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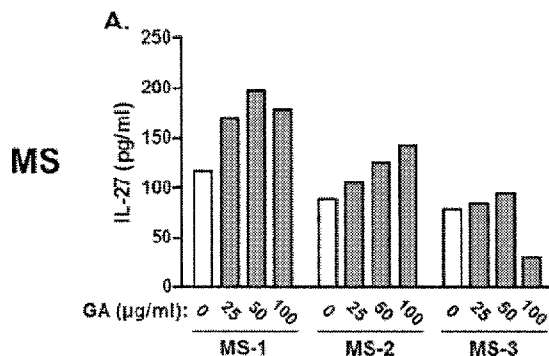
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(54) Title: METHOD OF USING IL-27 AS A PREDICTIVE BIOMARKER OF CLINICAL RESPONSE TO GLATIRAMER ACETATE THERAPY

Fig. 1A



(57) Abstract: The present invention provides a method for treating a subject afflicted with multiple sclerosis with a pharmaceutical composition comprising glatiramer acetate and a pharmaceutically acceptable carrier, comprising the steps of: a) administering a therapeutic amount of the pharmaceutical composition to the subject; b) determining whether the subject is a glatiramer acetate responder by evaluating IL-27 concentration in the blood of the subject; and c) continuing the administration of the pharmaceutical composition if the subject is identified as a glatiramer acetate responder, or modifying treatment of the subject if the subject is not identified as a glatiramer acetate responder.

METHOD OF USING IL-27 AS A PREDICTIVE BIOMARKER OF CLINICAL RESPONSE
TO GLATIRAMER ACETATE THERAPY

This application claims priority of U.S. Provisional Application No.
5 62/264, 149, filed December 7, 2015, the contents of which are hereby
incorporated by reference.

Throughout this application various publications are referenced by
Arabic numeral in parenthesis. The full citation of the corresponding
reference appears at the end of the specification before the claims.

10 The disclosures of these publications in their entireties are hereby
incorporated by reference into this application in order to more fully
describe the state of the art to which this invention pertains.

BACKGROUND OF THE INVENTION

Multiple Sclerosis

15 Multiple sclerosis (MS) is a chronic, debilitating autoimmune disease
of the central nervous system (CNS) with either relapsing-remitting
(RR) or progressive course leading to neurologic deterioration and
disability. At time of initial diagnosis, RRMS is the most common form
of the disease (1) which is characterized by unpredictable acute
20 episodes of neurological dysfunction (relapses), followed by variable
recovery and periods of clinical stability. The vast majority of RRMS
patients eventually develop secondary progressive (SP) disease with or
without superimposed relapses. Around 15% of patients develop a
sustained deterioration of their neurological function from the
25 beginning; this form is called primary progressive (PP) MS. Patients
who have experienced a single clinical event (Clinically Isolated
Syndrome or "CIS") and who show lesion dissemination on subsequent
magnetic resonance imaging (MRI) scans according to McDonald's
criteria, are also considered as having relapsing MS.(2)

30 With a prevalence that varies considerably around the world, MS is the

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most common cause of chronic neurological disability in young adults. (3,4) Anderson et al. estimated that there were about 350,000 physician-diagnosed patients with MS in the United States in 1990 (approx. 140 per 100,000 population). (5) It is estimated that about 5 2.5 million individuals are affected worldwide. (6) In general, there has been a trend toward an increasing prevalence and incidence of MS worldwide, but the reasons for this trend are not fully understood. (5)

Current therapeutic approaches consist of i) symptomatic treatment ii) treatment of acute relapses with corticosteroids and iii) treatment 10 aimed to modify the course of the disease. Currently approved therapies target the inflammatory processes of the disease. Most of them are considered to act as immunomodulators but their mechanisms of action have not been completely elucidated. Immunosuppressants or cytotoxic agents are also used in some patients after failure of 15 conventional therapies. Several medications have been approved and clinically ascertained as efficacious for the treatment of RR-MS; including BETASERON®, AVONEX® and REBIF®, which are derivatives of the cytokine interferon beta (IFNB), whose mechanism of action in MS is generally attributed to its immunomodulatory effects, antagonizing 20 pro-inflammatory reactions and inducing suppressor cells. (7)

Glatiramer Acetate

Glatiramer acetate (GA) is the active substance in Copaxone®, a marketed product indicated for reduction of the frequency of relapses in patients with RRMS. Its effectiveness in reducing relapse rate and 25 disability accumulation in RR-MS is comparable to that of other available immunomodulating treatments. (8,9,10) Glatiramer acetate consists of the acetate salts of synthetic polypeptides containing four naturally occurring amino acids: L-glutamic acid, L-alanine, L-tyrosine and L-lysine. The average molecular weight of glatiramer 30 acetate is between 5,000 and 9,000 Daltons. At a daily standard dose of 20 mg, GA is generally well tolerated, however response to the drug is variable. In various clinical trials, GA reduced relapse rates and progression of disability in patients with RR-MS. The therapeutic

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effect of GA is supported by the results of magnetic resonance imaging (MRI) findings from various clinical centers (11), however there are no validated predictive biomarkers of response to GA treatment.

5 A possible initial mode of action of GA is associated with binding to MHC molecules and consequent competition with various myelin antigens for their presentation to T cells.(12) A further aspect of its mode of action is the potent induction of T helper 2 (Th2) type cells that presumably can migrate to the brain and lead to in situ bystander
10 suppression.(13) It has been shown that GA treatment in MS results in the induction of GA-specific T cells with predominant Th2 phenotype both in response to GA and cross-reactive myelin antigens.(13,14) Furthermore, the ability of GA-specific infiltrating cells to express anti-inflammatory cytokines such as IL-10 and transforming growth
15 factor-beta (TGF- β) together with brain-derived neurotrophic factor (BDNF) seem to correlate with the therapeutic activity of GA in EAE.(15,16,17)

Clinical experience with GA consists of information obtained from completed and ongoing clinical trials and from post-marketing
20 experience. The clinical program includes three double-blind, placebo-controlled studies in RRMS subjects treated with GA 20 mg/day.(18,19,20) A significant reduction in the number of relapses, compared with placebo, was seen. In the largest controlled study, the relapse rate was reduced by 32% from 1.98 under placebo to 1.34 under
25 GA 20 mg. GA 20 mg has also demonstrated beneficial effects over placebo on MRI parameters relevant to RRMS. A significant effect in median cumulative number of Gd-enhancing lesions over 9 months of treatment (11 lesions in the 20 mg group compared to 17 lesions under placebo) was demonstrated.

30 The clinical program with GA also includes one double-blind study in chronic-progressive MS subjects,(21) one double-blind placebo-controlled study in primary progressive patients,(22) one double-blind

placebo-controlled study in CIS patients(23) and numerous open-label and compassionate use studies, mostly in RRMS. The clinical use of GA has been extensively reviewed and published in the current literature. (24,25,26,27)

SUMMARY OF THE INVENTION

The present invention provides a method for treating a subject afflicted with multiple sclerosis with a pharmaceutical composition comprising glatiramer acetate and a pharmaceutically acceptable carrier, comprising the steps of:

- a) administering a therapeutic amount of the pharmaceutical composition to the subject;
- b) determining whether the subject is a glatiramer acetate responder by evaluating IL-27 concentration in the blood of the subject; and
- 10 c) continuing the administration of the pharmaceutical composition if the subject is identified as a glatiramer acetate responder, or modifying treatment of the subject if the subject is not identified as a glatiramer acetate responder.

The present invention also provides a method for monitoring treatment of multiple sclerosis in a subject afflicted therewith, comprising:

- a) administering to the subject a therapeutic amount of a pharmaceutical composition comprising glatiramer acetate and a pharmaceutically acceptable carrier; and
- b) evaluating a change in IL-27 concentration in the blood of the subject.

