone of polyoxyethylated glycerol is the same backbone occurring naturally in, for example, animals and humans in mono-, di-, and triglycerides. Therefore, this branching would not necessarily be seen as a foreign agent in the body.

As an alternative to polyalkylene oxides, dextran, polyvinyl pyrrolidones, polyacrylamides, polyvinyl alcohols, carbohydrate-based polymers and the like may be used. Those of ordinary skill in the art will recognize that the foregoing list is merely illustrative and that all polymer materials having the qualities described herein are contemplated.

The polymer can be of any particular molecular weight, but it is preferred that the molecular weight be between about 300 and 100,000, more preferably between 10,000 and 40,000. In particular, sizes of 20,000 or more are best at preventing protein loss due to filtration in the kidneys.

Polyalkylene glycol derivatization has a number of advantageous properties in the formulation of polymer-IFN-β conjugates in the practice of the present invention, as associated with the following properties of polyalkylene glycol derivatives: improvement of aqueous solubility, while at the same time eliciting no antigenic or immunogenic

response; high degrees of biocompatibility; absence of *in vivo* biodegradation of the polyalkylene glycol derivatives; and ease of excretion by living organisms.

Moreover, in another aspect of the invention, one can utilize IFN-β covalently bonded to the polymer component in which the nature of the conjugation involves cleavable covalent chemical bonds. This allows for control in terms of the time course over which the polymer may be cleaved from the IFN-β. This covalent bond between the IFN-β drug and the polymer may be cleaved by chemical or enzymatic reaction. The polymer-IFN-β product retains an acceptable amount of activity. Concurrently, portions of polyethylene glycol are present in the conjugating polymer to endow the polymer-IFN-β conjugate with high aqueous solubility and prolonged blood circulation capability. As a result of these improved characteristics the invention contemplates parenteral, nasal, and oral delivery of both the active polymer-IFN-β species and, following hydrolytic cleavage, bioavailability of the IFN-β per se, in *in vivo* applications.

The reaction of the polymer with the IFN-β to obtain conjugates, e.g., N-terminal conjugated products, can be readily carried out using a wide variety of reaction schemes. The activity and stability of the IFN-β conjugates can be varied in several ways, by using a polymer of different molecular size. Solubilities of the conjugates can be varied by changing the proportion and size of the polyethylene glycol fragment incorporated in the polymer composition.

In one embodiment, conjugates according to the present invention are prepared by reacting a protein with an activated polyaklylene glycol compound (PCG). For example, IFN can be reacted with a PEG-aldehyde in the presence of a reducing agent (*e.g.*, sodium cyanoborohydride) via reductive alkylation to produce a PEG-protein conjugate, attached via an amine linkage. *See, e.g.*, European Patent 0154316 B1 and International Patent Application No. PCT/US03/01559.

In certain embodiments of the invention, human IFN-β is PEGylated with the following activated polyalkylene glycols: 20 kDa mPEG-O-2-methylpropionaldehyde, 20 kDa mPEG-O-*p*-methylphenyl-O-2-methylpropionaldehyde, 20 kDa mPEG-O-*m*-methylphenyl-O-2-methylpropionaldehyde, 20 kDa mPEG-O-*p*-phenylacetaldehyde, 20 kDa mPEG-O-*p*-phenylpropionaldehyde, and 20 kDa mPEG-O-*m*-phenylacetaldehyde to obtain 20 kDa mPEG-O-2-methylpropionaldehyde-modified IFN-β, 20 kDa mPEG-O-*p*-methylphenyl-O-2-methylpropionaldehyde-modified IFN-β, 20 kDa mPEG-O-*m*-

methylphenyl-O-2-methylpropionaldehyde-modified IFN-β, 20 kDa mPEG-O-*p*-phenylacetaldehyde-modified IFN-β, 20 kDa mPEG-O-*p*-phenylpropionaldehyde-modified IFN-β, and 20 kDa mPEG-O-*m*-phenylacetaldehyde-modified IFN-β, respectively. A detailed description of the preparation and characterization of human IFN-β modified with 20 kDa mPEG-O-2-methylpropionaldehyde and 20 kDa mPEG-O-*p*-phenylacetaldehyde is set forth below and is also provided in International Patent Application No. PCT/US03/01559.

In one embodiment, a pegylated IFN-β is prepared as follows. IFN-β, e.g., nonformulated AVONEX® (IFN-β-1a bulk intermediate, (a clinical batch of bulk drug that passed all tests for use in humans), at 250 μg/ml in 100 mM sodium phosphate pH 7.2, 200 mM NaCl) is diluted with an equal volume of 100 mM MES pH 5.0, and the pH was adjusted to 5.0 with HCl. The sample is loaded onto an SP-Sepharose® FF column (Pharmacia, Piscataway, NJ) at 6 mg IFN-β/ml resin. The column is washed with 5 mM sodium phosphate pH 5.5, 75 mM NaCl, and the product is eluted with 30 mM sodium phosphate pH 6.0, 600 mM NaCl. Elution fractions can be analyzed for their absorbance values at 280 nm and the concentration of interferon in the samples estimated from the absorbance using an extinction coefficient of 1.51 for a 1 mg/ml solution.

To a 1 mg/ml solution of the IFN-β from the SP eluate, 0.5 M sodium phosphate pH 6.0 is added to 50 mM, sodium cyanoborohydride (Aldrich, Milwaukee, WI) is added to 5 mM, and 20K PEG aldehyde (Shearwater Polymers, Huntsville, AL) is added to 5 mg/ml. The sample is incubated at room temperature for 20 hours. The pegylated interferon is purified from reaction products by sequential chromatography steps on a Superose® 6 FPLC sizing column (Pharmacia) with 5 mM sodium phosphate pH 5.5, 150 mM NaCl as the mobile phase and SP-Sepharose® FF. The sizing column results in base line separation of modified and unmodified IFN-β. The PEG-interferon beta-containing elution pool from gel filtration is diluted 1:1 with water and loaded at 2 mg interferon beta /ml resin onto an SP-Sepharose® column. The column is washed with 5 mM sodium phosphate pH 5.5, 75 mM NaCl and then the pegylated interferon beta is eluted from the column with 5 mM sodium phosphate pH 5.5, 800 mM NaCl. Elution fractions are analyzed for protein content by absorbance at 280 nm. The pegylated interferon concentration is reported in interferon equivalents as the PEG moiety did not contribute to absorbance at 280 nm. These method and characterization of the pegylated IFN-β obtained are further described in

WO 00/23114. PEG conjugation of IFN-β does not appear to alter its antiviral activity. In addition, the specific activity of pegylated IFN-β was found to be much greater (about 10 times) than that of the non-pegylated IFN-β (WO 00/23114).

IFN-β can also be pegylated with a 5K PEG-aldehyde moiety that can be purchased, e.g., from Fluka, Inc. (Cat. No. 75936, Ronkonkoma, NY) following the same protocol as described above for the 20K PEG aldehyde.

A 20 kDa mPEG-O-2-methylpropionaldehyde-modified IFN-β can be prepared as follows. 10 mL of nonformulated AVONEX® (IFN-β-1a bulk intermediate, (a clinical batch of bulk drug that passed all tests for use in humans), at 250 μg/mL in 100 mM sodium phosphate pH 7.2, 200 mM NaCl) is diluted with 12 mL of 165 mM MES pH 5.0 and 50 μL of 5 N HCl. The sample is loaded onto a 300 μL SP-Sepharose FF column (Pharmacia). The column is washed with 3 × 300 μL of 5 mM sodium phosphate pH 5.5, 75 mM NaCl, and the protein is eluted with 5 mM sodium phosphate pH 5.5, 600 mM NaCl. Elution fractions are analyzed for their absorbance at 280 nm and the concentration of IFN-β in the samples estimated using an extinction coefficient of 1.51 for a 1 mg/mL solution. The peak fractions are pooled to give an IFN-β concentration of 3.66 mg/mL, which is subsequently diluted to 1.2 mg/mL with water.

To 0.8 mL of the IFN-β from the diluted SP-Sepharose eluate pool, 0.5 M sodium phosphate pH 6.0 is added to 50 mM, sodium cyanoborohdride (Aldrich) is added to 5 mM, and 20 kDa mPEG-O-2-methylpropionaldehyde is added to 5 mg/mL. The sample is incubated at room temperature for 16 h in the dark. The PEGylated IFN-β is purified from the reaction mixture on a 0.5 mL SP-Sepharose FF column as follows: 0.6 mL of the reaction mixture is diluted with 2.4 mL 20 mM MES pH 5.0, and loaded on to the SP-Sepharose column. The column is washed with sodium phosphate pH 5.5, 75 mM NaCl and then the PEGylated IFN-β is eluted from the column with 25 mM MES pH 6.4, 400 mM NaCl. The PEGylated IFN-β is further purified on a Superose 6 HR 10/30 FPLC sizing column with 5 mM sodium phosphate pH 5.5, 150 mM NaCl as the mobile phase. The sizing column (25 mL) is run at 20 mL/h and 0.5 mL fractions are collected. The elution fractions are analyzed for protein content by absorbance at 280 nm, pooled, and the protein concentration of the pool determined. The PEGylated IFN-β concentration is reported in IFN equivalents as the PEG moiety does not contribute to absorbance at 280 nm. Samples of the pool are removed for analysis, and the remainder can be diluted to 30

μg/mL with HSA-containing formulation buffer, aliquoted at 0.25 mL/vial, and stored at −70 °C.

