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(54) **Title**: THERAPEUTIC INHIBITION OF GRANULOCYTE FUNCTION IN DEMYELINATING DISEASE

(57) **Abstract**: Compositions and methods are provided for the treatment of IL-17-type inflammatory demyelinating diseases with inhibitors of granulocyte function, e.g. elastase inhibitors. Diseases of interest include multiple sclerosis, neuromyelitis optica, animal models of such diseases, etc. In some embodiments pharmaceutical formulations comprising an elastase inhibitor in an effective dose for treatment of IL-17-type inflammatory demyelinating disease and a pharmaceutically acceptable excipient are provided. Patients may be classified into subtypes prior to treatment, which subtypes are informative of the patient's need for therapy and responsiveness to a therapy of interest.

THERAPEUTIC INHIBITION OF GRANULOCYTE FUNCTION IN DEMYELINATING DISEASE

BACKGROUND OF THE INVENTION

[01] There is a long-standing interest in manipulating cells of the immune system to achieve control of autoimmune disease. While targeted antigen-specific therapy remains of great interest, there has also been considerable development of polyclonal, or non-antigen specific therapies. In addition to general immunosuppression, *e.g.* through the use of agents such as hydrocortisone, many therapies are now being brought to the clinic that provide for a more selective modification of the immune system.

[02] A downside to this promising therapy is the diversity of responses in patient populations. While a significant proportion of patients may respond to a particular therapy, many do not. The clinician may therefore need to prescribe sequential expensive and time-consuming therapies in order to determine which is effective for the individual patient. Furthermore, it has been reported that IFN-β can exacerbate symptoms in some individuals.

[03] The use of disease-modifying therapies in autoimmune conditions is of great clinical interest; however these therapies suffer from the inability to determine *a priori* which patients will benefit. The present invention addresses this need.

[04] Publications of interest include Guo et al. *J Clin Invest* (2008); Nagai et al. *Scand J Immunol* 65, 107-17 (2007); McRae et al. *J Immunol* 160, 4298-304 (1998); Martin-Saavedra et al. *Mol Immunol* 45, 4008-19 (2008).

SUMMARY OF THE INVENTION

[05] Compositions and methods are provided for the treatment of IL-17-type inflammatory demyelinating diseases with inhibitors of granulocyte function, *e.g.* elastase inhibitors. Diseases of interest include multiple sclerosis, neuromyelitis optica, animal models of such diseases, *etc*. In some embodiments pharmaceutical formulations comprising an elastase inhibitor in an effective dose for treatment of IL-17-type inflammatory demyelinating disease and a pharmaceutically acceptable excipient are provided. Significant granulocyte infiltrates are shown herein to be characteristic of NMO and IL-17-type MS/EAE. Characteristic also of granulocytes are the presence of elastase and Gro-α.

[06] Assessment in an inflammatory demyelinating disease patient allows improved care, where patients classified according to responsiveness can be treated with an appropriate agent, *e.g.* patients classified as a predominantly TH17-type disease subtype can be treated with appropriate agents, *e.g.* inhibitors of granulocyte function, *etc*. Patients may be classified upon initial presentation of symptoms, and may be further monitored for status over the

course of the disease to maintain appropriate therapy, or may be classified at any appropriate stage of disease progression. A patient having high levels of markers indicative of a TH17 subtype, *e.g.* IL-17F, IL-23, β-IFN, and/or low levels of IL-7 *etc.* is classified as non-responder to TH1 subtype immunotherapy, and may appropriately be treated with an inhibitor of granulocyte function. Patients having an IL-17-type disease subtype are also characterized by the presence of excessive numbers of polymorphonuclear leukocytes (PMN or PML), a clinical factor. Classification of a patient may alternatively, or in combination with marker assessment, comprise determining the levels of one or more of PMN-associated markers, including elastase and Gro-α.

[07] Methods of determining responder status in a patient with an immune-related disease may comprise obtaining or preparing a cytokine measurement panel comprising one or more affinity reagents specific for markers, including for example, anti-IL-17, e.g. IL-17F, β-IFN, IL-7, etc.; physically contacting the panel with a patient sample such as blood, serum, cerebrospinal fluid, *etc.*; identifying the markers that bind to the panel; comparing the markers bound to the those bound with a control sample known to be one or more of: a non-diseased individual, an individual known to have a responder or non-responder phenotype, *etc.* Those patients non-responsive to TH1-therapy, such as β-IFN, may be characterized has having a level of IL-17, e.g. IL-17F, that is significantly higher than a non-diseased individual, while those patients responsive to such therapy have a level of IL-17 not significantly different than a non-diseased individual. Those patients responsive to TH1-therapy, such as β-IFN, may be characterized has having a level of IL-7 that is significantly higher than a non-diseased individual, while those patients non-responsive to such therapy have a level of IL-7 not significantly different than a non-diseased individual. The resulting data sets provide a signature pattern from which the prognostic classification can be determined.

BRIEF DESCRIPTION OF THE DRAWINGS

[08] Fig 1. Correlation of a) IL-17F vs IFN-β levels, b) IL-17F vs MIP1β levels and c) IFN-β vs MIP1β in serum from responders, non-responders and healthy controls. R^2 values close to 1 demonstrate that the cytokines are positively correlated.

[09] Fig. 2. Comparison of the effect of copaxone and control on TH1 and TH17 induced disease.

[10] Figure 3A-3C, differential expression of IL-17A and IL-17F in NMO and EAE.

[11] Figure 4A-4C, Role of granulocytes in NMO and effect of neutrophil elastase inhibition in the treatment of TH17 EAE.

DETAILED DESCRIPTION OF THE EMBODIMENTS

[12] Mammalian species that provide samples for analysis and that are amenable to treatment include canines; felines; equines; bovines; ovines; *etc.* and primates, particularly humans. Animal models, particularly small mammals, *e.g.* murine, lagomorpha, *etc.* may be used for experimental investigations. Animal models of interest include those for models of autoimmunity, graft rejection, and the like.

Definitions

[13] *Inflammatory demyelinating disease.* The term "inflammatory" response is the development of a humoral (antibody mediated) and/or a cellular (mediated by antigen-specific T cells or their secretion products) response. Inflammatory demyelinating diseases of the central nervous system are of particular interest and include, without limitation, multiple sclerosis (MS), neuromyelitis optica (NO), and experimental acquired encephalitis (EAE). Demyelinating inflammatory diseases of the peripheral nervous system include Guillain-Barre syndrome (GBS) with its subtypes acute inflammatory demyelinating polyradiculoneuropathy, acute motor axonal neuropathy, acute motor and sensory axonal neuropathy, Miller Fisher syndrome, and acute pandysautonomia; chronic inflammatory demyelinating polyneuropathy (CIDP) with its subtypes classical CIDP, CIDP with diabetes, CIDP/monoclonal gammopathy of undetermined significance (MGUS), sensory CIDP, multifocal motor neuropathy (MMN), multifocal acquired demyelinating sensory and motor neuropathy or Lewis-Sumner syndrome, multifocal acquired sensory and motor neuropathy, and distal acquired demyelinating sensory neuropathy.

[14] *Multiple sclerosis* is characterized by various symptoms and signs of CNS dysfunction, with remissions and recurring exacerbations. The most common presenting symptoms are paresthesias in one or more extremities, in the trunk, or on one side of the face; weakness or clumsiness of a leg or hand; or visual disturbances, *e.g.* partial blindness and pain in one eye (retrobulbar optic neuritis), dimness of vision, or scotomas. Other common early symptoms are ocular palsy resulting in double vision (diplopia), transient weakness of one or more extremities, slight stiffness or unusual fatigability of a limb, minor gait disturbances, difficulty with bladder control, vertigo, and mild emotional disturbances; all indicate scattered CNS involvement and often occur months or years before the disease is recognized. Excess heat may accentuate symptoms and signs.

[15] The course is highly varied, unpredictable, and, in most patients, remittent. At first, months or years of remission may separate episodes, especially when the disease begins with retrobulbar optic neuritis. However, some patients have frequent attacks and are rapidly incapacitated; for a few the course can be rapidly progressive (primary progressive MS,

PPMS). Relapsing remitting MS (RR MS) is characterized clinically by relapses and remissions that occur over months to years, with partial or full recovery of neurological deficits between attacks. Such patients manifest approximately 1 attack, or relapse, per year. Over 10 to 20 years, approximately 50% of RR MS patients develop secondary progressive MS (SP MS) which is characterized by incomplete recovery between attacks and accumulation of neurologic deficits resulting in increasing disability.