The present invention also provides a method for determining clinical responsiveness to glatiramer acetate therapy in a subject afflicted with multiple sclerosis and receiving glatiramer acetate, the method comprising evaluating IL-27 concentration in the blood of the subject, to thereby determine clinical responsiveness to glatiramer acetate treatment.

BRIEF DESCRIPTION OF THE FIGURES**Fig. 1A-1D**

Differential production of IL-27 by Multiple Sclerosis (MS) and healthy donors (HD) peripheral blood mononuclear cells (PBMCs) in response to GA stimulation in vitro.

(Fig. 1A-1C) Production of IL-27 derived from the supernatants of human MS PBMC samples which were cultured in the presence or absence of the indicated GA concentrations for 3 days. PBMCs derived from different MS patients appear to segregate into groups of (Fig. 1A) efficient IL-27 producers, (Fig. 1B) weak IL-27 producers, or (Fig. 1C) IL-27 non-producers in response to GA stimulation *in vitro*. (Fig. 1D) Production of IL-27 derived from the supernatants of human HD PBMC samples cultured in the presence or absence 50 µg/ml GA for 3 days. Data shown was pooled from at least three separate experiments carried out in triplicate or duplicate wells. BD; below detection.

Fig. 2A-2B

Differential expression of EBI3 and IL27p28 in efficient IL-27 producers versus non-producers.

Human MS PBMCs derived from efficient IL-27 producers (Fig. 2A) and IL-27 non-producers (Fig. 2B) were cultured in the presence or absence of 50 µg/ml GA overnight (16-17 h). Cells were harvested for quantitative real-time PCR analysis in order to analyze gene expression of the IL-27 heterodimer subunits, EBI3 and IL-27p28, in (Fig. 2A) efficient IL-27 producers versus (Fig. 2B) non-producers in response to GA stimulation *in vitro*. EBI3 and IL27p28 expression were both normalized to the gene expression of β-actin. The baseline expression of EBI3 or IL27p28 is designated at the 0 h time point. Data shown are representative from two experiments. BD; below detection threshold.

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Fig. 3A-3C

GA-mediated augmentation of IL-27 production by MS PBMCs in the presence of inflammation-inducing stimuli.

5 Production of IL-27 in PBMCs isolated from (Fig. 3A) efficient IL-27 producers, (Fig. 3B) weak IL-27 producers, and (Fig. 3C) IL-27 non-producers upon cultivation in the presence or absence of 50 µg/ml GA with/without ODN 684 or Pam3CSK4 at the indicated concentrations for 3 d. Data shown was pooled from three separate *in vitro* cell culture experiments carried out in triplicate wells. BD; below detection.

10 Figure 4

Correlation between serum IL-27 production and clinical response in GA-treated MS patients.

15 IL-27 production in the serum derived from total MS patients (n=30), GA responders (GA-R; n=15) or GA non-responders (GA-NR; n=15). All graphs indicate the mean ± standard error of mean (S.E.M.; error bars) for each time point throughout the administration of GA treatment.

Figure 5

Correlation between serum IL-10 production and clinical response in GA-treated MS patients.

20 Serum IL-10 production in MS patients grouped according to their clinical response to GA treatment as either GA responders (GA-R; n=17) or GA non-responders (GA-NR; n=14) for a minimum of at least two time points. All graphs indicate the mean ± standard error of mean (S.E.M.; error bars) for each time point.

25

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method for treating a subject afflicted with multiple sclerosis with a pharmaceutical composition comprising glatiramer acetate and a pharmaceutically acceptable carrier, comprising the steps of:

a) administering a therapeutic amount of the pharmaceutical composition to the subject;

b) determining whether the subject is a glatiramer acetate responder by evaluating IL-27 concentration in the blood of the subject; and

10 c) continuing the administration of the pharmaceutical composition if the subject is identified as a glatiramer acetate responder, or modifying treatment of the subject if the subject is not identified as a glatiramer acetate responder.

The present invention also provides a method for monitoring treatment
15 of multiple sclerosis in a subject afflicted therewith, comprising:

a) administering to the subject a therapeutic amount of a pharmaceutical composition comprising glatiramer acetate and a pharmaceutically acceptable carrier; and

b) evaluating a change in IL-27 concentration in the blood of the
20 subject.

The present invention also provides a method for determining clinical responsiveness to glatiramer acetate therapy in a subject afflicted with multiple sclerosis and receiving glatiramer acetate, the method comprising evaluating IL-27 concentration in the blood of the subject,
25 to thereby determine clinical responsiveness to glatiramer acetate treatment.

The present invention also provides a method of predicting clinical responsiveness to glatiramer acetate therapy in a subject afflicted

with multiple sclerosis and receiving glatiramer acetate, the method comprising evaluating IL-27 concentration in the blood of the subject, to thereby determine clinical responsiveness to glatiramer acetate treatment.

- 5 In some embodiments, the multiple sclerosis is relapsing-remitting multiple sclerosis.

In some embodiments, the IL-27 concentration is serum concentration.

In some embodiments, the IL-27 concentration is PBMC supernatant concentration.

- 10 In some embodiments, an increase in IL-27 concentration is associated with a subject being a responder to glatiramer acetate treatment.

In some embodiments, the increase in IL-27 concentration is observed at 3 months after the first administration of glatiramer acetate.

- 15 In some embodiments, the increase in IL-27 concentration is observed at 6 months after the first administration of glatiramer acetate.

In some embodiments, the increase in IL-27 concentration is observed at 9 months after the first administration of glatiramer acetate.

In some embodiments, the increase in IL-27 concentration is observed at 12 months after the first administration of glatiramer acetate.

- 20 In some embodiments, the increase in IL-27 concentration is observed at 24 months after the first administration of glatiramer acetate.

In some embodiments, at 6 months the serum IL-27 concentration is greater than or equal to 1500 pg/ml.

- 25 In some embodiments, at 6 months the serum IL-27 concentration is greater than or equal to 1000 pg/ml, 1100 pg/ml, 1200 pg/ml, 1300 pg/ml, 1400 pg/ml, 1600 pg/ml, 1700 pg/ml, 1800 pg/ml, 1900 pg/ml, 2000 pg/ml, 2100 pg/ml, 2200 pg/ml, 2300 pg/ml, 2400 pg/ml or 2500

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pg/ml.

In some embodiments, the invention further comprises evaluating IL-10 concentration in the blood of the subject.

In some embodiments, the IL-10 concentration is serum concentration.

- 5 In some embodiments, the IL-10 concentration is PBMC supernatant concentration.

In some embodiments, an increase in IL-10 concentration relative to baseline is associated with a subject being a responder to glatiramer acetate treatment.

- 10 In some embodiments, the increase in IL-10 concentration is observed at 3 months after the first administration of glatiramer acetate.

In some embodiments, the increase in IL-10 concentration is observed at 6 months after the first administration of glatiramer acetate.

- 15 In some embodiments, the increase in IL-10 concentration is observed at 9 months after the first administration of glatiramer acetate.

In some embodiments, the increase in IL-10 concentration is observed at 12 months after the first administration of glatiramer acetate.

In some embodiments, the increase in IL-10 concentration is observed at 24 months after the first administration of glatiramer acetate.

- 20 In some embodiments, at 6 months the serum IL-10 concentration is greater than or equal to 45 pg/ml.

In some embodiments, at 6 months the serum IL-10 concentration is greater than or equal to 35 pg/ml, 40 pg/ml, 50 pg/ml, 55 pg/ml, 60 pg/ml, 65 pg/ml, 70 pg/ml or 75 pg/ml.

- 25 In some embodiments, the invention further comprises evaluating the gene expression level of EBI3 or p28 in the blood of the subject.

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In some embodiments, administering the pharmaceutical composition comprises administering to the human subject three subcutaneous injections of the pharmaceutical composition over a period of seven days with at least one day between every subcutaneous injection.