20 kDa mPEG-O-*p*-phenylacetaldehyde-modified IFN-β can be prepared as follows. 20 mL of nonformulated AVONEX® (IFN-β bulk intermediate, a clinical batch of bulk drug that passed all tests for use in humans, at 250 μg/mL in 100 mM sodium phosphate pH 7.2, 200 mM NaCl) is diluted with 24 mL of 165 mM MES pH 5.0, 100 μL of 5 N HCl, and 24 mL water. The sample is loaded onto a 600 μL SP-Sepharose FF column (Pharmacia). The column is washed with 2 × 900 μL of 5 mM sodium phosphate pH 5.5, 75 mM NaCl, and the protein is eluted with 5 mM sodium phosphate pH 5.5, 600 mM NaCl. Elution fractions are analyzed for their absorbance at 280 nm and the concentration of IFN-β in the samples was estimated using an extinction coefficient of 1.51 for a 1 mg/mL solution. The peak fractions are pooled to give an IFN-β concentration of 2.3 mg/mL. To 1.2 mL of the IFN-β-1a from the SP-Sepharose eluate pool, 0.5 M sodium phosphate pH 6.0 is added to 50 mM, sodium cyanoborohdride (Aldrich) is added to 5 mM, and 20 kDa mPEG-O-*p*-phenylacetaldehyde, is added to 10 mg/mL. The sample is incubated at room temperature for 18 h in the dark. The PEGylated IFN-β can be purified from the reaction mixture on a 0.75 mL SP-Sepharose FF column as follows: 1.5 mL of reaction mixture is diluted with 7.5 mL 20 mM MES pH 5.0, 7.5 mL water, and 5 μL 5 N HCl, and loaded onto the SP-Sepharose column. The column is washed with sodium phosphate pH 5.5, 75 mM NaCl and then the PEGylated IFN-β is eluted from the column with 20 mM MES pH 6.0, 600 mM NaCl. The PEGylated IFN-β is further purified on a Superose 6 HR 10/30 FPLC sizing column with 5 mM sodium phosphate pH 5.5, 150 mM NaCl as the mobile phase. The sizing column (25 mL) is run at 20 mL/h and 0.5 mL fractions are collected. The elution fractions are analyzed for protein content by absorbance at 280 nm, pooled, and the protein concentration of the pool determined. The PEGylated IFN-β concentration is reported in IFN equivalents after adjusting for the contribution of the PEG (20 kDa mPEG-O-*p*-phenylacetaldehyde has an extinction coefficient at 280 nm of 0.5 for a 1 mg/mL solution) to the absorbance at 280 nm using an extinction coefficient of 2 for a 1 mg/mL solution of the PEGylated IFN-β. Samples of the pool can be removed for analysis, and the remainder can be diluted to 30 μg/mL with HSA-containing formulation buffer, aliquoted at 0.25 mL/vial, and stored at −70 °C.

Glycosylated IFN-β coupled to a non-naturally occurring polymer can be used in the methods of the invention. The polymer may comprise a polyalkylene glycol moiety. The polyalkylene moiety may be coupled to the interferon-beta by way of a group selected from an aldehyde group, a maleimide group, a vinylsulfone group, a haloacetate group, plurality of histidine residues, a hydrazine group and an aminothiol group. IFN-β may be coupled to a polyethylene glycol moiety, wherein the IFN-β is coupled to the polyethylene glycol moiety by a labile bond, wherein the labile bond is cleavable by biochemical hydrolysis and/or proteolysis. The polymer may have a molecular weight of from about 5 to about 40 kilodaltons. Another IFN-β that may be used is a physiologically active interferon-beta composition comprising a physiologically active glycosylated interferon-beta N-terminally coupled to a polymer comprising a polyalkylene glycol moiety, wherein the physiologically active interferon-beta and the polyalkylene glycol moiety are arranged such that the physiologically active interferon-beta in the physiologically active interferon-beta composition has substantially similar activity relative to physiologically active interferon-beta lacking said moiety, when measured by an antiviral assay.

Heterologous polypeptides or other molecules can be covalently or non-covalently linked to an IFN-β protein or variant thereof. "Covalently coupled" means that the different moieties of the invention are either directly covalently bonded to one another, or else are indirectly covalently joined to one another through an intervening moiety or moieties, such as a bridge, spacer, or linkage moiety or moieties. The intervening moiety or moieties are called a "coupling group." The term "conjugated" is used interchangeably with "covalently coupled."

IFN-βs for use in the invention can be glycosylated or non-glycosylated (or unglycosylated). Non-glycosylated IFN-βs can be produced, e.g., in a prokaryotic host cell.

IFN-β proteins or variants thereof can also be modified by attaching polysaccharides that are not normally present on IFN-βs.

3. Methods of producing IFN-β therapeutics

The IFN-β therapeutics of the present invention can be produced by any suitable methods, such as methods including constructing a nucleic acid encoding an IFN-β therapeutic and expressing this nucleic acid in a suitable transformed host. This method

will produce recombinant IFN-β therapeutics. IFN-β therapeutics may also be produced by chemical synthesis or a combination of chemical synthesis and recombinant DNA technology.

In one embodiment, a nucleic acid encoding an IFN-β therapeutic is constructed by isolating or synthesizing a DNA sequence encoding an IFN-β or variant thereof. For example, an IFN-β fusion protein can be produced as described, e.g., herein. A naturally-occurring IFN-β nucleic acid can be obtained according to methods well known in the art. For example, a nucleic acid can be isolated by reverse transcriptase-polymerase chain reaction (RT-PCR) using RNA obtained from a cell known to express IFN-β, e.g., a leukocyte, and primers based on the sequence of the IFN-β gene, e.g., SEQ ID NO: 1. Nucleic acids encoding IFN-β proteins can also be isolated by screening libraries, e.g., cDNA libraries made from cells expressing IFN-β, with a probe, e.g., an oligonucleotide comprising a portion of an IFN-β sequence.

Alternatively, the complete amino acid sequence may be used to construct a back-translated gene. A DNA oligomer containing a nucleotide sequence coding for IFN-β therapeutic may be synthesized. For example, several small oligonucleotides coding for portions of the desired polypeptide may be synthesized and then ligated together. The individual oligonucleotides typically contain 5' or 3' overhangs for complementary assembly.

Changes can be introduced into nucleic acids encoding IFN-β proteins by methods well known in the art. For example, changes can be made by site-specific mutagenesis, as described in, e.g., Mark et al., "Site-specific Mutagenesis Of The Human Fibroblast Interferon Gene", Proc. Natl. Acad. Sci. USA, 81, pp. 5662-66 (1984) and U.S. Pat. No. 4,588,585.

Another method of constructing a nucleic acid encoding an IFN-β therapeutic is via chemical synthesis. For example, a gene that encodes the desired IFN-β therapeutic may be synthesized by chemical means using an oligonucleotide synthesizer. Such oligonucleotides are designed based on the amino acid sequence of the desired IFN-β therapeutic.

When choosing a nucleic acid for expression in an expression system, it may be desirable to select those codons that are favored in the host cell or expression system in

which the recombinant IFN-β therapeutic will be produced. It is known, e.g., that certain codons are expressed preferably over others in prokaryotic cells ("codon preference").

A DNA sequence encoding an IFN-β therapeutic may or may not also include a DNA sequence that encodes a signal sequence. Such signal sequence, if present, should be one recognized by the cell chosen for expression of the IFN-β therapeutic. The signal sequence may be prokaryotic, eukaryotic or a combination of the two. Signal sequences are well known in the art, and several different ones are described in the art. The signal sequence may be that of a native (i.e., naturally-occurring) IFN-β. The inclusion of a signal sequence depends on whether it is desired to have the IFN-β therapeutic secreted from the recombinant cells in which it is produced. If the chosen cells are prokaryotic, it generally is preferred that the DNA sequence not encode a signal sequence. If the chosen cells are eukaryotic, it generally is preferred that a signal sequence be encoded and most preferably that the wild-type IFN-β signal sequence be used.