[16] Diagnosis is indirect, by deduction from clinical, radiographic (brain plaques on magnetic resonance [MR] scan), and to a lesser extent laboratory (oligoclonal bands on CSF analysis) features. Typical cases can usually be diagnosed confidently on clinical grounds. The diagnosis can be suspected after a first attack. Later, a history of remissions and exacerbations and clinical evidence of CNS lesions disseminated in more than one area are highly suggestive.

[17] MRI, the most sensitive diagnostic imaging technique, may show plaques. It may also detect treatable nondemyelinating lesions at the junction of the spinal cord and medulla (eg, subarachnoid cyst, foramen magnum tumors) that occasionally cause a variable and fluctuating spectrum of motor and sensory symptoms, mimicking MS. Gadolinium-contrast enhancement can distinguish areas of active inflammation from older brain plaques. MS lesions may also be visible on contrast-enhanced CT scans; sensitivity may be increased by giving twice the iodine dose and delaying scanning (double-dose delayed CT scan).

[18] Conventional treatments for MS include interferon β (Avonex, Betaseron, Rebif), Copaxone (Glatiramer acetate), and anti-VLA4 (Tysabri, natalizumab), which reduce relapse rate and to date have only exhibited a modest impact on disease progression. MS is also treated with immunosuppressive agents including methylprednisolone, other steroids, methotrexate, cladribine and cyclophosphamide. Many biological agents, such as anti-IFNgamma antibody, CTLA4-Ig (Abetacept), anti-CD20 (Rituxan), and other anti-cytokine agents are in clinical development for MS.

[19] *Neuromyelitis optica* (NMO), or Devic's disease, is an autoimmune, inflammatory disorder of the optic nerves and spinal cord. Although inflammation may affect the brain, the disorder is distinct from multiple sclerosis, having a different pattern of response to therapy, possibly a different pattern of autoantigens and involvement of different lymphocyte subsets.

[20] The main symptoms of Devic's disease are loss of vision and spinal cord function. As for other etiologies of optic neuritis, the visual impairment usually manifests as decreased visual acuity, although visual field defects, or loss of color vision may occur in isolation or prior to formal loss of acuity. Spinal cord dysfunction can lead to muscle weakness, reduced sensation, or loss of bladder and bowel control. The damage in the spinal cord can range from inflammatory demyelination to necrotic damage of the white and grey matter. The

inflammatory lesions in Devic's disease have been classified as type II lesions (complement mediated demyelinization), but they differ from MS pattern II lesions in their prominent perivascular distribution. Therefore, the pattern of inflammation is often quite distinct from that seen in MS.

[21] Attacks are conventionally treated with short courses of high dosage intravenous corticosteroids such as methylprednisolone IV. When attacks progress or do not respond to corticosteroid treatment, plasmapheresis may be used. Commonly used immunosuppressant treatments include azathioprine (Imuran) plus prednisone, mycophenolate mofetil plus prednisone, Rituximab, Mitoxantrone, intravenous immunoglobulin (IVIG), and Cyclophosphamide. The monoclonal antibody rituximab is under study.

[22] The disease can be monophasic, i.e. a single episode with permanent remission. However, at least 85% of patients have a relapsing form of the disease with repeated attacks of transverse myelitis and/or optic neuritis. In patients with the monophasic form the transverse myelitis and optic neuritis occur simultaneously or within days of each other. On the other hand, patients with the relapsing form are more likely to have weeks or months between the initial attacks and to have better motor recovery after the initial transverse myelitis event. Relapses usually occur early with about 55% of patients having a relapse in the first year and 90% in the first 5 years. Unlike MS, Devic's disease rarely has a secondary progressive phase in which patients have increasing neurologic decline between attacks without remission. Instead, disabilities arise from the acute attacks.

[23] *Polymorphonuclear leukocytes.* Polymorphonuclear leukocytes (PMN or PML) are a category of white blood cells characterized by the presence of granules in their cytoplasm, which are also referred to as granulocytes. There are three types of granulocytes, distinguished by their appearance under Wright's stain: neutrophil, eosinophil, and basophil.

[24] Neutrophils are normally found in the bloodstream and are the most abundant type of phagocyte, constituting 50% to 60% of the total circulating white blood cells. Neutrophils are professional phagocytes. Mature neutrophils are smaller than monocytes, and have a segmented nucleus with several sections. The intra-cellular granules of the human neutrophil have long been recognized for their protein-destroying and bactericidal properties. Neutrophils can secrete products that stimulate monocytes and macrophages; these secretions increase phagocytosis and the formation of reactive oxygen compounds involved in intracellular killing. Neutrophils have two types of granules. Primary granules contain cationic proteins and defensins that are used to kill bacteria, proteolytic enzymes and cathepsin G to breakdown (bacterial) proteins, lysozyme to break down bacterial cell walls, and myeloperoxidase. The secondary granules contain compounds that are involved in the formation of toxic oxygen compounds, lysozyme, and lactoferrin.

[25] Eosinophils also have lobed nuclei (two to four lobes). Eosinophils play a crucial part in the killing of parasites because their granules contain a unique, toxic basic protein and cationic protein; IgE receptors are involved in this process.

[26] Basophils are low abundance cells in bone marrow and blood. Like neutrophils and eosinophils they have lobed nuclei. Basophils express receptors for IgE, IgG, complement, and histamine. Granule contents of basophils include histamine, heparin, chondroitin sulfate, peroxidase, platelet activating factor. When basophils are injured they release prostaglandin and histamine; causing dilation and increased permeability of capillaries close to the basophil.

[27] Elastase. Neutrophil elastase (EC 3.4.21.37) is a serine protease of neutrophil and monocyte granules. Its key physiologic role is in innate host defense, but it can also participate in tissue remodeling and possesses secretagogue actions important to local inflammatory responses. The protein consists of 218 amino acid residues, contains 2 asparagine-linked carbohydrate side chains, and is joined together by 2 disulfide bonds.

[28] Elastase inhibitors find use in the treatment of NMO and IL-17-type MS. Inhibitors are known in the art, and include without limitation, sivelestat sodium hydrate (Ono Pharmaceutical); alpha1-antitrypsin; pafistatin-like protease inhibitors (de Marco (2010) Peptides 31(7):1280-1260); Clitocybin D (Kim et al. (2009) J Microbiol Biotechnol. 19(10):1139-41); marama bean inhibitor (Nadaraja et al. (2010) J Enzyme Inhib Med Chem. 25(3):377-82; AE-3763 (Inoue et al. (2009) Bioorg Med Chem. 17(21):7477-86); Isodeoxyhelicobasidin (Xu et al. (2009) J Antibiot 62(6):333-4); guamerin (Jo et al. (2008) Int Immunopharmacol. 8(7):959-66); elafin (Wang et al. (2008) Am J Respir Cell Mol Biol. 38(6):724-32); Bornyl (3,4,5-trihydroxy)-cinnamate (Steinbrecher et al. (2008) Bioorg Med Chem. 16(5):2385-90); and the like as known in the art. Other inhibitors of interest include antibodies specific for neutrophil elastase, anti-sense oligonucleotides, siRNA, shRNA, and the like.

[29] Gro-alpha inhibitors are also of interest, e.g. antileukinate (see, for example Fujisawa et al. (1999) Melanoma res. 9(2):105-114. Other inhibitors of interest include antibodies specific for gro-alpha, anti-sense oligonucleotides, siRNA, shRNA, and the like.

[30] *T helper 17 cells* (Th17) are a subset of T helper cells, characterized by their production of interleukin 17 (IL-17). They are considered developmentally distinct from Th1 and Th2 cells and excessive amounts of the cell are thought to play a key role in autoimmune disease.

[31] In humans, a combination of TGF-β, IL-1β and IL-23 induces Th17 differentiation from naive T cells. Both interferon gamma (IFNγ) and IL-4, the main stimulators of Th1 and Th2 differentiation respectively, negatively regulate Th17 differentiation.

[32] Th17 cells primarily produce two main members of the IL-17 family; IL-17A and IL-17F, which are involved in the recruitment, activation and migration of neutrophils. These cells also secrete IL-21 and IL-22.

[33] *T helper 1 cells (Th1).* Proliferating helper T cells that develop into effector T cells differentiate into two major subtypes of cells known as Th1 and Th2 cells. Th1 cells primarily produce IFN-γ and TNF-β cytokines. IFN-γ increases the production of interleukin-12 by dendritic cells and macrophages, and via positive feedback, IL-12 stimulates the production of IFN-γ in helper T cells, thereby promoting the Th1 profile. IFN-γ also inhibits the production of cytokines such as IL-4. Conditions that polarize to the TH1 type include antigen presenting cells and IL-12.