- 5 In some embodiments, the pharmaceutical composition is a unit dose of a 1 ml aqueous solution comprising 20 mg of glatiramer acetate.

In some embodiments, the pharmaceutical composition is a unit dose of a 1 ml aqueous solution comprising 40 mg of glatiramer acetate.

In some embodiments, the human subject is a naive patient.

- 10 In some embodiments, the human subject has been previously administered a multiple sclerosis drug other than glatiramer acetate.

- In some embodiments, if the human subject is identified as a glatiramer acetate responder, the human subject is thereafter administered the pharmaceutical composition comprising glatiramer
15 acetate and a pharmaceutically acceptable carrier as monotherapy.

In some embodiments, if the human subject is not identified as a glatiramer acetate responder, the human subject is thereafter administered a multiple sclerosis drug which is not glatiramer acetate.

- 20 The present invention also provides a pharmaceutical composition comprising glatiramer acetate and a pharmaceutically acceptable carrier for use in treating a human subject afflicted with multiple sclerosis, wherein the human subject has been administered a therapeutic amount of the pharmaceutical composition, and wherein the
25 human subject is determined as a glatiramer acetate responder as identified by having an increase of IL-27 concentration in the blood of the subject.

The present invention also provides a pharmaceutical composition comprising glatiramer acetate and a pharmaceutically acceptable

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carrier for the manufacture of a medicament for use in treating a human subject afflicted with multiple sclerosis, wherein the human subject has been administered a therapeutic amount of the pharmaceutical composition, and wherein the human subject is
5 determined as a glatiramer acetate responder as identified by having an increase of IL-27 concentration in the blood of the subject.

The present invention also provides a use of a pharmaceutical composition comprising glatiramer acetate and a pharmaceutically acceptable carrier for treating a human subject afflicted with
10 multiple sclerosis, wherein the human subject has been administered a therapeutic amount of the pharmaceutical composition, and wherein the human subject is determined as a glatiramer acetate responder as identified by having an increase of IL-27 concentration in the blood of the subject.

15 Definitions

Forms of Multiple Sclerosis:

There are five distinct disease stages and/or types of MS:

- 1) benign multiple sclerosis;
- 2) relapsing-remitting multiple sclerosis (RRMS);
- 20 3) secondary progressive multiple sclerosis (SPMS);
- 4) progressive relapsing multiple sclerosis (PRMS; and
- 5) primary progressive multiple sclerosis (PPMS)

Benign multiple sclerosis is a retrospective diagnosis which is
25 characterized by 1-2 exacerbations with complete recovery, no lasting disability and no disease progression for 10-15 years after the initial onset. Benign multiple sclerosis may, however, progress into

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other forms of multiple sclerosis.

Patients suffering from RRMS experience sporadic exacerbations or relapses, as well as periods of remission. Lesions and evidence of axonal loss may or may not be visible on MRI for patients with RRMS.

5 SPMS may evolve from RRMS. Patients afflicted with SPMS have relapses, a diminishing degree of recovery during remissions, less frequent remissions and more pronounced neurological deficits than RRMS patients. Enlarged ventricles, which are markers for atrophy of the corpus callosum, midline center and spinal cord, are visible on MRI of
10 patients with SPMS.

PPMS is characterized by a steady progression of increasing neurological deficits without distinct attacks or remissions. Cerebral lesions, diffuse spinal cord damage and evidence of axonal loss are evident on the MRI of patients with PPMS. PPMS has periods of acute
15 exacerbations while proceeding along a course of increasing neurological deficits without remissions. Lesions are evident on MRI of patients suffering from PRMS. (28)

A clinically isolated syndrome (CIS) is a single monosymptomatic attack compatible with MS, such as optic neuritis, brain stem
20 symptoms, and partial myelitis. Patients with CIS that experience a second clinical attack are generally considered to have clinically definite multiple sclerosis (CDMS). Over 80 percent of patients with a CIS and MRI lesions go on to develop MS, while approximately 20 percent have a self-limited process. (29,30)

25 Multiple sclerosis may present with optic neuritis, blurring of vision, diplopia, involuntary rapid eye movement, blindness, loss of balance, tremors, ataxia, vertigo, clumsiness of a limb, lack of coordination, weakness of one or more extremity, altered muscle tone, muscle stiffness, spasms, tingling, paraesthesia, burning sensations,
30 muscle pains, facial pain, trigeminal neuralgia, stabbing sharp pains, burning tingling pain, slowing of speech, slurring of words, changes

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in rhythm of speech, dysphagia, fatigue, bladder problems (including urgency, frequency, incomplete emptying and incontinence), bowel problems (including constipation and loss of bowel control), impotence, diminished sexual arousal, loss of sensation, sensitivity to heat, 5 loss of short term memory, loss of concentration, or loss of judgment or reasoning.

Relapsing Form of Multiple Sclerosis:

The term relapsing MS includes:

- 1) patients with RRMS;
- 10 2) patients with SPMS and superimposed relapses; and
- 3) patients with CIS who show lesion dissemination on subsequent MRI scans according to McDonald's criteria.

As used herein, relapsing forms of multiple sclerosis include: Relapsing-remitting multiple sclerosis (RRMS), characterized by 15 unpredictable acute episodes of neurological dysfunction (relapses), followed by variable recovery and periods of clinical stability;

Secondary Progressive MS (SPMS), wherein patients having RRMS develop sustained deterioration with or without relapses superimposed; and

20 Primary progressive-relapsing multiple sclerosis (PPRMS) or progressive-relapsing multiple sclerosis (PRMS), an uncommon form wherein patients developing a progressive deterioration from the beginning can also develop relapses later on.

As used herein, "a subject afflicted with multiple sclerosis" includes a subject who has been clinically diagnosed to have multiple sclerosis 25 or relapsing multiple sclerosis (RMS), which includes relapsing-remitting multiple sclerosis (RRMS) and Secondary Progressive multiple sclerosis (SPMS), or is a subject presenting a clinically isolated syndrome (CIS).

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Relapses are characterized by the occurrence of neurological dysfunction symptoms, appearing after a 30-day period of stability or improvement and lasting for more than 24 hours (no infection, no fever). The number of relapses are analyzed using a logistic regression model controlling for treatment and age.

"Relapse Rate" is the number of confirmed relapses per unit time. "Annualized relapse rate" (ARR) is the mean value of the number of confirmed relapses per each patient multiplied by 365 and divided by the number of days on study drug per each patient.

As used herein, the "Kurtzke Expanded Disability Status Scale (EDSS)" is a method of quantifying disability in multiple sclerosis. The EDSS replaced the previous Disability Status Scales which used to bunch people with MS in the lower brackets. The EDSS quantifies disability in eight Functional Systems (FS) and allows neurologists to assign a Functional System Score (FSS) in each of these. The Functional Systems are: pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual & cerebral (according to www.multiple-sclerosis.org/expandeddisabilitystatusscale).

A "pharmaceutically acceptable carrier" refers to a carrier or excipient that is suitable for use with humans and/or animals without undue adverse side effects (such as toxicity, irritation, and allergic response) commensurate with a reasonable benefit/risk ratio. It can be a pharmaceutically acceptable solvent, suspending agent or vehicle, for delivering the instant compounds to the subject.

As used herein, "therapeutic" when referring to an amount of the compound refers to the quantity of the compound that is sufficient to yield a desired therapeutic response, which can be measured by an improvement of a symptom. For multiple sclerosis, such symptoms can include a MRI-monitored multiple sclerosis disease activity, relapse rate, accumulation of physical disability, frequency of relapses, time to confirmed disease progression, time to confirmed relapse, frequency of clinical exacerbation, brain atrophy, neuronal

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dysfunction, neuronal injury, neuronal degeneration, neuronal apoptosis, risk for confirmed progression, visual function, fatigue, impaired mobility, cognitive impairment, brain volume, abnormalities observed in whole Brain MTR histogram, general health status, functional status, quality of life, and/or symptom severity on work.