Once assembled (by synthesis, site directed mutagenesis or another method), the nucleic acid encoding an IFN-β therapeutic is inserted into an expression vector, in which it is operatively linked to an expression control sequence appropriate for expression of the IFN-β therapeutic in the desired transformed host. Proper assembly may be confirmed by nucleotide sequencing, restriction mapping, and expression of a biologically active polypeptide in a suitable host or host cell. As is well known in the art, in order to obtain high expression levels of a transfected gene in a host or host cell, the gene must be operatively linked to transcriptional and translational expression control sequences that are functional in the chosen expression host.

The choice of expression control sequence and expression vector will depend upon the choice of host cell. A wide variety of expression host/vector combinations may be employed. Useful expression vectors for eukaryotic hosts, e.g., eukaryotic host cells, include, for example, vectors comprising expression control sequences from SV40, bovine papilloma virus, adenovirus and cytomegalovirus, e.g., the following vectors: pcDNAI/amp, pcDNAI/neo, pRc/CMV, pSV2gpt, pSV2neo, pSV2-dhfr, pTk2, pRSVneo, pMSG, pSVT7, pko-neo and pHyg derived vectors. Alternatively, derivatives of viruses such as the bovine papillomavirus (BPV-1), or Epstein-Barr virus (pHEBo, pREP-derived and p205) can be used for transient expression of proteins in eukaryotic cells. The various methods employed in the preparation of the plasmids and transformation of host organisms

are well known in the art. For other suitable expression systems, see Molecular Cloning A Laboratory Manual, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1989) Chapters 16 and 17.

Useful expression vectors for bacterial hosts include known bacterial plasmids, such as plasmids from E. coli, including col E1, pCR1, pBR322, pMB9 and their derivatives, wider host range plasmids, such as RP4, phage DNAs, e.g., the numerous derivatives of phage lambda, e.g., NM989, and other DNA phages, such as M13 and filamentous single stranded DNA phages. Useful expression vectors for yeast cells include the 2.mu. plasmid and derivatives thereof. Useful vectors for insect cells include pVL 941. See also, Cate et al., "Isolation Of The Bovine And Human Genes For Mullerian Inhibiting Substance And Expression Of The Human Gene In Animal Cells", Cell, 45, pp. 685-98 (1986).

In addition, any of a wide variety of expression control sequences may be used in these vectors. Such useful expression control sequences include the expression control sequences associated with structural genes of the foregoing expression vectors. Examples of useful expression control sequences include, for example, the early and late promoters of SV40 or adenovirus, the lac system, the trp system, the TAC or TRC system, the major operator and promoter regions of phage lambda, for example PL, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, e.g., Pho5, the promoters of the yeast alpha-mating system and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof.

Any suitable host may be used to produce IFN-β therapeutics, including bacteria, fungi (including yeasts), plant, insect, mammal, or other appropriate animal cells or cell lines, as well as transgenic animals or plants. Exemplary hosts include strains of E. coli, Pseudomonas, Bacillus, Streptomyces, fungi, yeast, insect cells such as Spodoptera fruaiperda (SF9), animal cells such as Chinese hamster ovary (CHO) and mouse cells such as NS/0, African green monkey cells such as COS 1, COS 7, BSC 1, BSC 40, and BMT 10, and human cells, as well as plant cells in tissue culture. Such cells can be obtained from the American Type Culture Collection (ATCC). Preferred host cells for animal cell expression include cultured CHO cells and COS 7 cells and particularly the CHO-DDUKY-β1 cell line.

It should of course be understood that not all vectors and expression control sequences will function equally well to express the DNA sequences described herein. Neither will all hosts function equally well with the same expression system. However, one of skill in the art may make a selection among these vectors, expression control sequences and hosts without undue experimentation. The vector's copy number, the ability to control that copy number, and the expression of any other proteins encoded by the vector, such as antibiotic markers, should also be considered. For example, preferred vectors for use in this invention include those that allow the DNA encoding the IFN-β therapeutic to be amplified in copy number. Such amplifiable vectors are well known in the art. They include, for example, vectors able to be amplified by DHFR amplification (see, e.g., Kaufman, U.S. Pat. No. 4,470,461, Kaufman and Sharp, "Construction Of A Modular Dihydrafolate Reductase cDNA Gene: Analysis Of Signals Utilized For Efficient Expression", Mol. Cell. Biol., 2, pp. 1304-19 (1982)) or glutamine synthetase ("GS") amplification (see, e.g., U.S. Pat. No. 5,122,464 and European published application 338,841).

In selecting an expression control sequence, a variety of factors should also be considered. These include, for example, the relative strength of the sequence, its controllability, and its compatibility with the actual DNA sequence encoding the IFN-β therapeutic, particularly as regards potential secondary structures. Hosts should be selected by consideration of their compatibility with the chosen vector, the toxicity of the product coded for by the DNA sequences of this invention, their secretion characteristics, their ability to fold the polypeptides correctly, their fermentation or culture requirements, and the ease of purification of the products coded for by the DNA sequences.

Within these parameters, one of skill in the art may select various vector/expression control sequence/host combinations that will express the desired DNA sequences on fermentation or in large scale animal culture, for example, using CHO cells or COS 7 cells. Use of the CHO cell line CHO-KUKX-B1 DHFR sup for expressing IFN-β variants is further described in U.S. Patent No. 6,127,332.

An IFN-β therapeutic can also be produced in an *in vitro* system, e.g., in a *in vitro* translation system, e.g., cell lysate, e.g., a reticulocyte lysate. The term "*in vitro* translation system", which is used herein interchangeably with the term "cell- free translation system" refers to a translation system which is a cell-free extract containing at least the minimum elements necessary for translation of an RNA molecule into a protein. *In vitro* translation

systems typically comprise macromolecules, such as enzymes, translation, initiation and elongation factors, chemical reagents, and ribosomes. For example, an *in vitro* translation system may comprise at least ribosomes, ^{t}RNAs, initiator methionyl-^{t}RNA^{Met}, proteins or complexes involved in translation, e.g., eIF_2, eIF_3, the cap-binding (CB) complex, comprising the cap-binding protein (CBP) and eukaryotic initiation factor 4F (eIF_{4F}). A variety of *in vitro* translation systems are well known in the art and include commercially available kits. Examples of *in vitro* translation systems include eukaryotic lysates, such as rabbit reticulocyte lysates, rabbit oocyte lysates, human cell lysates, insect cell lysates and wheat germ extracts. Lysates are commercially available from manufacturers such as Promega Corp., Madison, Wis.; Stratagene, La Jolla, Calif.; Amersham, Arlington Heights, Ill.; and GIBCO/BRL, Grand Island, N.Y. RNA for use in *in vitro* translation systems can be produced *in vitro*, e.g., using SP6 or T7 promoters, according to methods known in the art.

In another method, an IFN-β therapeutic is expressed from the endogenous gene in a host cell. The method may comprise inserting a heterologous promoter upstream of the coding region of the IFN-β gene, e.g., an inducible promoter, expressing the endogenous IFN-β gene and recovering the IFN-β produced. A heterologous promoter can be introduced into cells by "knock-in," according to methods known in the art, or alternatively, by insertion of the promoter within the IFN-β gene.

The IFN-β therapeutic obtained according to the present invention may be glycosylated or unglycosylated depending on the host organism used to produce the therapeutic. If bacteria are chosen as the host, then the IFN-β therapeutic produced will be unglycosylated. Eukaryotic cells, on the other hand, will glycosylate the IFN-β therapeutics.

The IFN-β therapeutic produced by the transformed host can be purified according to any suitable method. Various methods are known for purifying IFN-β. See, e.g., U.S. Pat. Nos. 4,289,689, 4,359,389, 4,172,071, 4,551,271, 5,244,655, 4,485,017, 4,257,938, 4,541,952 and 6,127,332. In a preferred embodiment, the IFN-β therapeutic is purified by immunoaffinity, as described, e.g., in Okamura et al., "Human Fibroblastoid Interferon: Immunosorbent Column Chromatography And N-Terminal Amino Acid Sequence." Biochem., 19, pp. 3831-35 (1980).

For example, the IFN-β proteins and variants thereof may be isolated and purified in accordance with conventional conditions, such as extraction, precipitation, chromatography, affinity chromatography, electrophoresis or the like. For example, the interferon proteins and fragments may be purified by passing a solution thereof through a column having an interferon receptor immobilized thereon (see U.S. Pat. No. 4,725,669). The bound interferon molecule may then be eluted by treatment with a chaotropic salt or by elution with aqueous acetic acid. The immunoglobulin fusion proteins may be purified by passing a solution containing the fusion protein through a column which contains immobilized protein A or protein G which selectively binds the Fc portion of the fusion protein. See, for example, Reis, K. J., et al., J. Immunol. 132:3098-3102 (1984); PCT Application, Publication No. W087/00329. The chimeric antibody may then be eluted by treatment with a chaotropic salt or by elution with aqueous acetic acid.