[34] *Interleukin-17 (IL-17)* refers to a group of cytokines called the IL-17 family. IL-17 shows high homology to viral IL-17 encoded by an open reading frame of the T lymphotropic rhadinovirus Herpesvirus saimiri. To elicit its functions, IL-17 binds to a type I cell surface receptor called IL-17R of which there are at least three variants IL17RA, IL17RB, and IL17RC.

[35] In response to β-IFN, human T cells produce increased amounts of IL-17F, but not IL-17A, which may be accounted for by the presence of putative Type I interferon elements upstream of IL-17F but not IL-17A. In some embodiments of the invention, classification of β-IFN responsiveness in NMO or MS relies on a determination of IL-17F levels, which are optionally compared with levels of IL-17A. Increased levels of Il-17F and baseline levels of IL-17A are indicative of NMO or β-IFN non-responsive MS.

[36] Members of the IL-17 family include IL-17B, IL-17C, IL-17D, IL-17E (also called IL-25), and IL-17F. All members of the IL-17 family have a similar protein structure, with four highly conserved cysteine residues critical to their 3-dimensional shape, although with no sequence similarity to any other known cytokines. IL-17A is a 155 amino acid protein that is a disulfide linked, homodimeric, secreted glycoprotein with a molecular mass of 35kDa. Each subunit of the homodimer is approximately 15-20 KDa. The structure of IL-17 consists of a signal peptide of 23 amino acids (aa) followed by a 123 aa chain region characteristic of the IL-17 family. IL-17F is structurally similar to the cysteine knot family of proteins that includes the neurotrophins. The cysteine knot fold is characterized by two sets of paired β-strands stabilized by three disulfide interactions. However, in contrast to the other cysteine knot proteins, IL-17F lacks the third disulfide bond. Instead, a serine replaces the cysteine at this position. This unique feature is conserved in the other IL-17 family members. IL-17F also dimerizes in a fashion similar to nerve growth factor (NGF) and other neurotrophins. The genetic sequence of IL-17F may be accessed at Genbank, NM_052872. Also see Ely et al.

(2009) Nat. Immunol. 10 (12), 1245-1251; Kawaguchi e al. (2002) J. Biol. Chem. 277 (18), 15229-15232; Starnes et al. (2001) J. Immunol. 167 (8), 4137-4140; and Hymowitz et al. (2001) EMBO J. 20 (19), 5332-5341, each herein specifically incorporated by reference.

[37] Numerous immune regulatory functions have been reported for the IL-17 family. Most notably, IL-17 is involved in inducing and mediating proinflammatory and allergic responses. IL-17 induces the production of many other cytokines and prostaglandins from various cell types (fibroblasts, endothelial cells, epithelial cells, keratinocytes and macrophages). Each member of the IL-17 family has a distinct pattern of cellular expression. The expression of IL-17A and IL-17F appear to be restricted to a small group of activated T cells, and upregulated during inflammation.

[38] An N-linked glycosylation site on the protein was first identified after purification of the protein revealed two bands, one at 15 KDa and another at 20 KDa. Comparison of different members of the IL-17 family revealed four conserved cysteines that form two disulfide bonds.[5] IL-17 is unique in that it bears no resemblance to other known interleukins. Furthermore, IL-17 bears no resemblance to any other known proteins or structural domains.[4]

[39] The IL-17 receptor family consists of five, broadly distributed receptors that present with individual ligand specificities. Within this family of receptors, IL-17R is the best described. IL-17R binds both IL-17A and IL-17F and is expressed in multiple tissues: vascular endothelial cells, peripheral T cells, B cell lineages, fibroblast, lung, myelomonocytic cells and marrow stromal cells.

[40] "Suitable conditions" shall have a meaning dependent on the context in which this term is used. That is, when used in connection with an antibody, the term shall mean conditions that permit an antibody to bind to its corresponding antigen. When used in connection with contacting an agent to a cell, this term shall mean conditions that permit an agent capable of doing so to enter a cell and perform its intended function. In one embodiment, the term "suitable conditions" as used herein means physiological conditions.

[41] The term "inflammatory" response is the development of a humoral (antibody mediated) and/or a cellular (mediated by antigen-specific T cells or their secretion products) response. An "immunogen" is capable of inducing an immunological response against itself on administration to a mammal or due to autoimmune disease.

[42] Unless otherwise apparent from the context, all elements, steps or features of the invention can be used in any combination with other elements, steps or features.

[43] General methods in molecular and cellular biochemistry can be found in such standard textbooks as Molecular Cloning: A Laboratory Manual, 3rd Ed. (Sambrook et al., Harbor Laboratory Press 2001); Short Protocols in Molecular Biology, 4th Ed. (Ausubel et al. eds.,

John Wiley & Sons 1999); Protein Methods (Bollag et al., John Wiley & Sons 1996); Nonviral Vectors for Gene Therapy (Wagner et al. eds., Academic Press 1999); Viral Vectors (Kaplift & Loewy eds., Academic Press 1995); Immunology Methods Manual (I. Lefkovits ed., Academic Press 1997); and Cell and Tissue Culture: Laboratory Procedures in Biotechnology (Doyle & Griffiths, John Wiley & Sons 1998). Reagents, cloning vectors, and kits for genetic manipulation referred to in this disclosure are available from commercial vendors such as BioRad, Stratagene, Invitrogen, Sigma-Aldrich, and ClonTech.

[44] The present invention has been described in terms of particular embodiments found or proposed by the present inventor to comprise preferred modes for the practice of the invention. It will be appreciated by those of skill in the art that, in light of the present disclosure, numerous modifications and changes can be made in the particular embodiments exemplified without departing from the intended scope of the invention. Due to biological functional equivalency considerations, changes can be made in protein structure without affecting the biological action in kind or amount. All such modifications are intended to be included within the scope of the appended claims.

[45] The subject methods are used for prophylactic or therapeutic purposes. As used herein, the term "treating" is used to refer to both prevention of relapses, and treatment of pre-existing conditions. For example, the prevention of autoimmune disease may be accomplished by administration of the agent prior to development of a relapse. The treatment of ongoing disease, where the treatment stabilizes or improves the clinical symptoms of the patient, is of particular interest.

THERAPEUTIC AGENTS

[46] In one embodiment of the invention, modulators of T cell and/or granulocyte activity are used in the treatment of inflammatory demyelinating disease of an IL-17 subtype, including subtypes of MS and NO. Patients may be classified according to cytokine subtype prior to administration of a granulocyte inhibitor, particularly MS patients. NO patients generally have an IL-17 type disease, and may be treated with a granulocyte inhibitor in the absence of cytokine profiling.

[47] In some embodiments of the invention, the therapeutic agent is an elastase inhibitor, e.g. a small molecule inhibitor which may include without limitation, sivelestat sodium hydrate; alpha1-antitrypsin; pafistatin-like protease inhibitors; Clitocybin D); marama bean inhibitor; AE-3763; Isodeoxyhelicobasidin; guamerin; elafin; Bornyl (3,4,5-trihydroxy)-cinnamate; and the like as known in the art. Alternatively the therapeutic agent is an inhibitor of gro-alpha, e.g. peptides, small molecules, and the like.

[48] In some embodiments the therapeutic agents are antibodies specific for a granulocyte marker, e.g. elastase, gro-alpha, *etc.* The term "antibody" is used in the broadest sense and

specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired biological activity. "Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules which lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas.

[49] The method also provide for combination therapy, where the combination may provide for additive or synergistic benefits. Combinations of agents may be obtained with a second agent selected from one or more of the general classes of drugs commonly used in the non-antigen specific treatment of autoimmune disease, which include corticosteroids and disease modifying drugs; or from an antigen-specific agent. Corticosteroids have a short onset of action, but many disease modifying drugs take several weeks or months to demonstrate a clinical effect. These agents include methotrexate, leflunomide (Arava™), etanercept (Enbrel™), infliximab (Remicade™), adalimumab (Humira™), anakinra (Kineret™), rituximab (Rituxan™), CTLA4-Ig (abatacept), antimalarials, gold salts, sulfasalazine, d-penicillamine, cyclosporin A, cyclophosphamide azathioprine; and the like.