As used herein, "modifying treatment" includes stopping treatment with glatiramer acetate, stopping treatment of glatiramer acetate in favor of an alternative treatment, adding a further treatment to be used in conjunction with glatiramer acetate, changing the dose of glatiramer acetate, or any combination thereof.

As used herein, "in the blood of the subject" is represented by "serum" and also the "supernatant" of peripheral blood mononuclear cells (PBMCs) derived from the subject's blood.

As used herein, the "supernatant" at "3 months", "6 months", "9 months", "12 months" or "24 months" refer to supernatants collected from PBMCs purified from subject blood samples taken at 3, 6, 9, 12 or 24 months, respectively, and incubated in the presence of antigen i.e., glatiramer acetate, as described in the methods hereinbelow.

As used herein, "concentration observed at" a certain month refers to a concentration in the supernatant of PBMC derived from the subject's blood at that month. The concentration may be measured in freshly isolated cells or in cryopreserved cells after thawing.

As used herein, a subject at "baseline" is a subject prior to administration of the compound.

"Administering to the subject" or "administering to the (human) patient" means the giving of, dispensing of, or application of medicines, drugs, or remedies to a subject/patient to relieve, cure, or reduce the symptoms associated with a condition, e.g., a pathological condition. The administration can be periodic administration. The period of time between administrations is

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preferably consistent from time to time. Periodic administration can include administration, e.g., once daily, twice daily, three times daily, four times daily, weekly, twice weekly, three times weekly, four times a week and so on, etc.

5 "Treating" as used herein encompasses, e.g., inducing inhibition, regression, or stasis of a disease or disorder, e.g., Relapsing MS (RMS), or alleviating, lessening, suppressing, inhibiting, reducing the severity of, eliminating or substantially eliminating, or ameliorating a symptom of the disease or disorder. "Treating" as
10 applied to patients presenting CIS can mean delaying the onset of clinically definite multiple sclerosis (CDMS), delaying the progression to CDMS, reducing the risk of conversion to CDMS, or reducing the frequency of relapse in a patient who experienced a first clinical episode consistent with multiple sclerosis and who has a high
15 risk of developing CDMS.

"Inhibition" of disease progression or disease complication in a subject means preventing or reducing the disease progression and/or disease complication in the subject.

A "symptom" associated with MS or RMS includes any clinical or
20 laboratory manifestation associated with MS or RMS and is not limited to what the subject can feel or observe.

As used herein, a "naive patient" is a subject that has not been treated with any multiple sclerosis drug.

As used herein, a "multiple sclerosis drug" is a drug or an agent
25 intended to treat clinically defined MS, CIS or symptoms of any of the above mentioned diseases. "Multiple sclerosis drugs" may include but are not limited to antibodies, immunosuppressants, anti-inflammatory agents, immunomodulators, cytokines, cytotoxic agents and steroids and may include approved drugs, drugs in clinical trial, or alternative
30 treatments, intended to treat clinically defined MS, CIS or any form of neurodegenerative or demyelinating diseases. "Multiple sclerosis

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drugs" include but are not limited to Interferon and its derivatives (including BETASERON®, AVONEX® and REBIF®), Mitoxantrone and Natalizumab. Agents approved or in-trial for the treatment of other autoimmune diseases, but used in a MS or CIS patient to treat MS or CIS are also defined as multiple sclerosis drugs.

For the purpose of the present invention, "glatiramer acetate or a glatiramer acetate-related peptide or polypeptide" is intended to include any peptide or polypeptide, including a random copolymer, that cross-reacts functionally with myelin basic protein (MBP) and is able to compete with MBP on binding to the MHC class II in the antigen presentation.

A copolymer for use as active agent in the present invention may be a random copolymer comprising a suitable quantity of a positively charged amino acid such as lysine (K) or arginine (R), in combination with a negatively charged amino acid (preferably in a lesser quantity) such as glutamic acid (E) or aspartic acid (D), optionally in combination with a non-charged neutral amino acid such as alanine (A) or glycine (G), serving as a filler, and optionally with an amino acid adapted to confer on the copolymer immunogenic properties, such as an aromatic amino acid like tyrosine (Y) or tryptophan (W).

The copolymers for use in the present invention can be composed of L- or D-amino acids or mixtures thereof. As is known by those of skill in the art, L-amino acids occur in most natural proteins. However, D-amino acids are commercially available and can be substituted for some or all of the amino acids used to make the copolymers used in the present invention. The present invention contemplates the use of copolymers containing both D- and L-amino acids, as well as copolymers consisting essentially of either L- or D-amino acids.

In one embodiment, the active agent for use in the present invention comprises at least one random three- or four-amino acid copolymer comprising one amino acid selected from each of the four following groups: (a) lysine (K) and arginine (R); (b) glutamic acid (E) and

aspartic acid (D); (c) alanine (A) and glycine (G); and (d) tyrosine (Y) and tryptophan (W).

In one preferred embodiment, the copolymer comprises a combination of the amino acids tyrosine, glutamic acid, alanine, and lysine, herein
5 designated poly-YEAK, of net overall positive electrical charge, and is most preferably GA, of the following molar ratio of the amino acids: about 0.14 glutamic acid, about 0.43 alanine, about 0.10 tyrosine, and about 0.34 lysine. It may be a low molecular weight or high molecular weight copolymer being a polypeptide from about 15 to
10 about 100, preferably from about 40 to about 80, amino acids in length. The copolymer has an average molecular weight of about 2,000-40,000 Da, preferably of about 2,000-13,000 Da, more preferably of about 4,700-13,000 Da, most preferably of about 5,000-9,000 Da, and mostly preferred of about 6,000-8,000 Da. Preferred molecular weight
15 ranges and processes for making a preferred form of GA are described in U.S. Patent No. 5,800,808, the entire contents of which are hereby incorporated by reference in their entirety as if fully disclosed herein.

It is clear that this is given by way of example only, and that the
20 active agent can be varied both with respect to the constituents and relative proportions of the constituents, thus obtaining poly-YEAK copolymers different from GA.

In another embodiment, the active agent of the present invention is a GA-related polypeptide that is a random copolymer containing four
25 different amino acids, each from a different one of the groups (a) to (d), but excluding GA. The activity exhibited by GA is expected to remain if one or more of the following substitutions is made in the amino acid composition of the copolymer: aspartic acid (D) for glutamic acid (E), glycine (G) for alanine (A), arginine (R) for
30 lysine (K), and tryptophan (W) for tyrosine (Y).

Thus, in another embodiment, the GA-related polypeptide of the invention may include any of those copolymers disclosed in WO

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00/05250, the entire contents of which being hereby incorporated herein by reference as if fully disclosed herein, and other synthetic amino acid copolymers such as the random four-amino acid copolymers described by Fridkis-Hareli et al.(31) as candidates for treatment of multiple sclerosis, namely copolymers (14-, 35- and 50-mers) containing the amino acids phenylalanine, glutamic acid, alanine and lysine (poly-FEAK), or tyrosine, phenylalanine, alanine and lysine (poly-YFAK), and any other similar copolymer to be discovered that can be considered a universal antigen similar to GA.

10 In another embodiment, the GA-related polypeptide of the invention is a copolymer containing a combination of three different amino acids each from a different one of three groups of the groups (a) to (d). These copolymers are herein referred to as terpolymers. In a more preferred embodiment, the mole fraction of amino acids of the terpolymers is about what is preferred for GA.