Alternatively the interferon proteins and immunoglobulin-fusion molecules may be purified on anti-interferon antibody columns, or on anti-immunoglobulin antibody columns to give a substantially pure protein. By the term "substantially pure" is intended that the protein is free of the impurities that are naturally associated therewith. Substantial purity may be evidenced by a single band by electrophoresis.

IFN-β that has been produced and purified can be characterized, e.g., by peptide mapping. For example, an IFN-β therapeutic sample can be digested with endoproteinase Lys-C and analyzed on a reverse phase HPLC, as described, e.g., in U.S. Patent No. 6,127,332.

In a preferred embodiment, the IFN-β therapeutic is substantially free of other cellular material, e.g., proteins. The terms "substantially pure" or "purified preparations of an IFN-β therapeutic" refers to preparations of the an IFN-β therapeutic having less than about 20% (by dry weight) contaminating cellular material, e.g., nucleic acids, proteins, and lipids, and preferably having less than about 5% contaminating cellular material. Preferred preparations of the IFN-β therapeutic have less than about 2% contaminating cellular material; even more preferably less than about 1% contaminating cellular material and most preferably less than about 0.5; 0.2; 0.1; 0.01; 0.001% contaminating cellular material.

Preferred IFN-β therapeutic compositions are also substantially free of other cellular proteins (also referred to herein as "contaminating proteins"), i.e., the compositions have less than about 20% (by dry weight) contaminating protein, and preferably having less than

about 5% contaminating protein. Preferred preparations of the subject polypeptides have less than about 2% contaminating protein; even more preferably less than about 1% contaminating protein and most preferably less than about 0.5; 0.2; 0.1; 0.01; 0.001% contaminating proteins.

The purity and concentration of IFN-β preparations can be determined according to methods known in the art, e.g., by subjecting samples to gel electrophoresis, and as described, e.g., in Robert K. Scopes, Protein Purification, Principles and Practice, Third Ed., Springer Verlag New York, 1993, and references cited therein.

The biological activity of IFN-β therapeutics can be assayed by any suitable method known in the art, e.g., antibody neutralization of antiviral activity, induction of protein kinase, oligoadenylate 2,5-A synthetase or phosphodiesterase activities, e.g., as described in EP-B1-41313 and WO 00/23472. Such assays also include immunomodulatory assays (see, e.g., U.S. Pat. No. 4,753,795), growth inhibition assays, and measurement of binding to cells that express interferon receptors. Exemplary antiviral assays are further described in U.S. Patent 6,127,332 and WO 00/23472.

The ability of IFN-β therapeutics to treat glomerulonephritis can also be assessed in animal models, e.g., those described in the Examples and further herein. The testing can be conducted, e.g., as described in the Examples.

IFN-β therapeutics can also be purchased commercially under the following brand names: AVONEX® (IFN-β-1a) (Biogen, Inc., Cambridge, MA); REBIF® (IFN-β-1a) (Serono, S.A., Geneva, Switzerland); and BETAFERON® (IFN-β-1b) (Schering Aktiengesellschaft, Berlin, Germany), which is also marketed as BETASERON® (Berlex, Montville, NJ; IFN-β-1b). AVONEX® and REBIF® are recombinant wild-type human glycosylated IFN-β produced in Chinese hamster ovary cells. BETAFERON® is produced in bacteria.

4. Methods of treatment with IFN-β therapeutics

The invention provides methods for treating or preventing a neuropathy in a subject, comprising administering to the subject a therapeutically effective amount of an IFN-β therapeutic, optionally as an adjunct to another therapy. In one embodiment, the neuropathy is a demyelinating neuropathy, such as a chronic demyelinating neuropathy.

Examples of chronic demyelinating neuropathies include CIDP and multifocal motor neuropathy. In a preferred embodiment, the neuropathy is CIDP.

The subject may be a subject who has been identified as having a neuropathy. A subject can be diagnosed with a neuropathy according to methods known in the art. In particular, diagnosis of CIDP can be done according to methods known in the art, e.g., as further described herein. The treatment with an IFN-β therapeutic can be started at any time in a person diagnosed with the neuropathy. A treatment can also be started in a subject that does not appear to have the neuropathy, but is likely to develop it. Such subjects can be identified, e.g., by genetic criteria. Subjects likely to develop the neuropathy also include subjects having some but not all symptoms typically associated with the neuropathy, such that in certain instances it may not be clear whether the subject will in fact develop the neuropathy. Treatments can be conducted for at least about 1 month, at least about 3 months, at least about 6 months, at least about 1 year, at least about 3 years, at least about 5 years or longer.

A subject can be an animal, such as a mammal. Examples of mammals include humans, bovines, ovines, porcines, equines, canines, felines, non-human primates, mice and rats.

In certain embodiments, an IFN-β therapeutic is administered to a subject as an adjunct therapy, i.e., to a subject who is also receiving another treatment. For example, a person having CIDP may be treated by the administration of IVIg; steroids, such as prednisolone; or an immunosuppressive agent, such as azothioprine, cyclosporin or cyclophosphamide; or by plasma exchange, in addition to receiving an IFN-β therapeutic. Combination therapy with an IFN-β therapeutic may minimize the use of the other treatment, which other treatment may be more harmful (e.g., steroids), more expensive (e.g., IVIg) or more inconvenient (plasma exchange). Accordingly, in certain embodiments, administration of an IFN-β therapeutic to a subject permits one to decrease the dose and/or frequency of the other treatment. For example, doses and/or frequencies can be reduced by at least about 10%, 30%, 50%, 75%, 100% (i.e., two fold), five fold, 10 fold or more. Set forth below, are current standards of care for CIDP.

Accordingly, in one embodiment, the invention provides a method for treating a chronic demyelinating neuropathy, e.g., CIDP in a subject receiving a first CIDP treatment selected from the group consisting of administration of a steroid; administration of an anti-

inflammatory drug; administration of IVIg; and plasmapheresis, the improvement comprising administering to the subject, in addition to the first CIDP treatment, a dose of an IFN-β therapeutic in an amount effective to significantly reduce the dose or frequency of the first CIDP treatment, to provide effective relief from symptoms of CIDP.

Immunosuppressants are currently used for treating CIDP. For example, steroids have been found to be beneficial in CIDP. A favorable response is usually seen within 4 weeks. One steroid that is commonly used is Prednisone (Deltasone, Orasone, Meticorten). Prednisone is an oral corticosteroid that suppresses inflammation and immune responses and is believed to alter mediator function at site of inflammation and suppressing immune responses in CIDP. Although doses vary; most adult patients are started on 0.5 to 1 mg/kg/day PO (i.e., by mouth) initially (about 30-40 to 60-80 mg/day). Improvement can be anticipated within the next 2 months. Later, the dosing may be converted to alternate-day treatment and then titrated to lowest effective dose that allows maintaining a patient in remission.

Another immunosuppressant that have been found effective in treating CIDP is Azathioprine (Imuran), which is a purine analog that decreases metabolism of purines and also may inhibit DNA and RNA synthesis. Azathioprine is believed to reduce disability and symptoms of CIDP by suppressing immune-mediated damage to nerves. The initial dose is about 50 mg PO qd (by mouth, daily), which is usually increased gradually to a total daily dosage of 2-3 mg/kg/day PO. Although therapeutic doses of azathioprine are difficult to determine for each patient; some evidence suggests that elevations of red blood cell volume (MCV) indicate therapeutic dosing. Therapeutic responses may take more than 6 months to become apparent.

Yet another immunosuppressant that is currently used for treating CIDP is Mycophenolate (CellCept), which is a prodrug for immunosuppressive agent mycophenolic acid. Mycophenolate is believed to Inhibit lymphocyte purine synthesis by inhibiting enzyme inosine monophosphate dehydrogenase. The typical dose for an adult is 250 mg to 3 g/day, with an adjustment of the dose depending on clinical effect.

Cyclosporine (Sandimmune, Neoral), which is a cyclic polypeptide consisting of 11 amino acids can also be used for treating CIDP. Cyclosporine inhibits first phase of T cell activation and does not affect humoral immunity. It is believed that by suppressing T cells, cyclosporine may inhibit cell-mediated nerve damage at site of inflammatory/immune

reaction. Usually, it is administered at 5 mg/kg/day PO divided bid (by mouth twice a day) initially and the dose is increased according to the response. Trough and peak levels should be monitored to register efficacy and avoid toxicity; although no definitive desirable trough level has been identified specifically for CIDP, usual trough levels utilized for immunologic disorders are between 100 and 250.

Another immunosuppressant is cyclophosphamide (Cytoxan), which is a cell-cycle phase-nonspecific antineoplastic agent and immunosuppressant that acts as alkylating agent. The dose is typically 1-2 mg/kg/d PO.