[50] Antigen specific therapeutic methods include administration of an antigen or epitope specific therapeutic agent. One method to induce immune tolerance is tolerizing DNA vaccines (Garren et al. (2001) Immunity, 15:15-22; Robinson et al. (2003) Nature Biotechnology 21:1033-9). Tolerizing DNA vaccines are DNA plasmids containing the regulatory regions necessary for expression of the encoded cDNA in mammalian cells, and would be engineered to contain cDNA sequence encoding all or a portion of a targeted antigen in order to induce immune tolerance to the encoded epitopes. To enhance the ability of such plasmids to induce immune tolerance, the immunostimulatory CpG sequences (Krieg et al. (1998) Trends Microbiol. 6:23-27) can be reduced in number or completely removed from the plasmid vector. Additionally, immunoinhibitory GpG sequences can be added to the vector (see Ho et al. (2005) J. Immunology, 175:6226-34). Tolerizing DNA plasmids are delivered intramuscularly to induce immune tolerance to an antigen, thereby reducing T cell and autoantibody responses to reduce autoimmune destruction of the myelin sheath.

[51] As an alternative, or in addition to DNA tolerization, specific peptides, altered peptides, or proteins may be administered therapeutically to induce antigen-specific tolerance to treat autoimmunity. Native peptides targeted by the autoimmune response can be delivered to induce antigen-specific tolerance (Science 258:1491-4). Native peptides have been delivered intravenously to induce immune tolerance (J Neurol Sci. 152:31-8). Delivery of peptides that are altered from the native peptide, is also known in the art. Alteration of native peptides with

selective changes of crucial residues (altered peptide ligands or "APL") can induce unresponsiveness or change the responsiveness of antigen-specific autoreactive T cells. In another embodiment, whole protein antigens targeted by the autoimmune response can be delivered to restore immune tolerance to treat autoimmunity (Science 263:1139).

[52] Active ingredients in pharmaceutical compositions formulated for the treatment of various disorders are as described above. The active ingredient is present in a therapeutically effective amount, *i.e.*, an amount sufficient when administered to substantially modulate the effect of the targeted protein or polypeptide to treat a disease or medical condition mediated thereby. The compositions can also include various other agents to enhance delivery and efficacy, *e.g.* to enhance delivery and stability of the active ingredients.

[53] Thus, for example, the compositions can also include, depending on the formulation desired, pharmaceutically-acceptable, non-toxic carriers or diluents, which are defined as vehicles commonly used to formulate pharmaceutical compositions for animal or human administration. The diluent is selected so as not to affect the biological activity of the combination. Examples of such diluents are distilled water, buffered water, physiological saline, PBS, Ringer's solution, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or formulation can include other carriers, adjuvants, or non-toxic, nontherapeutic, nonimmunogenic stabilizers, excipients and the like. The compositions can also include additional substances to approximate physiological conditions, such as pH adjusting and buffering agents, toxicity adjusting agents, wetting agents and detergents. The composition can also include any of a variety of stabilizing agents, such as an antioxidant.

[54] When the pharmaceutical composition includes a polypeptide as the active ingredient, the polypeptide can be complexed with various well-known compounds that enhance the *in vivo* stability of the polypeptide, or otherwise enhance its pharmacological properties (*e.g.,* increase the half-life of the polypeptide, reduce its toxicity, enhance solubility or uptake). Examples of such modifications or complexing agents include sulfate, gluconate, citrate and phosphate. The polypeptides of a composition can also be complexed with molecules that enhance their *in vivo* attributes. Such molecules include, for example, carbohydrates, polyamines, amino acids, other peptides, ions (*e.g.,* sodium, potassium, calcium, magnesium, manganese), and lipids.

[55] Further guidance regarding formulations that are suitable for various types of administration can be found in Remington's Pharmaceutical Science*s*, Mace Publishing Company, Philadelphia, PA, 17th ed. (1985). For a brief review of methods for drug delivery, see, Langer, Science 249:1527-1533 (1990).

[56] The pharmaceutical compositions can be administered for prophylactic and/or therapeutic treatments. Toxicity and therapeutic efficacy of the active ingredient can be determined according to standard pharmaceutical procedures in cell cultures and/or experimental

animals, including, for example, determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Compounds that exhibit large therapeutic indices are preferred.

[57] The data obtained from cell culture and/or animal studies can be used in formulating a range of dosages for humans. The dosage of the active ingredient typically lies within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized.

[58] The pharmaceutical compositions described herein can be administered in a variety of different ways. Examples include administering a composition containing a pharmaceutically acceptable carrier via oral, intranasal, rectal, topical, intraperitoneal, intravenous, intramuscular, subcutaneous, subdermal, transdermal, intrathecal, or intracranial method.

[59] For oral administration, the active ingredient can be administered in solid dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, and suspensions. The active component(s) can be encapsulated in gelatin capsules together with inactive ingredients and powdered carriers, such as glucose, lactose, sucrose, mannitol, starch, cellulose or cellulose derivatives, magnesium stearate, stearic acid, sodium saccharin, talcum, magnesium carbonate. Examples of additional inactive ingredients that may be added to provide desirable color, taste, stability, buffering capacity, dispersion or other known desirable features are red iron oxide, silica gel, sodium lauryl sulfate, titanium dioxide, and edible white ink. Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a period of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric-coated for selective disintegration in the gastrointestinal tract. Liquid dosage forms for oral administration can contain coloring and flavoring to increase patient acceptance.

[60] The active ingredient, alone or in combination with other suitable components, can be made into aerosol formulations (*i.e.*, they can be “nebulized”) to be administered via inhalation. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen.

[61] Suitable formulations for rectal administration include, for example, suppositories, which are composed of the packaged active ingredient with a suppository base. Suitable suppository bases include natural or synthetic triglycerides or paraffin hydrocarbons. In addition, it is also possible to use gelatin rectal capsules, which are composed of a

combination of the packaged active ingredient with a base, including, for example, liquid triglycerides, polyethylene glycols, and paraffin hydrocarbons.

[62] Formulations suitable for parenteral administration, such as, for example, by intraarticular (in the joints), intravenous, intramuscular, intradermal, intraperitoneal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives.

[63] The components used to formulate the pharmaceutical compositions are preferably of high purity and are substantially free of potentially harmful contaminants (*e.g.*, at least National Food (NF) grade, generally at least analytical grade, and more typically at least pharmaceutical grade). Moreover, compositions intended for *in vivo* use are preferably sterile. To the extent that a given compound must be synthesized prior to use, the resulting product is preferably substantially free of any potentially toxic agents, such as any endotoxins, which may be present during the synthesis or purification process. Compositions for parental administration are also preferably sterile, substantially isotonic and made under GMP conditions.

[64] The compositions may be administered in a single dose, or in multiple doses, usually multiple doses over a period of time, *e.g.* daily, every-other day, weekly, semi-weekly, monthly *etc.* for a period of time sufficient to reduce severity of the inflammatory disease, which may comprise 1, 2, 3, 4, 6, 10, or more doses.

[65] Determining a therapeutically or prophylactically effective amount an agent can be done based on animal data using routine computational methods. In one embodiment, the therapeutically or prophylactically effective amount contains between about 0.1 mg and about 1 g of nucleic acid or protein, as applicable. In another embodiment, the effective amount contains between about 1 mg and about 100 mg of protein, as applicable. In a further embodiment, the effective amount contains between about 10 mg and about 50 mg of the nucleic acid or protein, as applicable. The effective dose will depend at least in part on the route of administration. The agents may be administered orally, in an aerosol spray; by injection, e.g. i.m., s.c., i.p., i.v., etc. In some embodiments, administration by other than i.v. may be preferred. The dose may be from about 0.1 μg/kg patient weight; about 1 μg/kg; about 10 μg/kg; to about 100 μg/kg.

[66] The compositions are administered in a pharmaceutically acceptable excipient. The term "pharmaceutically acceptable" refers to an excipient acceptable for use in the pharmaceutical and veterinary arts, which is not toxic or otherwise inacceptable. The concentration of compositions of the invention in the pharmaceutical formulations can vary widely, i.e. from

less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

[67] Treating, treatment, or therapy of a disease or disorder shall mean slowing, stopping or reversing the disease's progression by administration of treatment according to the present invention. In the preferred embodiment, treating a disease means reversing the disease's progression, ideally to the point of eliminating the disease itself. As used herein, ameliorating a disease and treating a disease are equivalent. Preventing, prophylaxis or prevention of a disease or disorder as used in the context of this invention refers to the administration of an αBC composition to prevent the occurrence or onset of a disease or disorder or some or all of the symptoms of a disease or disorder or to lessen the likelihood of the onset of a disease or disorder.