In one embodiment, the terpolymers for use in the present invention contain tyrosine (Y), alanine (A), and lysine (K), hereinafter designated poly-YAK. The average molar fraction of the amino acids in these terpolymers can vary. For example, tyrosine can be present in a mole fraction of about 0.005-0.250; alanine can be present in a mole fraction of about 0.3-0.6; and lysine can be present in a mole fraction of about 0.1-0.5, but preferably the molar ratios of tyrosine, alanine and lysine are about 0.10 to about 0.54 to about 0.35. The average molecular weight of poly-YAK is about 2,000-40,000 Da, preferably about 3,000-35,000 Da, more preferably about 5,000-25,000 Da. It is possible to substitute arginine (R) for lysine(K), glycine (G) for alanine (A), and or tryptophan (W) for tyrosine (Y).

In another embodiment, the terpolymers for use in the present invention contain tyrosine (Y), glutamic acid (E), and lysine (K), hereinafter designated poly-YEK. The average mole fraction of the amino acids in these terpolymers can vary: glutamic acid can be present in a mole fraction of about 0.005-0.300, tyrosine can be present in a mole fraction of about 0.005-0.250, and lysine can be

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present in a mole fraction of about 0.3-0.7, but preferably the molar ratios of glutamic acid, tyrosine, and lysine are about 0.26 to about 0.16 to about 0.58. The average molecular weight of poly-YEK is about 2,000-40,000 Da, preferably about 3,000-35,000 Da, more preferably about 5,000-25,000 Da. It is possible to substitute arginine (R) for lysine (K), aspartic acid (D) for glutamic acid (E), and/or tryptophan (W) for tyrosine (Y).

In a further embodiment, the terpolymers for use in the present invention contain lysine (K), glutamic acid (E), and alanine (A), hereinafter designated poly-KEA. The average molar fraction of the amino acids in these polypeptides can also vary. For example, glutamic acid can be present in a mole fraction of about 0.005-0.300, alanine in a mole fraction of about 0.005-0.600, and lysine can be present in a mole fraction of about 0.2-0.7, but preferably the molar ratios of glutamic acid, alanine and lysine are about 0.15 to about 0.48 to about 0.36. The average molecular weight of YEK is about 2,000-40,000 Da, preferably about 3,000-35,000 Da, more preferably about 5,000-25,000 Da. It is possible to substitute arginine (R) for lysine (K), aspartic acid (D) for glutamic acid (E), and/or glycine (G) for alanine (A).

In still another embodiment, the terpolymers for use in the present invention contain tyrosine (Y), glutamic acid (E), and alanine (A), hereinafter designated poly-YEA. The average molar fraction of the amino acids in these polypeptides can vary. For example, tyrosine can be present in a mole fraction of about 0.005-0.250, glutamic acid can be present in a mole fraction of about 0.005-0.300, and alanine can be present in a mole fraction of about 0.005-0.800, but preferably the molar ratios of glutamic acid, alanine, and tyrosine are about 0.21 to about 0.65 to about 0.14. The average molecular weight of poly-YEA is about 2,000-40,000 Da, preferably about 3,000-35,000 Da, and more preferably about 5,000-25,000 Da. It is possible to substitute tryptophan (W) for tyrosine (Y), aspartic acid (D) for glutamic acid (E), and/or glycine (G) for alanine (A).

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The terpolymers can be made by any procedure available to one of skill in the art for example as described in the above-mentioned publications WO 01152878 and WO 01/93893.

As binding motifs of GA to MS-associated HLA-DR molecules are known,
5 polypeptides of fixed sequence can readily be prepared and tested for binding to the peptide-binding groove of the HLA-DR molecules as described in Fridkis-Hareli et al.(32) Examples of such peptides are those disclosed in WO 005249, the entire contents of which are hereby incorporated by reference as if fully disclosed herein.

10 As used herein, "about" with regard to a stated number encompasses a range of +10 percent to -10 percent of the stated value. By way of example, about 100 mg/kg therefore includes the range 90-100 mg/kg and therefore also includes 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100,
101, 102, 103, 104, 105, 106, 107, 108, 109 and 110 mg/kg.
15 Accordingly, about 100 mg/kg includes, in an embodiment, 100 mg/kg.

It is understood that where a parameter range is provided, all integers within that range, tenths thereof, and hundredths thereof, are also provided by the invention. For example, "0.2-5 mg/kg" is a disclosure of 0.2 mg/kg, 0.21 mg/kg, 0.22 mg/kg, 0.23 mg/kg etc. up to 0.3 mg/kg, 0.31
20 mg/kg, 0.32 mg/kg, 0.33 mg/kg etc. up to 0.4 mg/kg, 0.5 mg/kg, 0.6 mg/kg etc. up to 5.0 mg/kg.

All combinations of the various elements described herein are within the scope of the invention.

Experimental Details

EXAMPLE 1

Among the numerous immunomodulatory effects of GA discovered thus far, a linkage between GA and the immune-regulating cytokine, interleukin (IL)-27, has not yet been studied. IL-27 is a heterodimeric cytokine belonging to the IL-6/IL-12 family, which is composed of the p28 and p40-related Epstein-barr virus-induced gene 3 (EBI3) subunits (Yoshida and Miyazaki, 2008). So far, the main source of IL-27 appears to be APCs upon stimulation of the Toll-like receptor (TLR) signaling pathway through myeloid differentiation primary response gene (MYD)88 (Pflanz et al., 2002; Wirtz et al., 2005; Liu et al., 2007). While various reports show opposing roles for IL-27 to promote or inhibit Th cell differentiation, the induction of IL-27 has proved to be beneficial in the context of detrimental CNS inflammation including EAE (Batten et al., 2006; Stumhofer et al., 2006; Fitzgerald et al., 2007a; Fitzgerald et al., 2007b; Diveu et al., 2009; Ilarregui et al., 2009; Murugaiyan et al., 2010; Mascanfroni et al., 2013). Therefore, the goal was to determine whether GA might be able to exhibit its beneficial effects in MS by mediating the production of IL-27.

Methods

Subjects

Thirty two patients (Female; 23 and Male; 9) diagnosed with relapsing-remitting MS (RRMS) according to the McDonald criteria (McDonald, 2001) were enrolled in this study as described previously (Dhib-Jalbut et al., 2013). The mean age was 41.1 years (range: 24-60), annualized relapse rate; 0.8 (range: 0.5-2) in the 2 years preceding enrollment, and, expanded disability status scale (EDSS); 2.1 (range; 1-4).

Classification of clinical responders and non-responders

Patients were classified as GA-responder (GA-R) or GA-non-responder (GA-NR) after at least 2 year treatment with GA as described

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previously (Dhib-Jalbut et al, 2013). A GA-R is a patient with no relapses and no evidence of disease progression as measured by EDSS at the end of treatment with GA. A GA-NR is a patient with one or more relapses or with progression in the EDSS of at least 1 point sustained for 6 months.

Analysis of IL-27 production in PBMCs

Human PBMCs used in this study were isolated from the whole blood of HD (NBAH blood center, New Brunswick, NJ) and MS patients by Ficoll-Hypaque gradient as previously described (Dhib-Jalbut et al., 2013). All cells were cultured with GA (Teva Pharmaceutical Industries Ltd., Israel) in the presence or absence of TLR ligands; Pam3CSK4 (TLR-2) and ODN 684 (TLR-9) (InvivoGen) for 3 days. Cytokine production of IL-27 and IL-10 was evaluated by ELISA kits (R&D Systems).

Quantitative Real-Time PCR

Total RNA was isolated from human PBMC cultures by RNEASY PLUS UNIVERSAL MINI KIT (Qiagen) according to the manufacturer's protocol. Then, cDNA was synthesized using Taqman® REVERSE TRANSCRIPTION REAGENTS and qRT-PCR analysis was performed on individual cDNA samples with the ABI 7500 REAL-TIME PCR SYSTEM using Taqman® GENE EXPRESSION MASTER MIX and Taqman® GENE EXPRESSION ASSAYS (Applied Biosystems by Life Technologies). All sample reactions were performed in triplicate with β -actin as the gene of reference.