Another standard method of treatment of CIDP patients is by intravenous immunoglobulin (IVIg) administration. The solution for IV infusion is typically composed mostly of heterogenous human IgG but also small amounts of IgA and IgM. In certain embodiments, the IV solution is heterogeneous with regard to the epitopes recognized by the antibodies, e.g., it is not a preparation of antibody obtained from immunization of an animal with a particular antigen. Its proposed mechanism of action is based on the thought that IVIg contains random sets of antibodies that would neutralize immune factors, causing damage to peripheral nerve in CIDP. On average, improvement is seen by day 10 and continues through day 42. The serum half-life approximately 21-29 days. Patients usually require repeated treatments every few weeks or months to maintain remission or treat recurrences. The common does is between 0.4 and 2 g/kg, usually divided into as many as 5 daily doses of 400 mg/kg. Treatment may be initiated at a higher dose, e.g., 2g/kg and later reduced to a lower dose, e.g., 0.5 to 1 g/kg. The frequency of administration of these doses varies, with most patients receiving doses every 2 to 8 weeks. For example, IVIG can be administered at 0.4 g/kg over several days to 1 to 2 g/kg every 1 to 4 weeks.

Another approved method of treatment of CIDP is plasmapheresis (or plasma exchange). This treatment is believed to remove antibodies and complement components that are responsible for immune-mediated damage of peripheral nerves. The plasma is removed from the blood through a method similar to dialysis. Its efficacy appears to be similar to that of IVIg in treatment of CIDP. Commonly, patients undergo 3 plasma exchanges per week for the first two weeks; after that, the number and frequency of treatments is determined by clinical response.

Accordingly, an IFN-β therapeutic can be administered to a subject together with administration of an immunosuppressant, e.g., a steroid; with administration of IVIg and/or

with plasmapheresis. The IFN-β therapeutic and the adjunct drug can be administered simultaneously or consecutively. If consecutively, it may be administered the same day or on different days. When an IFN-β therapy is combined with plasmapheresis, the IFN-β therapeutic may be administered after the plasmapheresis, such that no IFN-β therapeutic is removed during the plasmapheresis. When an IFN-β therapy is combined with an IVIg therapy, administration of the two different drugs is preferably separated by at least one or two hours.

In another embodiment, an IFN-β therapeutic is administered to subjects who were found to be refractory to another CIDP therapy, such as those set forth above. In other situations, an IFN-β therapeutic is administered to subjects who were not found to be refractory to another CIDP therapy. For example, an IFN-β therapeutic may be administered to subjects who were responsive to one or more other therapies. In yet other embodiments, an IFN-β therapeutic is administered to subjects who are naïve, i.e., have not had any treatment for CIDP previously. When a subject had not previously received treatment for CIDP, the methods of treatment with an IFN-β therapeutic may comprise first identifying a subject as having CIDP or as likely to develop CIDP.

A treatment may also be as follows: an IFN-β therapeutic is administered to a person receiving a first CIDP treatment, such as IVIG, and the combination treatment is continued for a certain amount of time, following which the first CIDP treatment is discontinued. The first CIDP treatment may be discontinued progressively, such as by reducing the dose and/or frequency of the first CIDP treatment. In an illustrative embodiment, a subject is receiving IVIG once every two weeks or once every four weeks. The subject then receives, in combination with the IVIG treatment, an IFN treatment, such as weekly or biweekly administration of an IFN-β therapeutic. The combined treatment may be continued for about 10-20 weeks, e.g., about 16 weeks. During the combined treatment, it may be preferable to separate the administrations of IVIg and the IFN-β therapeutic by at least about 1 to 3 hours, such by at least about 2 hours. After about 16 weeks, the first CIDP treatment is interrupted or reduced. For example, the subject may then receive smaller doses of IVIg or less frequent administrations. In individuals who do not improve following the discontinuation or reduction of the first CIDP treatment, the first CIDP treatment may be reinstated and a combination treatment with an IFN-β therapeutic pursued.

Generally, IFN-β therapeutics may be administered by any route. For example, IFN-β therapeutics may be provided to an individual directly, e.g., locally, as by injection or topical administration to a tissue locus or systemically, e.g., parenterally or orally. Local administration includes, e.g., administration directly into an affected muscle. Parenteral administration includes aerosol, subcutaneous, intravenous, intramuscular, intra-articular, intrasynovial, intraperitoneal, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. Administration may be by periodic injections of a bolus of IFN-β therapeutic, or it may be made more continuous by intravenous or intraperitoneal administration from a reservoir which is external (e.g., an i.v. bag) or internal (e.g., a bioerodable implant or implanted pump).

The IFN-β therapeutics are preferably administered as a sterile pharmaceutical composition containing a pharmaceutically acceptable carrier. The term "carrier" as used herein includes acceptable adjuvants and vehicles. Pharmaceutically acceptable carriers that may be used in the pharmaceutical compositions of this invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as prolamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol, wool fat, dextrose, glycerol, ethanol and the like or combinations thereof. IFN-β therapeutics may be prepared in a composition comprising one or more other proteins, e.g., for stabilizing the IFN-β therapeutic. For example, IFN-β therapeutics can be mixed with albumin.

Where the IFN-β therapeutic is to be provided parenterally the agent preferably comprises part of an aqueous solution. The solution is physiologically acceptable so that in addition to delivery of the desired IFN-β therapeutic to the subject, the solution does not otherwise adversely affect the subject's electrolyte and/or volume balance. The aqueous medium for the IFN-β therapeutic thus may comprise normal physiologic saline (e.g., 0.9% NaCl, 0.15M, pH 7-7.4). Useful solutions for parenteral administration may be prepared by

any of the methods well known in the pharmaceutical art, described, for example, in Remington's Pharmaceutical Sciences (Gennaro, A., ed.), Mack Pub., 1990.

Pharmaceutical compositions may be in the form of a sterile injectable preparation, for example a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as do natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant.

Pharmaceutical compositions comprising IFN-β therapeutics may also be given orally. For example, they can be administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers that are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added. Topically-transdermal patches may also be used.

The pharmaceutical compositions of this invention may also be administered by nasal aerosol or inhalation through the use of a nebulizer, a dry powder inhaler or a metered dose inhaler. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

IFN-β therapeutics can be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles.

Liposomes can be formed from a variety of phospholipids, containing cholesterol, stearylamine, or phosphatidylcholines. In some embodiments, a film of lipid components is hydrated with an aqueous solution of drug to a form lipid layer encapsulating the drug, as described in U.S. Pat. No. 5,262,564. Liposomes may contain surface molecules that direct them to particular cells or tissues. Such modified liposomes can be prepared according to methods known in the art.

IFN-βs or variants thereof may also be coupled to soluble polymers as targetable drug carriers. Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxypropyl-methacrylamide-phenol, polyhydroxyethylaspanamidephenol, or polyethyleneoxidepolylysine substituted with palmitoyl residues. IFN-βs or variants thereof can also be coupled to proteins, such as, for example, receptor proteins and albumin. Furthermore, the IFN-βs or variants thereof may be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates and cross-linked or amphipathic block copolymers of hydrogels.

An IFN-β therapeutic may also be provided as a liquid composition comprising a stabilizing agent. The stabilizing agent may be present at an amount of between 0.3% and 5% by weight of the IFN-β therapeutic. The stabilizing agent may be an amino acid, such as an acidic amino acid (e.g., glutamic acid and aspartic acid) or arginine or glycine. If the stabilizing agent is arginine-HCl, its concentration will preferably range between 0.5% (w/v) to 5% and is most preferably 3.13% (equivalent to 150 mM arginine-HCl). If the stabilizing agent is glycine, its concentration will preferably range between 0.5% (w/v) to 2.0% and most preferably 0.52% (equivalent to 66.7 mM to 266.4 mM, and most preferably 70 mM). If the stabilizing agent is glutamic acid, its concentration will preferably range between 100 mM to 200 mM, and is most preferably 170 mM (equivalent to a w/v percent ranging from 1.47% to 2.94% and most preferably 2.5%). In certain embodiments, the range of concentrations of IFN-β therapeutics in the liquid formulations is from about 30 μg/ml to about 250 μg/ml, such as 48 to 78 μg/ml, e.g., about 60 μg/ml. This amount will depend, e.g., on the specific activity of the particular IFN-β therapeutic. Generally, the range of doses will be from about 1 million international unit (MIU) to about 50 MIU, e.g., about 3, 6, 9, or 12 MIU per dose.

In one embodiment, the amino acid stabilizing agent is arginine, which is incorporated as its acidic form (arginine-HCl) in about pH 5.0 solutions. Accordingly, poly-ionic excipients are preferred in this instance. The liquid composition may be contained within a vessel, e.g., a syringe, in which the vessel has a surface in contact with the liquid that is coated with a material that is inert to IFN-β, e.g., silicone or polyetrafluoroethylene. Preferred compositions have a pH between 4.0 and 7.2. In certain embodiments, the solution comprising the stabilizing agent has not been lyophilized and/or has not been subject to oxygen containing gas during preparation and storage.