Patient Classification

[68] Cytokines are messenger molecules produced by B cells, T cells, macrophage, dendritic cells and other immune and host cells. Cytokines play roles in the pathogenesis of multiple sclerosis and other autoimmune diseases. Cytokines include chemokines, lymphokines, growth factors, angiogenesis factors, and other secreted and cell surface molecules that transmit signals to other cells. Cytokines of interest for the methods of the invention include, without limitation, IL-17F, IL-17A, IL-5, IL-8, β-IFN, γ-IFN and IL-23. While serum levels of IL-17F are found to be increased in certain MS and NO patients, in some cases serum levels of IL-17A may be normal.

[69] In addition, greater infiltration of granulocytes (PMN) are found in lesions of NO and IL-17-type MS or EAE, and markers of granulocytes such as elastase and GroA find use in disease classification, where a patient sample, e.g. CSF, is analyzed for the presence of increased levels of granulocytes relative to a normal control sample are indicative of NO or IL-17-type MS or EAE; and/or the presence of markers indicative of granulocytes, where increased levels of granulocytes markers relative to a normal control sample are indicative of NO or IL-17-type MS or EAE.

[70] In one embodiment of the invention, a method is provided for determining which immunomodulatory treatment a multiple sclerosis patient will be non-responsive to, the method comprising determining circulating levels of at least one cytokine in a patient, where the cytokine(s) is indicative of the TH1/TH17 status of the patient. A patient having high levels of cytokines indicative of a TH17 subtype, *e.g.* IL-17F, IL-5, IL-8, IL-23, β-IFN, *etc.* or granulocytes or markers thereof, or low levels of a cytokine indicative of a TH1-subtype, e.g. IL-7 relative to a non-diseased individual or a patient with a known responder phenotype is classified as non-responder to TH1 subtype immunotherapy. Such non-responder patients

may be treated with inhibitors of granulocyte function, e.g. elastase inhibitors, gro-alpha inhibitors, and the like.

[71] A variety of different assays can be utilized to quantitate the presence of such markers. Many such methods are known to one of skill in the art, including ELISA, protein arrays, eTag system, bead based systems, tag or other array based systems etc. Examples of such methods are set forth in the art, including, inter alia, chip-based capillary electrophoresis: Colyer *et al.* (1997) J Chromatogr A. 781(1-2):271-6; mass spectroscopy: Petricoin *et al.* (2002) Lancet 359: 572–77; eTag systems: Chan-Hui *et al.* (2004) Clinical Immunology 111:162-174; microparticle-enhanced nephelometric immunoassay: Montagne *et al.* (1992) Eur J Clin Chem Clin Biochem. 30(4):217-22; antigen arrays: Robinson *et al.* (2002) Nature Medicine, 8:295-301; the Luminex XMAP bead array system (www.luminexcorp.com); and the like, each of which are herein incorporated by reference. Detection may utilize one or a panel of specific binding members, e.g. specific for one, two, three, four, five or more cytokines.

[72] The signature pattern may be generated from a biological sample using any convenient protocol, for example as described below. The readout may be a mean, average, median or the variance or other statistically or mathematically-derived value associated with the measurement. The cytokine readout information may be further refined by direct comparison with the corresponding reference or control pattern. A binding pattern may be evaluated on a number of points: to determine if there is a statistically significant change at any point in the data matrix; whether the change is an increase or decrease in the binding; whether the change is specific for one or more physiological states, and the like. The absolute values obtained for each cytokine under identical conditions will display a variability that is inherent in live biological systems and also reflects the variability inherent between individuals.

[73] Following obtainment of the signature pattern from the sample being assayed, the signature pattern is compared with a reference or control profile to make a prognosis regarding the phenotype of the patient from which the sample was obtained/derived. Typically a comparison is made with a sample or set of samples from an unaffected, normal source. Additionally, a reference or control signature pattern may be a signature pattern that is obtained from a sample of a patient known to be responsive or non-responsive to the therapy of interest, and therefore may be a positive reference or control profile.

[74] In certain embodiments, the obtained signature pattern is compared to a single reference/control profile to obtain information regarding the phenotype of the patient being assayed. In yet other embodiments, the obtained signature pattern is compared to two or more different reference/control profiles to obtain more in depth information regarding the phenotype of the patient. For example, the obtained signature pattern may be compared to a positive and negative reference profile to obtain confirmed information regarding whether the patient has the phenotype of interest.

[75] Samples can be obtained from the tissues or fluids of an individual. For example, samples can be obtained from whole blood, tissue biopsy, serum, *etc.* Other sources of samples are body fluids such as lymph, cerebrospinal fluid, and the like. Also included in the term are derivatives and fractions of such cells and fluids. Diagnostic samples are collected any time after an individual is suspected to have an autoimmune disease or has exhibited symptoms that predict such a disease.

[76] Various immunoassays designed to quantitate cytokines may be used in screening. Measuring the concentration of the target protein in a sample or fraction thereof may be accomplished by a variety of specific assays. For example, a conventional sandwich type assay may be used in an array, ELISA, RIA, *etc.* format.

[77] Other immunoassays are known in the art and may find use as diagnostics. Ouchterlony plates provide a simple determination of antibody binding. Western blots may be performed on protein gels or protein spots on filters, using a detection system specific for the cytokines as desired, conveniently using a labeling method.

[78] For multiplex analysis of cytokines, arrays containing one or more anti-cytokine affinity reagents, e.g. antibodies can be generated. Such an array may be constructed comprising antibodies against cytokines.

[79] Arrays provide a high throughput technique that can assay a large number of polypeptides in a sample. Arrays can be created by spotting a probe onto a substrate (*e.g.*, glass, nitrocellulose, *etc.*) in a two-dimensional matrix or array having bound probes. The probes can be bound to the substrate by either covalent bonds or by non-specific interactions, such as hydrophobic interactions. Techniques for constructing arrays and methods of using these arrays are described in, for example, Schena *et al.* (1996) Proc Natl Acad Sci U S A. **93**(20):10614-9; Schena *et al.* (1995) Science **270**(5235):467-70; Shalon *et al.* (1996) Genome Res. **6**(7):639-45, USPN 5,807,522, EP 799 897; WO 97/29212; WO 97/27317; EP 785 280; WO 97/02357; USPN 5,593,839; USPN 5,578,832; EP 728 520; USPN 5,599,695; EP 721 016; USPN 5,556,752; WO 95/22058; and USPN 5,631,734.

[80] It is to be understood that this invention is not limited to the particular methodology, protocols, cell lines, animal species or genera, and reagents described, as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

[81] As used herein the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. All technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs unless clearly indicated otherwise.

[82] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the subject invention, and are not intended to limit the scope of what is regarded as the invention. Efforts have been made to ensure accuracy with respect to the numbers used (e.g. amounts, temperature, concentrations, etc.) but some experimental errors and deviations should be allowed for. Unless otherwise indicated, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees centigrade; and pressure is at or near atmospheric.

[83] All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

EXPERIMENTAL

[84] Cytokine Profiles Before Treatment of RRMS patients responding to IFN-β versus non-responders. We analyzed the pre-treatment levels of 26 cytokines/chemokines in the serum of RRMS patients, 12 were classified as responders and 14 as non-responders to IFN-β treatment. The median relapse rate in the two years following initiation of IFN-β treatment in the non-responders was 2, while it was 0 in the responder population. Likewise, the median number of steroid interventions was 2 in the non-responders and 0 in the responders. Cluster analysis of the cytokine profiles grouped 5 non-responders together. This group of non-responders had significantly elevated serum concentrations of both IL-17F and IFN-β compared to the responders. Furthermore, we found there was a significant correlation with IL-17F and IFN-β levels found in the serum of responders, non-responders and healthy individuals (Fig. 1a). This strong correlation was not found in comparing IL-17F or IFN-β to MIP1β or other analytes.

[85] We analyzed cytokine and chemokine levels in serum from RRMS patients prior to the initiation of IFN-β treatment. After treatment began, disease course was monitored for two years or longer and the patients were classified as responders and non-responders. All non-responders had relapses during first 2 years of IFN-β treatment where the responders had none. During relapses, the non-responders received steroids therapy which has powerful immune suppressive activity that would attenuate disease progression in these patients. Therefore, it is quite reasonable to describe the clinical course of the non-responders as exacerbated compared to the responders. Strikingly, we found a subset of non-responders that have high serum levels of the TH17 cytokine, IL-17F. IL-17F has been shown to be produced by TH17 cells in EAE suggesting that this group of MS patient is skewed towards a TH17 disease. Furthermore, these patients have high levels of endogenous IFN-β in their serum compared to the responders. These data show that there is striking correlation in the

concentration IL-17F and IFN-β in serum, demonstrating that these two cytokines are associated biologically.