Statistics

GRAPHPAD PRISM SOFTWARE was used throughout this study for statistical analyses. P-values for significant production of serum IL-27 and IL-10 amongst GA-treated patients enlisted in the study were calculated by Student's t-test. All p-values ≤ 0.05 were considered statistically significant.

Results

Heterogeneous production of IL-27 in Multiple Sclerosis (MS) patient and healthy donor (HD) peripheral blood mononuclear cells (PBMCs) in response to in vitro stimulation with GA

5 To examine whether GA can induce IL-27 production in PBMCs, PBMCs from MS patients (prior to starting GA therapy) were isolated and cultured in the absence or presence of increasing concentrations of GA (25, 50, and 100 µg/ml) (Fig. 1A-1C). The production of IL-27 appeared to be heterogeneous amongst MS patients in response to GA, as MS-1, MS-2, 10 and MS-3 appeared to be more efficient producers of IL-27 (Fig. 1A); in contrast, MS-4, MS-5, and MS-6 appeared to weakly produce IL-27 (Fig. 1B), while MS-7 and MS-8 did not increase their production of IL-27 in response to GA stimulation *in vitro* (Fig. 1C).

To check whether heterogeneous GA-mediated IL-27 production across all 15 MS patients could be a consequence of MS disease activity, the production of IL-27 in healthy donors (HD) by culturing HD PBMCs in the presence or absence of increasing concentrations of GA (25, 50, and 100 µg/ml) was analyzed. Similar to MS patients, the ability of HD PBMCs to produce IL-27 in response to GA was also heterogeneous across 20 all HD, whereby some HD were also efficient IL-27 producers (HD-1 through HD-4), weak IL-27 producers (HD-5 through HD-7), or IL-27 non-producers (HD-8) (Fig. 1D). Thus, the ability to produce IL-27 in response to GA varies between individuals, and not necessarily related to MS disease activity.

25 Since IL-27 is composed of *EBI3* and *p28* subunits, the induction of the *EBI3* and *p28* subunits within the PBMCs of an efficient IL-27 producer and IL-27 non-producer MS patient, respectively was analyzed. Though the induction of *EBI3* gene expression was slightly up-regulated in the efficient IL-27 producer and IL-27 non-producer (~2.2-fold and ~1.6- 30 fold, respectively), the induction of *p28* gene expression was much greater in the efficient IL-27 producer (~5.9-fold) (Fig. 2A-2B). On the other hand, the level of *p28* gene expression in the IL-27 non-

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producer was similar to the expression level of the *EBI3* subunit (~1.5-fold) (Fig. 2A-2B). Therefore, GA can induce *p28* gene more efficiently compared to *EBI3* gene and efficient IL-27 producers are more likely to induce greater *p28* gene expression in response to GA, while IL-27 non-producers may fail to up-regulate *p28* gene expression after GA stimulation.

Augmentation of IL-27 production via GA stimulation in the presence of TLR stimuli depends on initial IL-27 production in response to GA

Signaling through TLRs can lead to inflammation associated with the activation of innate and adaptive immunity. TLRs not only recognize pathogen-associated molecular patterns, but endogenous damage-associated molecular patterns which can be released into the host during inflammatory responses as well. In addition, TLRs are also found up-regulated on both infiltrating and resident CNS cells in MS and EAE. (33) To assess whether stimulation with GA may augment IL-27 production in response to inflammation-inducing TLR stimuli, MS PBMCs in the presence or absence of GA and increasing amounts of TLR9 agonist (ODN BW006), or TLR2 agonist (Pam3CSK4) were cultured, and assessed IL-27 production. It appeared that efficient producers of IL-27 in response to GA stimulation alone could augment the production of IL-27 in response to TLR stimuli (Fig. 3A). Weak GA-mediated IL-27 producers, however, showed only a modest augmentation of IL-27 (Fig. 3B), whereas IL-27 non-producers could not augment IL-27 production in response to the TLR stimuli (Fig. 3C). This data suggests that GA can augment the TLR-mediated IL-27 production and efficient IL-27 producers may be more likely to respond to inflammatory insults triggered through the TLR signaling pathway.

Correlation between serum IL-27 and IL-10 in clinical responders to GA therapy

To examine whether GA therapy can induce the production of IL-27 in MS patients, the serum of MS patients for the IL-27 cytokine prior to the start of GA therapy (Pre-Rx) and at time points of 3-, 6-, 9- 12-, and

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24-months following the start of GA therapy were evaluated (Figure. 4). During GA therapy, there was an increase in serum IL-27 amongst all patients at the 3- and 6-month time points, which became statistically significant for the 6-month time point ($p=0.0197$). When
5 MS patients were grouped into clinical GA responders (GA-R) or GA non-responders (GA-NR), however, a significant increase in serum IL-27 at 6-month time point ($p=0.0237$), was only observed amongst GA-R in comparison to GA-NR (Figure. 4). At the 9-12- and 24-month time
10 points, no change in serum IL-27 was detected regardless of patient grouping. Thus, these data suggest that clinical responders to GA therapy show an increase in the production of IL-27 in the serum.

Since the effects of IL-27 include the induction of IL-10-producing Tr-1 cells, which are immunosuppressive and able to ameliorate disease in EAE studies (34, 35 36), whether a correlation between serum IL-10
15 and serum IL-27 production could be observed in GA-R versus GA-NR was tested. Therefore, the production of serum IL-10 in GA-R and GA-NR was measured and an increase in serum IL-10 over the 3- and 6-month time points was found, which reached statistical significance at 6-months for GA-R ($p=0.0315$) in comparison to GA- NR (Fig. 5). In fact, a
20 decreasing trend for serum IL-10 was observed over 3- and 6-months in GA-NR.

Discussion

In recent years, studies have revealed the therapeutic mechanisms of GA to alter more than just T cell antigen reactivity at the level of the T cell receptor and MHC class II; it is now known that GA can deviate *in vivo* immune responses toward a Th2 bias, promote increased anti-inflammatory cytokines and Treg function, and influence the phenotype and reactivity of APCs (37). Moreover, several effects of GA have been found to confer neuroprotection and augment neuronal repair (37, 38).

In the present study, IL-27 production upon stimulation of human MS and HD PBMCs with GA *in vitro* were identified. GA stimulation could

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also augment the production of IL-27 from MS PBMCs cultured in the presence of TLR agonists. In addition, IL-27 and IL-10 were also increased in the serum of GA-R in comparison to GA-NR. This indicates that IL-27 induction by GA may be one of the unidentified links between GA and its beneficial immunomodulatory effects on immune system in MS treatment. However, future experiments are required to screen the GA-R and GA-NR by measuring the GA-mediated IL-27 production in PBMCs.

In this study, GA-mediated production of IL-27 in PBMCs was various among MS patients and HD, and this difference was not only reflected by measuring the levels of IL-27 cytokine production in cell culture supernatants, but was also confirmed by analyzing the gene expression levels of the IL-27 heterodimeric subunits. Since only efficient IL-27-producing MS PBMCs could augment the production of IL-27 when stimulated by GA in the presence of TLR agonists, it appears that only certain individuals may have the potential to modulate immune responses through IL-27 induction when exposed to inflammatory insults or stimuli.

One possible explanation for the differential production of IL-27 by GA could be due to the promiscuous binding of GA to MHC class II (39, 40, 41); in this case, GA may bind certain MHC class II haplotypes more efficiently, which may affect signaling through this molecule. However, it is still unclear as to which binding partner(s) GA can interact with to produce IL-27. Furthermore, upon grouping patients as GA-R and GA-NR and analyzing levels of serum IL-27 and IL-10 throughout clinical GA therapy, for the first time it was clearly shown that significant elevations in serum IL-27 and IL-10 could be evidenced in individuals responding to GA therapy. Therefore, the data herein suggest that IL-27 is a potentially promising biomarker for MS responders to GA therapy.