Organic acid and phosphate buffers to be used in the present invention to maintain the pH in the range of about 4.0 to about 7.2, such as from about 4.5 to about 5.5, e.g., 5,0, can be conventional buffers of organic acids and salts thereof such as citrate buffers (e.g., monosodium citrate-disodium citrate mixture, citric acid-trisodium citrate mixture, citric acid-monosodium citrate mixtures, etc.), succinate buffers (e.g., succinic acid-monosodium succinate mixture, succinic acid-sodium hydroxide mixture, succinic acid-disodium succinate mixture, etc.), tartrate buffers, fumarate buffers, gluconate buffers, oxalate buffers, lactate buffers, phosphate buffers, and acetate buffers, as further described in WO 98/28007.

Exemplary formulations, which can be prepared as described in WO 98/38007, include:

(i) a 20 mM acetate buffer at pH 5.0, the buffer having preferably not previously been lyophilized, in which the buffer includes IFN-β and at least one ingredient selected from (a) 150 mM arginine-HCl; (b) 100 mM sodium chloride and 70 mM glycine; (c) 150 mM arginine-HCl and 15 mg/ml human serum albumin; (d) 150 mM arginine-HCl and 0.1% Pluronic F-68; (e) 140 mM sodium chloride; (f) 140 mM sodium chloride and 15 mg/ml human serum albumin; and (g) 140 mM sodium chloride and 0.1% Pluronic F-68;

(ii) a liquid at pH 5.0 that includes IFN-β or a variant thereof, 170 mM L-glutamic acid, and 150 mM sodium hydroxide, the liquid preferably not having previously been lyophilized; and

(iii) a 20 mM phosphate buffer at pH 7.2, the buffer having preferably not previously been lyophylized, wherein the buffer includes IFN-β and least one ingredient selected from: (a) 140 mM arginine-HCl and (b) 100 mM sodium chloride and 70 mM glycine.

Prefered compositions also include polysorbate, e.g., at 0.005% w/v polysorbate 20.

IFN-β or a variant thereof can also be administered together with a soluble IFN type I receptor or portion thereof, such as an IFN-binding chain of the receptor, as described, e.g., in U.S. Patent No. 6,372,207. As described in the patent, administration of an IFN type I in the form of a complex with an IFN binding chain of the receptor improves the stability of the IFN and enhances the potency of the IFN. The complex may be a non-covalent complex or a covalent complex.

IFN-βs can be formulated in dry powder form, which may or may not be solubilized or suspended prior to administration to a subject. In particular, it has been shown that IFN-βs conjugated to a polymer, e.g., PEG are particularly stable in dry form (see, e.g., WO 00/23114 and PCT/US/95/06008).

The formulated compositions may contain therapeutically effective amounts of an IFN-β therapeutic, i.e., they may contain amounts of an IFN-β therapeutic that provides appropriate concentrations of the IFN-β therapeutic to the muscular tissues or other appropriate tissues for a time sufficient to prevent, inhibit, delay or alleviate permanent or progressive loss of muscular function, or otherwise provide therapeutic efficacy. As will be appreciated by those skilled in the art, the concentration of the IFN-β therapeutics in a therapeutic composition may vary depending upon a number of factors, including the biological efficacy of the selected IFN-β therapeutic, the chemical characteristics (e.g., hydrophobicity) of the IFN-β therapeutic employed, the formulation of the IFN-β therapeutic excipients, the administration route, and the treatment envisioned, such as whether the IFN-β therapeutic will be administered directly into a tissue or whether it will be administered systemically. The preferred dosage to be administered may also depend on such variables as the conditions of the tissues of the subject, the extent of muscular loss, and the overall health status of the particular subject. It may also depend on the age, weight, sex, general health, diet rate of excretion of the patient, as well as the sensitivity of the subject to side effects and whether the IFN-β therapeutic is coadministered with other drugs. An ordinarily skilled physician or veterinarean can readily determine and prescribe the effective amount of the IFN-β therapeutic required to prevent, counter or arrest the progres of the condition.

Dosages may be administered continuously, or daily, but it is currently preferred that dosages be administered once, twice or three times per week for as long as a

satisfactory response persists (as measured, for example, by stabilization and/or improvement of the disease by appropriate medical markers and/or quality of life indices). Less frequent dosages, for example monthly dosages, may also be employed. In order to facilitate frequent infusions, implantation of a semi-permanent stent (e.g., intravenous, intraperitoneal or intracapsular) may be advisable.

Any of the above pharmaceutical compositions may contain 0.1-99%, 1-70%, or, preferably, 1-50% of IFN-β therapeutic as active ingredients.

For any route of administration, divided or single doses may be used. For example, IFN-β therapeutics may be administered daily or weekly, in a single dose, or the total dosage may be administered in divided doses of two, three or four.

IFN-β therapeutics may be administered at dosage levels of between about 0.001 and about 100 mg/kg body weight per dose, e.g., between about 0.1 and about 50 mg/kg body weight; between about 0.1 mg/kg body weight and about 20 mg/kg body weight; or between about 1 mg/kg body weight and about 3 mg/kg body weight. These doses may be administered at intervals of every 1-14 days, such as every day, every other day, every third day, every fifth day, weekly, or biweekly. Depending in particular on the specific activity of the IFN-β therapeutic, it may also be administered at a dose ranging from about 10 to about 100 μg/dose, such as from about 20 to about 50μg/dose, e.g., at about 30μg/dose.

In terms of international units, an IFN-β therapeutic may be administered at a dose of between about 1 and 30 MIU/dose, such as between 3 and 20 MIU/dose, e.g., between 3 and 12 MIU. Preferred doses include about 3 MIU, 6 MIU and 12 MIU per dose. Such doses are preferably administered about once or twice weekly. Optimization of dosages can be determined, e.g., by administration of the IFN-β therapeutics, followed by assessment of the circulating or local concentration of the IFN-β therapeutic.

In certain embodiments, an IFN-β therapeutic is administered by subcutaneous injection to deliver 0.01-100 μg/kg, or more preferably 0.01-10 μg/kg of IFN-β, e.g., PEGylated IFN-β, over one week, two injections of 0.005-50 μg/kg, or more preferably 0.005-5 μg/kg, respectively, may be administered at 0 and 72 hours. Additionally, one approach for parenteral administration employs the implantation of a slow-release or sustained-released system, which assures that a constant level of dosage is maintained, according to U.S. Pat. No. 3,710,795.

Oral dosages of the present invention, preferably for pegylated IFN-β therapeutics, will range between about 0.01-100 μg/kg/day orally, or more preferably 0.01-10 μg/kg/day orally. The compositions are preferably provided in the form of scored tablets containing 0.5-5000 μg, or more preferably 0.5-500 μg of IFN-β therapeutic. In a preferred embodiment, a subject having a neuropathy, e.g., CIDP is treated with an IFN-β therapeutic by intramuscular administration of the IFN-β therapeutic. The IFN-β therapeutic may be administered weekly. In an even more preferred embodiment, the IFN-β therapeutic is administered weekly to the subject at a dose of about 6 MIU. In other embodiments, a subject is treated by weekly administration of about 6 MIU of IFN-β therapeutic, which administration is not necessarily intramuscular.

In certain embodiments, an IFN-β therapeutic is administered according to one regimen for a certain interval of time, and is then administered according to another regimen. For example, a subject may receive an IFN-β therapeutic weekly for two months and then twice weekly for the following months. A subject can also be treated by subcutaneous administration first and then by intramuscular administration. In other regimens, a particular dose, mode of administration or IFN-therapeutic is alternated with a different dose, mode of administration or IFN-therapeutic for every other administration.

In a most preferred embodiment, the IFN-β therapeutic is AVONEX®. AVONEX® is sold as a lyophilized powder consisting of the following:

Formulation per 1ml dose:
30 mcg interferon-b-1a (6 million international units (MIU))
50mM sodium phosphate
100mM sodium chloride
15mg Human Serum Albumin
pH 7.2

The specific activity of AVONEX® interferon is 2 x 10^8 units/mg, i.e., 200 MU of antiviral activity per milligram of IFN-b-1a protein. The patient reconstitues the powder with sterile water prior to intramuscular injection of the 1ml once per week. AVONEX® can also be prepared as a liquid formulation consisting of the following:

Formulation per 0.5ml dose:
30 mcg (μg) IFN-b-1a (6 million international units (MIU))
20 mM acetate (sodium acetate and acetic acid)

150 mM arginine HCl
0.005% w.v polysorbate 20
water for injection
pH 4.8

This formulation can be packaged in a pre-filled syringe. The patient may either manually use the syringe as provided or use in conjunction with an autoinjector. The dosing schedule is 6 MIU (i.e., 30 mcg) intramuscular once per week.