[86] Without limiting the invention, it may be hypothesized that IFN-β is pro-inflammatory during TH17 biased disease. Therefore, not only would IFN-β treatment be ineffective, it could worsen symptoms. This is supported by observations in EAE, where symptoms were worsened by IFN-β treatment in TH17 induced EAE, and in RRMS, where patients with high IL-17F have exacerbated disease.

[87] In addition, neuromyelitis optica (NMO), another demyelinating disorder closely related to MS, also provides evidence for this hypothesis. IFN-β treatment of individuals with NMO induces severe relapses. The main cellular component of NMO lesions are granulocytes and the function of a TH17 response is to mobilize and attract granulocytes from the bone marrow to the site of inflammation. It has been shown that there are high levels of IL-17 found in the CSF of patients with NMO. The disease processes of NMO and the group of non-responders with high levels of IL-17F are likely similar.

[88] Manipulating the effects of cytokines has been a popular and at times effective strategy for the development of treatments for MS. However, our data demonstrate that IFN-β is a double-edged sword. In the context of a TH1 response with high IFN-γ levels, IFN-β is anti-inflammatory and is effective in attenuating disease. However, in the context of a TH17 response, with high levels of IL-17A/F, IFN-β is pro-inflammatory and IFN-β can exacerbate disease.

[89] A direct result of this work is the utility of a test for IL-17F prior to embarking with therapy using IFN-β. This will reduce the morbidity of IFN-β, as it would preclude those whose clinical symptoms are likely to worsen when it is used. And it would select those patients who would benefit from the drug. In an contemporary environment where economic impacts of medical decision making is more and more important, the virtues of excluding certain patients has further urgency. And here the exclusion would save not only the unnecessary expenditure of funds, it would also save individuals from undesired complications. IL-17F is thus one of the first biomarkers that could govern whether or not a high priced recombinant drug is used in the clinic.

Materials and Methods

[90] MS Patients Clinical Classification and Serum Collection. Twenty-six closely monitored RRMS patients receiving IFNβ treatment for at least 12 months were identified from the outpatient clinic as responders or non-responders to IFNβ therapy at the MS Center Amsterdam, the Netherlands. The patients were classified based on EDSS (Extended Disability Status Scale) progression and the number of relapses and steroid interventions (3 days of 1000 mg/day i.v. methylprednisolone) in the two years before initiation of treatment as

compared to the first two years after starting treatment (see Table 1). Two MS neurologists, blinded to the laboratory data, independently classified the selected patients as responder or non-responder. In case of disagreement there was a consensus meeting afterwards. Serum samples were obtained at a fixed time of the day just before starting IFNβ therapy. The study design received approval from the institutional Medical Ethics Board of the VU University Medical Center, Amsterdam, the Netherlands. All patients signed written informed consent.

[91] ELISA and Multiplex Analysis of Human cytokines. Supernatants from TH differentiation cultures were assayed for IL-17, IL-10 and IFN-γ by ELISA (Ebioscience). Analysis of cytokines in the sera from MS patients and healthy controls was performed by multiplex bead analysis (Panomics) according to the protocol recommended by the manufacturer. Multiplex results were analysed using Gene Cluster software to identify features with significant differences in antibody reactivity and the patient samples were ordered using a hierarchical clustering algorithm and the results presented as a heat map using TreeView software.

[92] Statistical analysis. EAE data are presented as means ± SEM and statistical significance was determined using a two tailed Mann-Whitney test with a value of $P < 0.05$ was considered significant. STAT1 activation and ELISA data are presented as means ± 1 standard deviation and statistics significance was determined using a two tailed student T-test.

Table 1. Demographic and clinical characteristics of patients with relapsing remitting multiple sclerosis and their clinical response to IFNβ therapy

	Responder	Non-responder
Number	12	14
Female/Male (n)	10/2	11/3
Median age at onset (yr)	27.6 [24.5; 35.8]	26.7 [19.3; 36.0]
Median age at start IFNβ (yr)	33.5 [30.3; 39.5]	33.0 [23.0; 37.8]
Median EDSS score around start IFNβ	2.5 [2.0; 3.5]	2.5 [1.8; 4.3]
Relapse rate in 2 yrs before start IFNβ	2 [2-3]	2 [1-3]
Relapse rate in 2 yrs after start IFNβ	0 [0; 0]	2 [1.5; 2.0]
Steroid interventions before start IFNβ (n)	0 [0; 2]	1 [0; 3]
Steroid interventions after start IFNβ (n)	0 [0; 0.5]	2 [1; 3]
Duration of IFNβ treatment (mnths)	80 [46; 141]	56 [38; 104]
Avonex	4	5
Rebif	2	8
Betaferon	6	1

Median values are shown with 25 and 75 percentiles.
IFNb = Interferon-beta.
EDSS = Expanded Disability Status Scale

Example 2

Increased Granulocytes are indicative of NMO and TH17 EAE

[93] As shown in Figure 4A, markers indicative of granulocytes are found at increased levels in NMO and TH17EAE. In plasma, increased levels of neutrophil elastase is found in patients with NMO (Figure 4B).

[94] Remarkably, an elastase inhibitor attenuated TH17 EAE, shown in Figure 4C, indicating the utility of this treatment for β-interferon resistant MS and NMO.

What is Claimed is:

1. A method for treating a patient suffering from an inflammatory demyelinating disease having an IL-17 subtype, the method comprising:

administering to said patient an inhibitor of granulocyte function in an amount effective to decrease disease symptoms or progression.

2. The method of Claim 1, wherein said inhibitor of granulocyte function is an inhibitor of neutrophil elastase.

3. The method of Claim 2, wherein the elastase inhibitor is sivelestat sodium hydrate.

4. The method of Claim 1, wherein said patient is a human.

5. The method of Claim 4, wherein said inflammatory demyelinating disease having an IL-17 subtype is neuromyelitis optica.

6. The method of Claim 4, wherein said inflammatory demyelinating disease having an IL-17 subtype is multiple sclerosis.

7. The method of any one of Claim 1-6, wherein prior to said administering step the patient is classified for responsiveness by the method comprising:

determining circulating levels of at least one cytokine or granulocyte marker in a patient to provide a marker signature pattern, where the marker is indicative of the TH1/TH17 status of the patient;

comparing said marker signature pattern with a control signature pattern;

wherein a statistically significant match with a responder pattern for said therapy of interest or a statistically significant difference from a non-responder pattern for said therapy of interest is indicative that said multiple sclerosis patient has a positive prognosis for being responsive to said therapy of interest;

directing therapeutic intervention for said patient based on said prognosis.

8. The method according to Claim 7, wherein said at least one marker includes IL-17F.

9. The method according to Claim 8, wherein said at least one marker further includes one or more of IL-17A, β-IFN, IL-7, IL-5, IL-8, IL-23, elastase, Gro-alpha, and γ-IFN.

10. The method of Claim 9, wherein said determining step comprises:

preparing a measurement panel comprising one or more affinity reagents specific for markers indicative of the TH1/TH17 status of the patient;

physically contacting the panel with a patient sample;

quantifying the markers that bind to the panel.