REFERENCES

1. Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. Multiple sclerosis. N Engl J Med 2000; 343:938-52.
2. Guideline on clinical investigation of medicinal products for the treatment of multiple sclerosis EMEA, London 16 September 2006.
3. Bjartmar C, Fox RJ. Pathological mechanisms and disease progression of multiple sclerosis: therapeutic implications. Drugs of Today 2002; 38:17-29.
4. Fleming JO. Diagnosis and management of multiple sclerosis. 1st ed. New York: Professional communications, Inc., 2002.
5. Anderson DW, Ellenberg JH, Leventhal CM et al. Revised estimate of the prevalence of multiple sclerosis in the United States. Ann Neurol 1992; 31:333-36.
6. Compston A, Lassmann H, McDonald I. The story of multiple sclerosis. In: Compston A, Confavreux C, Lassman H, McDonald I, Miller D, Noseworthy JH, Smith K, Wekerle H, editors. McAlpine's Multiple Sclerosis. London: Churchill Livingstone; 2006. p. 3-68.
7. Revel M., Pharmacol. Ther., 100(1):49-62 (2003).
8. Martinelli BF, Rovaris M, Johnson KP, Miller A, Wolinsky JS, Ladkani D, Shifroni G, Comi G, Filippi M. Effects of glatiramer acetate on relapse rate and accumulated disability in multiple sclerosis: meta-analysis of three double-blind, randomized, placebo-controlled clinical trials. Mult Scler. 2003 Aug; 9(4):349-55.
9. Mikol DD, Barkhof F, Chang P, Coyle PK, Jeffery DR, Schwid SR, Stubinski B, Uitdehaag BM; REGARD study group. Lancet Neurol. 2008 Oct;7(10):903-14. Epub 2008 Sep 11.
10. BECOME TRIAL, Presented at the 23rd Congress of the European

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Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS) in Prague, Czech Republic.

11. Comi G, Filippi M and Wolinsky JS. European /Canadian multi-center, double-blind randomized, placebo controlled study of the effects of glatiramer acetate on magnetic resonance imaging-measured disease activity and burden in patients with relapsing-remitting multiple sclerosis. *Ann Neurol* 2001; (49):290-297.
12. Fridkis HM, Aharoni R, Teitelbaum D, Arnon R, Sela M, Strominger JL. Binding of random copolymers of three amino acids to class II MHC molecules. *Int. Immunol.* 1999 May;11(5):635-41.
13. Dhib-Jalbut SS, Zhan M, Johnson KP, Martin R. Glatiramer acetate reactive blood mononuclear cells respond to myelin antigens with a Th-2 biased phenotype. *J Neuroimmunology* 2003; 140 :163-171.
14. Chen M, Gran B, Costello K, Johnson KP, Martin R, Dhib-Jalbut S. Glatiramer acetate induces a Th-2 biased response and cross-reactivity with myelin basic protein in patients with MS. *Multiple Sclerosis* 2001; 7:209-219.
15. Weber MS, Prod'homme T, Youssef S, Dunn SE, Rundle CD, Lee L, Patarroyo JC, Stüve O, Sobel RA, Steinman L, Zamvil SS. Type II monocytes modulate T cell-mediated central nervous system autoimmune disease. *Nat Med* (2007) 13:935-943.
16. Aharoni R, Kayhan B, Eilam R, Sela M, and Arnon R. Glatiramer acetate-specific T cells in the brain express T_H helper 2/3 cytokines and brain-derived neurotrophic factor in situ. *PNAS* Aug 2003;100(24):14157-62.
17. Sarchielli P, Zaffaroni M, Floridi A, Greco L, Candelieri A, Mattioni A, Tenaglia S, Di Filippo M, Calabresi P. Production of brain-derived neurotrophic factor by mononuclear cells of patients with multiple sclerosis treated with glatiramer acetate, interferon-beta 1a, and high doses of immunoglobulins. *Mult Scler*

2007 Apr;13(3):313-31. Epub 2007 Jan 29.

18. Bornstein, MB, Miller, A, Slagle, S, et al. A pilot trial of Cop 1 in exacerbating relapsing multiple sclerosis. *New Eng J Med* 1987; 317: 408-14.
19. Comi, G, Fillippi, M, Wolinsky, JS, et al. European/Canadian multicenter, double-blind, randomized, placebo-controlled study of the effects of glatiramer acetate on magnetic resonance imagine-measured disease activity and burden in patients with relapsing multiple sclerosis. *Ann Neurol* 2001; 49: 290-7.
20. Johnson, KP, Brooks, BR, Cohen, JA, et al. Extended use of glatiramer acetate (Copaxone) is well tolerated and maintains its clinical effect on multiple sclerosis relapse rate and degree of disability. *Neurology* 1998; 50:701-8.
21. Bornstein, MB, Miller, A, Slagle, S, et al. A placebo-controlled, double-blind, randomized, two-center, pilot trial of Cop-1 in chronic progressive multiple sclerosis. *Neurology* 1991; 41: 533-39.
22. Wolinsky, JS, Narayana, PA, O'Conner, P, et al. Glatiramer acetate in primary progressive multiple sclerosis: Results of a multinational, multicenter, double-blind, placebo-controlled trial. *Ann Neurol* 2007; 61:14-24.
23. Comi G, Filippi M, Treatment with glatiramer acetate delays conversion to clinically definite multiple sclerosis (CDMS) in patients with clinically isolated syndromes (CIS). *Neurology* 2008; 71 (2): 153.
24. Tselis, A, Khan, O, Lisak, RP, Glatiramer acetate in the treatment of multiple sclerosis. *Neuropsychiatric Dis Treat* 2007;3(2):259-67.

25. Wolinsky, JS, The use of glatiramer acetate in the treatment of multiple sclerosis. *Adv Neurol* 2006; 273-92.
26. Comi G, Cohen JA, Filippi M, Results from a phase III, one-year, randomized, double-blind, parallel-group, dose-comparison study with glatiramer acetate in relapsing-remitting multiple sclerosis. *Mult Scler* 2008; 14(suppl 1):S299.
27. Comi G, Filippi M. Presented at: 60th Annual Meeting of the American Academy of Neurology: April 12-19; Chicago, IL. Abstract LBS.003.
28. Vieira, P.L., Heystek, H.C., Wormmeester, J., Wierenga, E.A., Kapsenberg, M.L., 2003. Glatiramer acetate (copolymer-1, copaxone) promotes Th2 cell development and increased IL-10 production through modulation of dendritic cells. *J Immunol* 170, 4483-4488.
29. Kim, H.J., Ifergan, I., Antel, J.P., Seguin, R., Duddy, M., Lapierre, Y., Jalili, F., Bar-Or, A., 2004. Type 2 monocyte and microglia differentiation mediated by glatiramer acetate therapy in patients with multiple sclerosis. *J Immunol* 172, 7144-7153.
30. Weber, M.S., Prod'homme, T., Youssef, S., Dunn, S.E., Rundle, C.D., Lee, L., Patarroyo, J.C., Stuve, O., Sobel, R.A., Steinman, L., Zamvil, S.S., 2007. Type II monocytes modulate T cell-mediated central nervous system autoimmune disease. *Nat Med* 13, 935-943.
31. Fridkis-Hareli M, Santambrogio L, Stern JN, Fugger L, Brosnan C, Strominger JL. Novel synthetic amino acid copolymers that inhibit autoantigen-specific T cell responses and suppress experimental autoimmune encephalomyelitis. *J Clin Invest* 109: 1635-1643 (2002).
32. Fridkis-Hareli M, Neveu JM, Robinson RA, Lane WS, Gauthier L, Wucherpfennig KW, Sela M, Strominger JL. Binding motifs of