In another embodiment, the IFN-β is REBIF®, which is provided as a lyophilized powder and as a liquid formulation. The lyophilized powder consists of the following:

Formulation per 2.0ml dose:
3 MIU of IFN-b-1a
mannitol
HSA
Sodium acetate
pH 5.5

The specific activity of REBIF® interferon is 2.7×10^8 units/mg, i.e., 270 MU of antiviral activity per milligram of IFN-b-1a protein. The patient reconstitutes the powder with a sodium chloride solution (0.9% NaCl) prior to injection subcutaneously three times a week. The formulation of liquid REBIF® is as follows:

Formulation per 0.5 ml dose:
6 or 12 MIU IFN-b-1a
4 or 2 mg HSA
27.3 mg mannitol
0.4 mg sodium acetate
water for injection

The liquid formulation is packaged in a pre-filled syringe and administered with or without use of an autoinjector device (Rebiject) 3 times (6 or 12 MIU, corresponding to 66 µg/week or 132 µg/week, respectively) per week subcutaneously.

In yet another embodiment, the IFN-β is BETASERON® (from Berlex), an IFN-β containing a cys-17 to ser mutation that is produced in *E. coli*. This non-glycosylated IFNβ is less potent than AVONEX® or REBIF® which are both produced in CHO cells. Doses are sold as 250 mcg (8 MIU) doses, both in lyophilized and liquid formulations, for injection subcutaneously every other day. BETAFERON® is another commercially

available IFN-β, which can be administered subcutaneously, according to the manufacturer's instructions.

In addition to an IFN-beta therapeutic, and optionally another therapy, subjects may also receive medication for the treatment of neuropathic pain, e.g., antiepileptic medications. The two most frequently used medications are gabapentin (Neurontin) and carbamazepine (Tegretol). Alternatively, tricyclic antidepressants, e.g., amitriptyline (Elavil), can also be used for the treatment of neuropathic pain.

The course of the disease and its response to drug treatments may be followed by clinical examination and laboratory findings. The effectiveness of the therapy of the invention is determined by the extent to which the previously described signs and symptoms of the disease are alleviated and the extent to which the normal side effects of interferon (*i.e.*, flu-like symptoms such as fever, headache, chills, myalgia, fatigue, etc. and central nervous system related symptoms such as depression, paresthesia, impaired concentration, etc.) are eliminated or substantially reduced.

The present invention is further illustrated by the following examples, which should not be construed as limiting in any way. The contents of all cited references (including literature references, issued patents, published patent applications as cited throughout this application) are hereby expressly incorporated by reference.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Molecular Cloning A Laboratory Manual, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1989); DNA Cloning, Volumes I and II (D. N. Glover ed., 1985); Oligonucleotide Synthesis (M. J. Gait ed., 1984); Mullis et al. U.S. Patent No: 4,683,195; Nucleic Acid Hybridization(B. D. Hames & S. J. Higgins eds. 1984); Transcription And Translation (B. D. Hames & S. J. Higgins eds. 1984); Culture Of Animal Cells (R. I. Freshney, Alan R. Liss, Inc., 1987); Immobilized Cells And Enzymes (IRL Press, 1986); B. Perbal, A Practical Guide To Molecular Cloning (1984); the treatise, Methods In Enzymology (Academic Press, Inc., N.Y.); Gene Transfer Vectors For Mammalian Cells (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); Methods In Enzymology, Vols. 154 and 155 (Wu et al. eds.),

Immunochemical Methods In Cell And Molecular Biology (Mayer and Walker, eds., Academic Press, London, 1987); Handbook Of Experimental Immunology, Volumes I-IV (D. M. Weir and C. C. Blackwell, eds., 1986); Manipulating the Mouse Embryo, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986).

Examples

Example 1: Treatment of CIDP patients with IFN-β 1a

CIDP patients that are being treated with IVIg will be switched to an IFN-β 1a treatment as follows.

Patients having an established diagnosis for CIDP and who are being treated with a stable once every two weeks or once every four weeks regimen of IVIgIVIGIVIg will be given one of the following regimens of IFN-β 1a via intramuscular injection: 30 mcg (6 MIU) of AVONEX® once weekly; 30 mcg (6 MIU) of AVONEX® twice weekly; 60 mcg (12 MIU) of AVONEX® once weekly; or 60 mcg (12 MIU) of AVONEX® twice weekly. When administration of IVIg and IFN-β 1a fall on the same day, the administrations will be separated by at least a two hour period. The patients will receive this combination treatment for 16 weeks, during which time, the disease state of the patients will be assessed about every four weeks. At week 16, the IVIg will be discontinued and the IFN-β 1a treatment will be continued following the same regimen as before.

The disease will continue to be monitored in the patients. If patients were doing better with the combination treatment, the IVIg treatment will be reinstated. IVIg may later be reduced by progressively decreasing the amount or frequency of IVIg administrations.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

The claims defining the invention are as follows:

1. A method of treating a chronic demyelinating motor neuropathy in a mammal, comprising administering to the mammal a therapeutically effective amount of an IFN-β therapeutic, wherein the IFN-β therapeutic is administered via a non-subcutaneous parenteral route.

2. The method of claim 1, wherein the IFN-β therapeutic comprises mature IFN-β.

3. The method of claim 1 or claim 2, wherein the IFN-β therapeutic lacks the first methione.

4. The method of any one of claims 1 to 3, wherein the IFN-β is human IFN-β.

5. The method of claim 4, wherein the IFN-β is at least about 95% identical to full length mature human IFN-β having SEQ ID NO:4.

6. The method of claim 5, wherein the IFN-β comprises SEQ ID NO:4.

7. The method of any one of claims 1 to 6, wherein the IFN-β is glycosylated.

8. The method of any one of claims 1 to 6, wherein the IFN-β is not glycosylated.

9. The method of claim 4, wherein the IFN-β is IFN-β-1a.

10. The method of claim 4, wherein the IFN-β is IFN-β-1b.

11. The method of any one of claims 1 to 10, wherein the IFN-β therapeutic comprises IFN-β fused to the constant domain of an immunoglobulin molecule.

12. The method of claim 11, wherein the immunoglobulin molecule is a human immunoglobulin molecule.

13. The method of claim 12, wherein the immunoglobulin molecule is the heavy chain of IgG1.

14. The method of claim 13, wherein the IFN-β comprises SEQ ID NO: 14.

15. The method of any one of claims 1 to 14, wherein the IFN-β therapeutic comprises a pegylated IFN-β.

16. The method of any one of claims 1 to 15, wherein the IFN-β therapeutic comprises a stabilizing agent.

17. The method of claim 16, wherein the stabilizing agent is an acidic amino acid.

18. The method of claim 17, wherein the stabilizing agent is arginine.

19. The method of any one of claims 1 to 18, wherein the IFN-β therapeutic has a pH between about 4.0 and 7.2.

20. The method of any one of claims 1 to 19, comprising administering to the mammal several doses of an IFN-β therapeutic.

21. The method of claim 20, wherein the IFN-β therapeutic is administered weekly at a dose of about 6 MIU.

22. The method of claim 20, wherein the IFN-β therapeutic is administered twice a week at a dose of about 6 MIU.

23. The method of claim 20, wherein the IFN-β therapeutic is administered weekly at a dose of about 12 MIU.

24. The method of claim 20, wherein the IFN-β therapeutic is administered twice a week at a dose of about 12 MIU.

25. The method of any one of claims 1 to 24, wherein the IFN-β therapeutic is administered intramuscularly.

26. The method of any one of claims 1 to 24, wherein the IFN-β therapeutic is administered intravenously (i.v.).

27. The method of any one of claims 1 to 26, wherein the chronic demyelinating motor neuropathy is chronic inflammatory demyelinating neuropathy (CIDP).

28. The method of any one of claims 1 to 27, wherein the mammal is a human.

29. A method of treating CIDP, comprising administering to a subject having CIDP a pharmaceutically effective amount of an IFN-β therapeutic and further administering to the subject an immunosuppressant or subjecting the subject to plasmapheresis, wherein the IFN-β therapeutic is administered via a non-subcutaneous parenteral route.

30. The method of claim 29, comprising administering to the subject an immunosuppressant selected from the group consisting of a steroid, azothioprine, cyclosporin, cyclophosphamide, and mycophenolate.

31. A method of treating CIDP, comprising administering to a subject having CIDP a pharmaceutically effective amount of an IFN-β therapeutic in combination with a second CIDP treatment, wherein administration of the IFN-β therapeutic is via a non-subcutaneous parenteral route.

32. The method of claim 31, wherein the second CIDP treatment is selected from the group consisting of administration IVIg; administration of a steroid; administration of an anti-inflammatory drug and plasmapheresis.

33. A method of treating CIDP, comprising administering to a subject having CIDP a pharmaceutically effective amount of an IFN-β therapeutic in combination with a second CIDP treatment, wherein administration of the IFN-β therapeutic is weekly, and wherein the IFN-β therapeutic is administered via a non-subcutaneous parenteral route.