11. The method of any one of Claims 7-10 wherein the patient sample is blood or a derivative thereof.

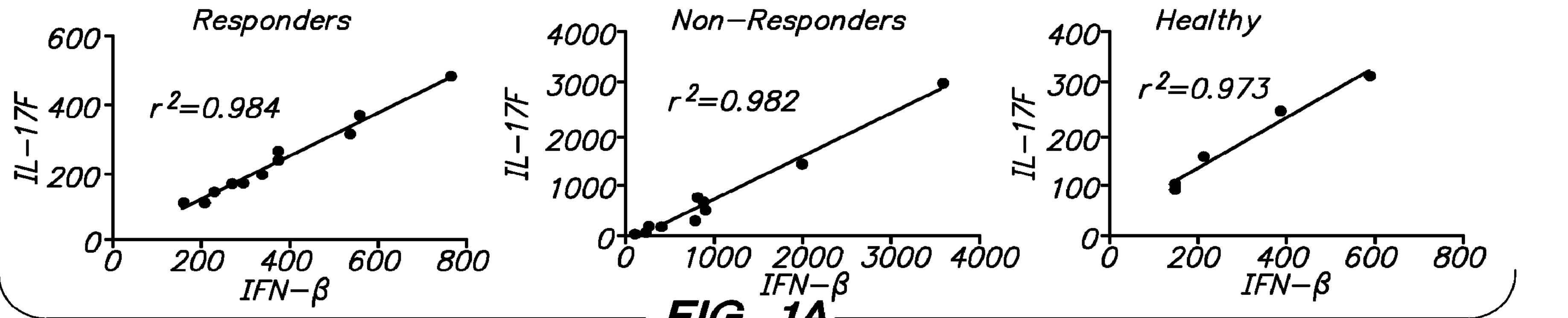


FIG. 1A

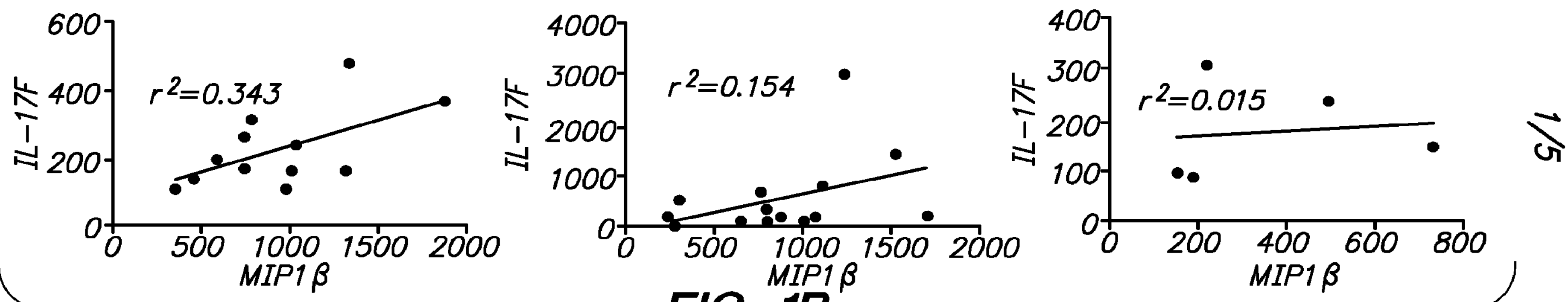


FIG. 1B

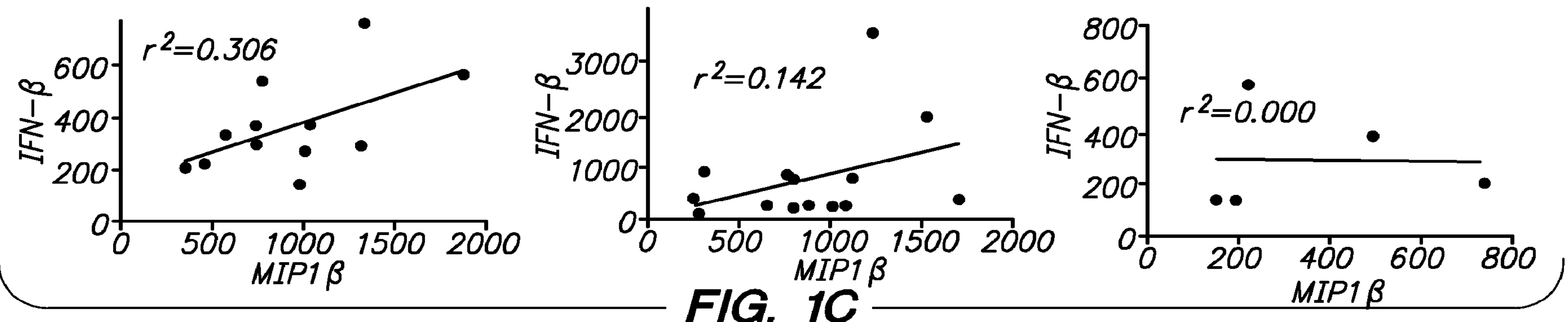
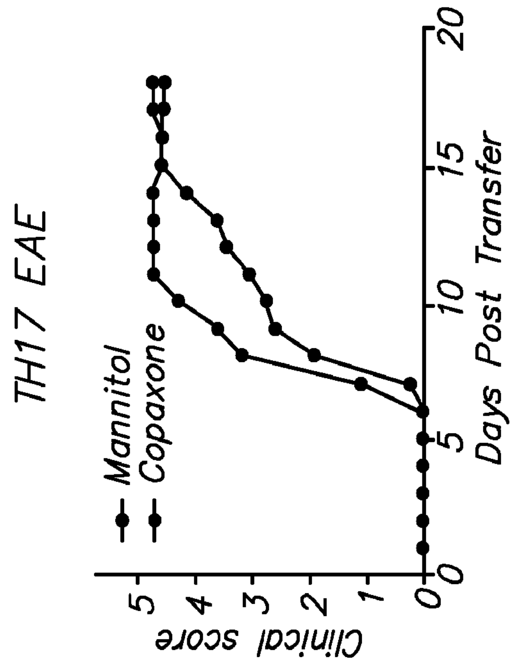