34. The method of claim 33, wherein the second CIDP treatment is selected from the group consisting of administration of IVIg; administration of a steroid; administration of an anti-inflammatory drug and plasmapheresis.

35. A method of treating CIDP in a subject receiving a first CIDP treatment selected from the group consisting of administration of a steroid; administration of an anti-inflammatory drug; administration of IVIG and plasmapheresis, the improvement comprising administering to the subject, in addition to the first CIDP treatment, a dose of an IFN-β therapeutic in an amount effective to significantly reduce the dose or frequency of the first CIDP treatment, wherein administration of the IFN-β therapeutic is via a non-subcutaneous parenteral route, to provide effective relief from symptoms of CIDP.

36. A method of treating CIDP in a subject receiving a first CIDP treatment selected from the group consisting of administration of a steroid; administration of an anti-inflammatory drug; administration of IVIG and plasmapheresis, the improvement comprising administering to the subject, in addition to the first CIDP treatment, once a week a dose of an IFN-β therapeutic in an amount effective to significantly reduce the dose or frequency of the first CIDP treatment, wherein the IFN-β therapeutic is administered via a non-subcutaneous parenteral route, to provide effective relief from symptoms of CIDP.

37. A method of treating CIDP in a subject receiving a first CIDP treatment selected from the group consisting of administration of a steroid; administration of an anti-inflammatory drug; and plasmapheresis, the improvement comprising administering to the subject, in addition to the first CIDP treatment, a dose of an IFN-β therapeutic in an amount effective to significantly reduce the dose or frequency of the first CIDP treatment, wherein the IFN-β therapeutic is administered via a non-subcutaneous parenteral route, to provide effective relief from symptoms of CIDP.

Date: 10 November 2009

IFNβ G162C-Ig direct fusion construct open reading frame

```
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  61 GCTTCTCAAGCCATGAGCTACAACTTGCTTGGATTCCTACAAAGAAGCAGCAATTTTCAG 120
     A  S  Q  A  M  S  Y  N  L  L  G  F  L  Q  R  S  S  N  F  Q

 121 TGTCAGAAGCTCCTGTGGCAATTGAATGGGAGGCTTGAATACTGCCTCAAGGACAGGATG 180
     C  Q  K  L  L  W  Q  L  N  G  R  L  E  Y  C  L  K  D  R  M

 181 AACTTTGACATCCCTGAGGAGATTAAGCAGCTGCAGCAGTTCCAGAAGGAGGACGCCGCA 240
     N  F  D  I  P  E  E  I  K  Q  L  Q  Q  F  Q  K  E  D  A  A

 241 TTGACCATCTATGAGATGCTCCAGAACATCTTTGCTATTTTCAGACAAGATTCATCTAGC 300
     L  T  I  Y  E  M  L  Q  N  I  F  A  I  F  R  Q  D  S  S  S

 301 ACTGGCTGGAATGAGACTATTGTTGAGAACCTCCTGGCTAATGTCTATCATCAGATAAAC 360
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 361 CATCTGAAGACAGTCCTGGAAGAAAACTGGAGAAGAAGATTTCACCAGGGGAAAACTC 420
     H  L  K  T  V  L  E  E  K  L  E  K  E  D  F  T  R  G  K  L

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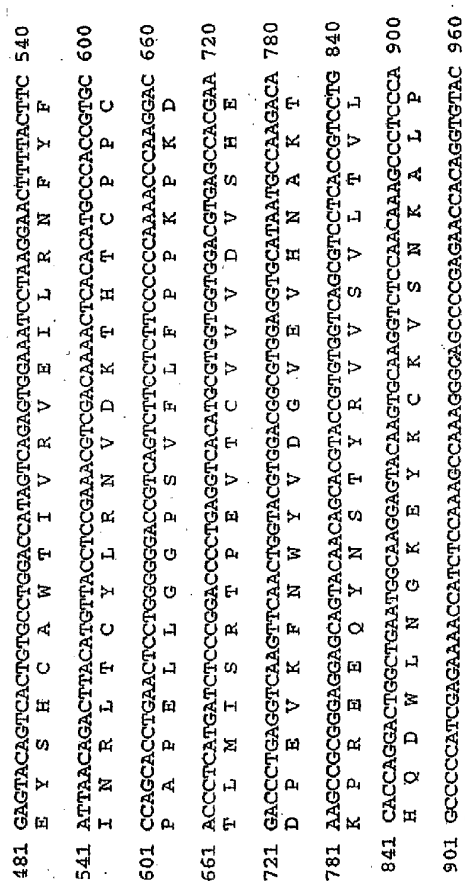
FIG. 1 A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K P R E E Q Y N S T Y R V V S V L T V L
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H Q D W L N G K E Y K C K V S N K A L P
901 GCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTAC 960
A P I E K T I S K A K G Q P R E P Q V Y

FIG. 1 B

```
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1021 AAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC 1080
       K  G  F  Y  P  S  D  I  A  V  E  W  E  S  N  G  Q  P  E  N

1081 AACTACAAGACCACGCCTCCCGTGTTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAG 1140
       N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F  L  Y  S  K

1141 CTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCAT 1200
       L  T  V  D  K  S  R  W  Q  Q  G  N  V  F  S  C  S  V  M  H

1201 GAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCCGGGAAATGA    1257
       E  A  L  H  N  H  Y  T  Q  K  S  L  S  L  S  P  G  K  *
```

FIG. 1 C

IFNβ G162C-Ig fusion G4S linker construct open reading frame

```
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 121 TGTCAGAAGCTCCTGTGGCAATTGAATGGGAGGCTTGAATACTGCCTCAAGGACAGGATG 180
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 181 AACTTTGACATCCCTGAGGAGATTAAGCAGCTGCAGCAGTTCCAGAAGGAGGACGCCGCA 240
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 241 TTGACCATCTATGAGATGCTCCAGAACATCTTTGCTATTTTCAGACAAGATTCATCTAGC 300
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 301 ACTGGCTGGAATGAGACTATTGTTGAGAACCTCCTGGCTAATGTCTATCATCAGATAAAC 360
     T  G  W  N  E  T  I  V  E  N  L  L  A  N  V  Y  H  Q  I  N
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FIG. 2A

```
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481 GAGTACAGTCACTGTGCCTGGACCATAGTCAGAGTGGAAATCCTAAGGAACTTTTACTTC 540
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541 ATTAACAGACTTACATGTTACCTCCGAAACGGCGGTGGTGGCAGCGTCGACAAAACTCAC 600
    I  N  R  L  T  C  Y  L  R  N  G  G  G  G  S  V  D  K  T  H

601 ACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCC 660
    T  C  P  P  C  P  A  P  E  L  L  G  G  P  S  V  F  L  F  P

661 CCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTG 720
    P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V  V

721 GACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTG 780
    D  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V

781 CATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGC 840
    H  N  A  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S

841 GTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCC 900
    V  L  T  V  L  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S

901 AACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA 960
    N  K  A  L  P  A  P  I  E  K  T  I  S  K  A  K  G  Q  P  R
```

FIG. 2B

```
961  GAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGC 1020
       E  P  Q  V  Y  T  L  P  P  S  R  D  E  L  T  K  N  Q  V  S

1021 CTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAAT 1080
       L  T  C  L  V  K  G  F  Y  P  S  D  I  A  V  E  W  E  S  N

1081 GGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGTTGGACTCCGACGGCTCCTTC 1140
       G  Q  P  E  N  N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F

1141 TTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCA 1200
       F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q  G  N  V  F  S

1201 TGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCT 1260
       C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K  S  L  S  L  S

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       P  G  K  *
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FIG. 2C

SEQUENCE LISTING

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Ala Leu Thr Ile Tyr Glu Met Leu Gln Asn Ile Phe Ala Ile Phe Arg
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Gln Asp Ser Ser Ser Thr Gly Trp Asn Glu Thr Ile Val Glu Asn Leu
95 100 105

ctg gct aat gtc tat cat cag ata aac cat ctg aag aca gtc ctg gaa 447
Leu Ala Asn Val Tyr His Gln Ile Asn His Leu Lys Thr Val Leu Glu
110 115 120

gaa aaa ctg gag aaa gaa gat ttc acc agg gga aaa ctc atg agc agt 495
Glu Lys Leu Glu Lys Glu Asp Phe Thr Arg Gly Lys Leu Met Ser Ser
125 130 135 140

ctg cac ctg aaa aga tat tat ggg agg att ctg cat tac ctg aag gcc 543
Leu His Leu Lys Arg Tyr Tyr Gly Arg Ile Leu His Tyr Leu Lys Ala
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175 180 185

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Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

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Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

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His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

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            20                  25                  30

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        35                  40                  45

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    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
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