FIG. 1C



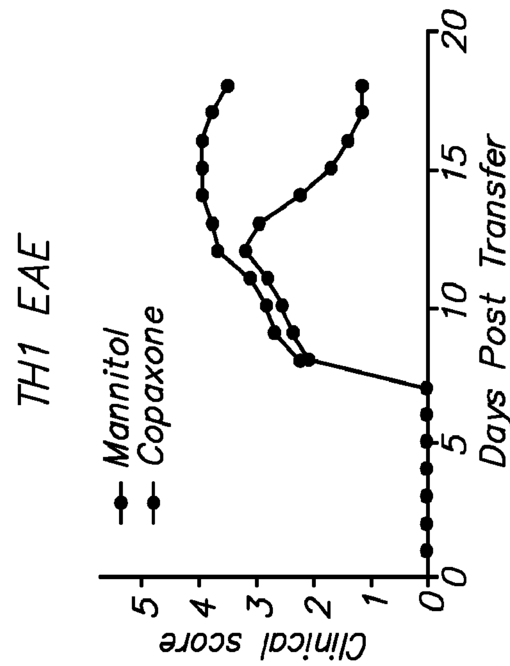


FIG. 2

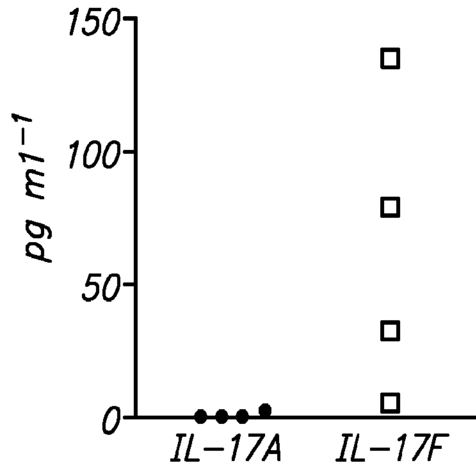


FIG. 3A

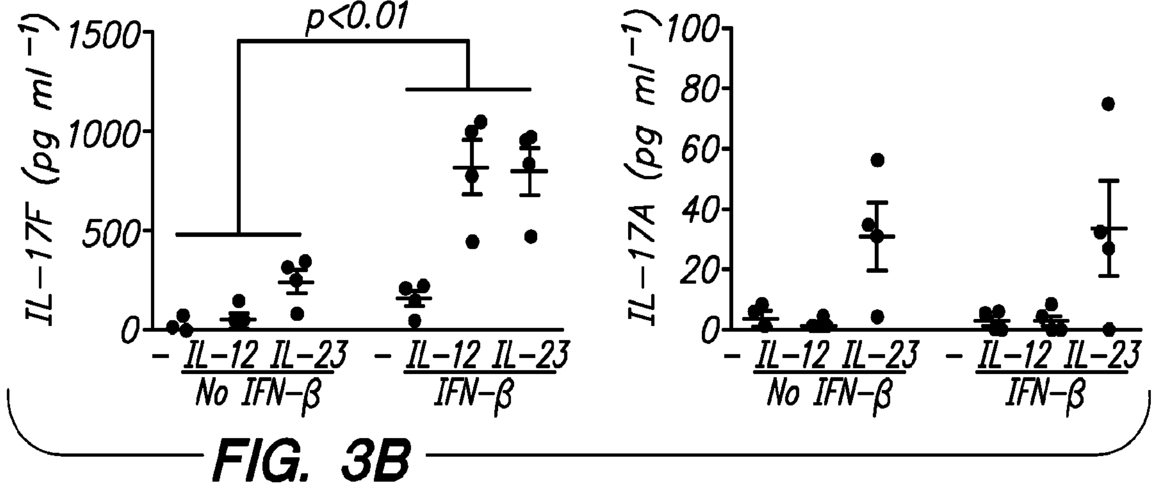


FIG. 3B

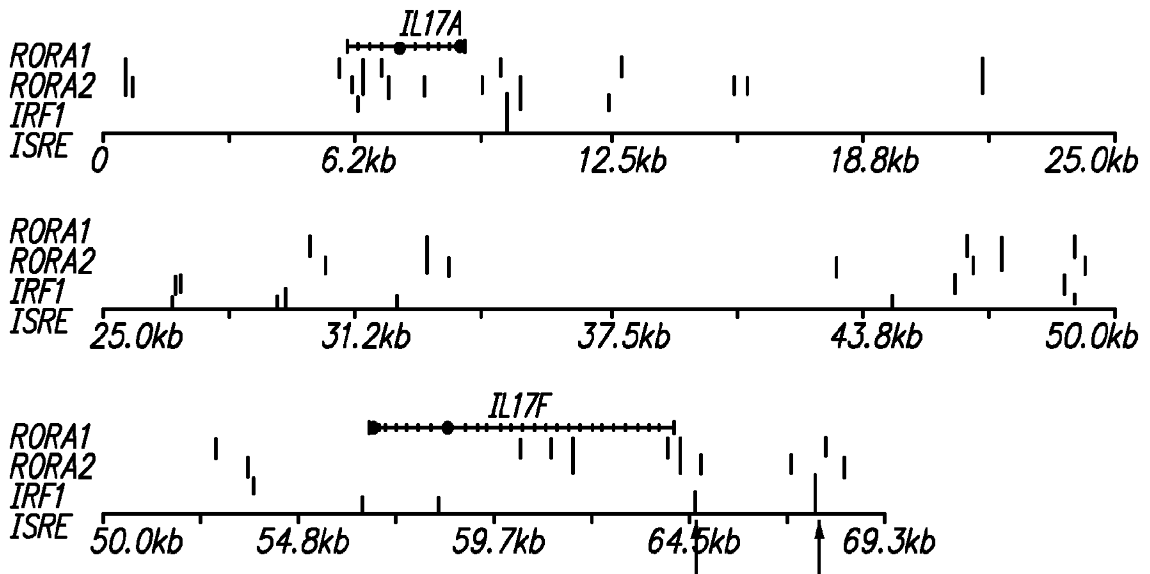


FIG. 3C

4/5

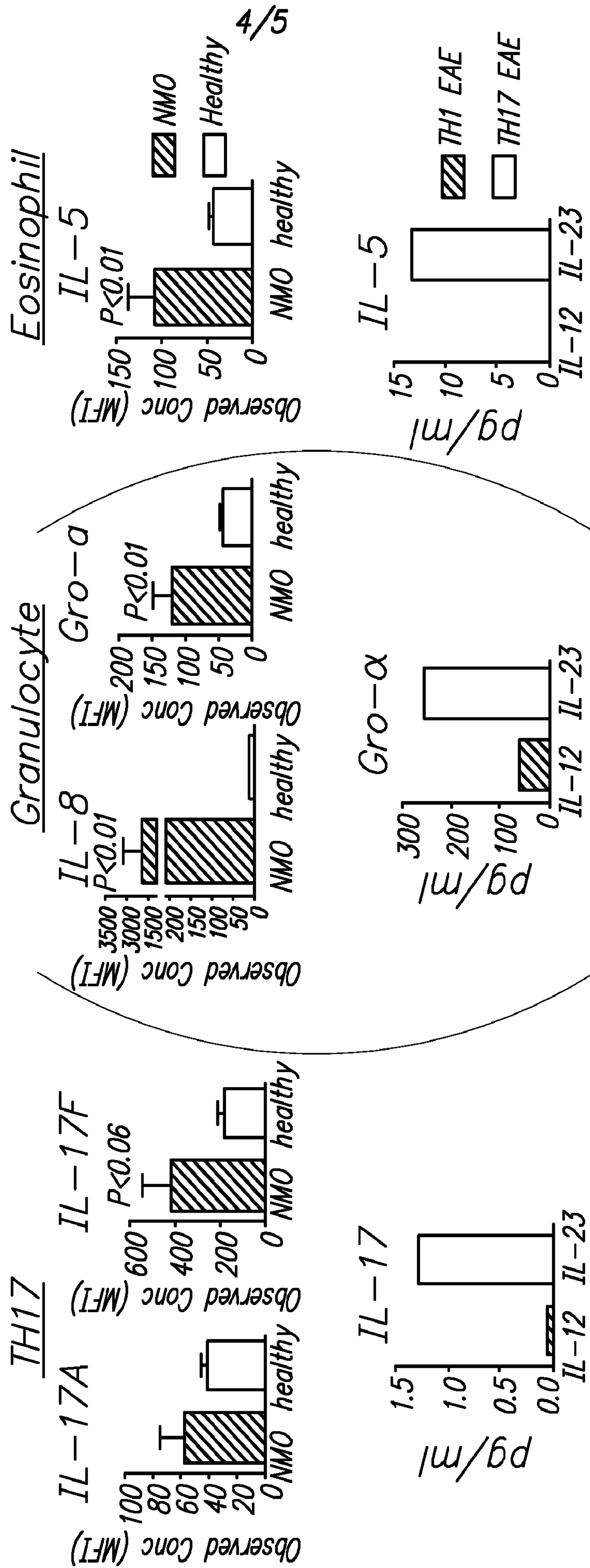


FIG. 4A

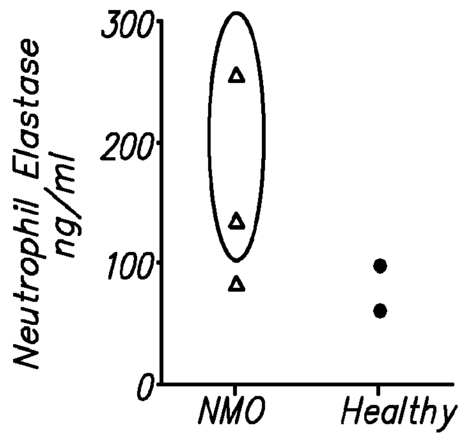


FIG. 4B

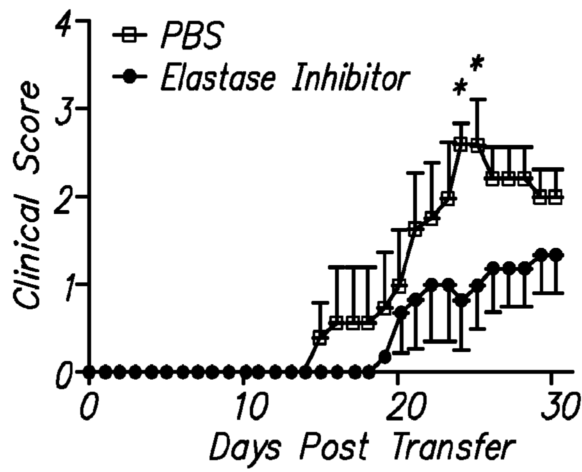


FIG. 4C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/24557

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01N 41/06; A61K 31/18 (2011.01)
USPC - 514/601
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC: 514/601

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 414/400, 722; 514/23, 117 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (PGPB, USPT, EPAB, JPAB)
inflammatory demyelinating disease , IL-17 subtype, administering, administer, administered,inhibitor of granulocyte function ,neutrophil elastase, sivelestat sodium hydrate, human, mammal, neuromyelitis optica, multiple sclerosis, circulating levels, blood levels,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2005/0186245 A1 (HUNTER et al.) 25 August 2005 (25.08.2005) para [0013], [0126]-[0125], [0146], [0157], [0174], [0293]	1-10
Y	US 2007/0269428 A1 (CHRISTIE et al.) 22 November 2007 (22.11.2007) abstract; para [0011], [0013], [0018]-[0021], [0075]	1-10
Y	US 6,849,719 B2 (SHI et al.) 1 February 2005 (01.02.2005) col 121, ln 54-55; col 122, ln 18-19	5-10
Y	BD PHARMINGEN. HumanTh1/Th17 Phenotyping Kit. Technical Data Sheet, 2008, pp 1-4; fig 1; pg 1, para 3 http://www.bdbiosciences.com/external_files/pm/doc/tds/human/live/web_enabled/560752.pdf	7-10
Y	HAAK et al. IL-17A and IL-17F do not contribute vitally to autoimmune neuro-inflammation in mice. The Journal of Clinical Investigation, Jan 2009, Vol 119 No 1, pp 61-69	8-10

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 March 2011 (28.03.2011)	12 APR 2011

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/24557

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 11
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.