- copolymer 1 to multiple sclerosis-and rheumatoid arthritis-associated HLA-DR molecules. *J Immunol* 162 : 4697-4704 (1999).
33. Miranda-Hernandez, S., Baxter, A.G., 2013. Role of toll-like receptors in multiple sclerosis. *Am J Clin Exp Immunol* 2, 75-93.
 34. Awasthi, A., Carrier, Y., Peron, J.P., Bettelli, E., Kamanaka, M., Flavell, R.A., Kuchroo, V.K., Oukka, M., Weiner, H.L., 2007. A dominant function for interleukin 27 in generating interleukin 10-producing anti-inflammatory T cells. *Nat Immunol* 8, 1380-1389.
 35. Fitzgerald, D.C., Zhang, G.X., El-Behi, M., Fonseca-Kelly, Z., Li, H., Yu, S., Saris, C.J., Gran, B., Ciric, B., Rostami, A., 2007b. Suppression of autoimmune inflammation of the central nervous system by interleukin 10 secreted by interleukin 27-stimulated T cells. *Nat Immunol* 8, 1372-1379.
 36. Pot, C., Jin, H., Awasthi, A., Liu, S.M., Lai, C.Y., Madan, R., Sharpe, A.H., Karp, C.L., Miaw, S.C., Ho, I.C., Kuchroo, V.K., 2009. Cutting edge: IL-27 induces the transcription factor c-Maf, cytokine IL-21, and the costimulatory receptor ICOS that coordinately act together to promote differentiation of IL-10-producing Tr1 cells. *J Immunol* 183, 797-801.
 37. Schrempf, W., Ziemssen, T., 2007. Glatiramer acetate: mechanisms of action in multiple sclerosis. *Autoimmun Rev* 6, 469-475.
 38. Blanchette, F., Neuhaus, O., 2008. Glatiramer acetate: evidence for a dual mechanism of action. *J Neurol* 255 Suppl 1, 26-36.
 39. Fridkis-Hareli, M., Neveu, J.M., Robinson, R.A., Lane, W.S., Gauthier, L., Wucherpfennig, K.W., Sela, M., Strominger, J.L., 1999. Binding motifs of copolymer 1 to multiple sclerosis- and rheumatoid arthritis-associated HLA-DR molecules. *J Immunol* 162, 4697-4704.
 40. Fridkis-Hareli, M., Strominger, J.L., 1998. Promiscuous binding

-34-

of synthetic copolymer 1 to purified HLA-DR molecules. J Immunol 160, 4386-4397.

41. Fridkis-Hareli, M., Teitelbaum, D., Gurevich, E., Pecht, I., Brautbar, C., Kwon, O.J., Brenner, T., Arnon, R., Sela, M., 1994. Direct binding of myelin basic protein and synthetic copolymer 1 to class II major histocompatibility complex molecules on living antigen-presenting cells--specificity and promiscuity. Proc Natl Acad Sci U S A 91, 4872-4876.

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What is claimed is:

1. A method for treating a subject afflicted with multiple sclerosis with a pharmaceutical composition comprising glatiramer acetate and a pharmaceutically acceptable carrier, comprising the steps of:
 - a) administering a therapeutic amount of the pharmaceutical composition to the subject;
 - b) determining whether the subject is a glatiramer acetate responder by evaluating IL-27 concentration in the blood of the subject; and
 - c) continuing the administration of the pharmaceutical composition if the subject is identified as a glatiramer acetate responder, or modifying treatment of the subject if the subject is not identified as a glatiramer acetate responder.
2. A method for monitoring treatment of multiple sclerosis in a subject afflicted therewith, comprising:
 - a) administering to the subject a therapeutic amount of a pharmaceutical composition comprising glatiramer acetate and a pharmaceutically acceptable carrier; and
 - b) evaluating a change in IL-27 concentration in the blood of the subject.
3. A method for determining clinical responsiveness to glatiramer acetate therapy in a subject afflicted with multiple sclerosis and receiving glatiramer acetate, the method comprising evaluating IL-27 concentration in the blood of the subject, to thereby determine clinical responsiveness to glatiramer acetate treatment.
4. The method of any one of claims 1-3, wherein the multiple sclerosis is relapsing-remitting multiple sclerosis.

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5. The method of any one of claims 1-4, wherein the IL-27 concentration is serum concentration.
6. The method of any one of claims 1-4, wherein the IL-27 concentration is PBMC supernatant concentration.
7. The method of any one of claims 1-6, wherein an increase in IL-27 concentration is associated with a subject being a responder to glatiramer acetate treatment.
8. The method of claim 7, wherein the increase in IL-27 concentration is observed at 3 months after the first administration of glatiramer acetate.
9. The method of claim 7, wherein the increase in IL-27 concentration is observed at 6 months after the first administration of glatiramer acetate.
10. The method of claim 7, wherein the increase in IL-27 concentration is observed at 9 months after the first administration of glatiramer acetate.
11. The method of claim 7, wherein the increase in IL-27 concentration is observed at 12 months after the first administration of glatiramer acetate.
12. The method of claim 7, wherein the increase in IL-27 concentration is observed at 24 months after the first administration of glatiramer acetate.
13. The method of claim 9, wherein at 6 months the serum IL-27 concentration is greater than or equal to 1500 pg/ml.
14. The method of any one of claims 1-13 further comprising evaluating IL-10 concentration in the blood of the subject.
15. The method of claim 14, wherein the IL-10 concentration is serum concentration.

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16. The method of claim 14, wherein the IL-10 concentration is PBMC supernatant concentration.
17. The method of any one of claims 14-16, wherein an increase in IL-10 concentration relative to baseline is associated with a subject being a responder to glatiramer acetate treatment.
18. The method of claim 17, wherein the increase in IL-10 concentration is observed at 3 months after the first administration of glatiramer acetate.
19. The method of claim 17, wherein the increase in IL-10 concentration is observed at 6 months after the first administration of glatiramer acetate.
20. The method of claim 17, wherein the increase in IL-10 concentration is observed at 9 months after the first administration of glatiramer acetate.
21. The method of claim 17, wherein the increase in IL-10 concentration is observed at 12 months after the first administration of glatiramer acetate.
22. The method of claim 17, wherein the increase in IL-10 concentration is observed at 24 months after the first administration of glatiramer acetate.
23. The method of claim 19, wherein at 6 months the serum IL-10 concentration is greater than or equal to 45 pg/ml.
24. The method of any one of claims 1-23 further comprising evaluating the gene expression level of EBI3 or p28 in the blood of the subject.
25. The method of any one of claims 1-2 or 4-24, wherein administering the pharmaceutical composition comprises administering to the human subject three subcutaneous injections of the pharmaceutical composition over a period of seven days with at least one day between every subcutaneous injection.

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26. The method of any one of claims 1-2 or 4-25, wherein the pharmaceutical composition is a unit dose of a 1 ml aqueous solution comprising 20 mg of glatiramer acetate.
27. The method of any one of claims 1-2 or 4-25, wherein the pharmaceutical composition is a unit dose of a 1 ml aqueous solution comprising 40 mg of glatiramer acetate.
28. The method of any one of claims 1-2 or 4-27, wherein the human subject is a naive patient.
29. The method of any one of claims 1-2 or 4-27, wherein the human subject has been previously administered a multiple sclerosis drug other than glatiramer acetate.
30. The method of any one of claims 1 or 4-29, wherein if the human subject is identified as a glatiramer acetate responder, the human subject is thereafter administered the pharmaceutical composition comprising glatiramer acetate and a pharmaceutically acceptable carrier as monotherapy.
31. The method of any one of claims 1 or 4-29, wherein if the human subject is not identified as a glatiramer acetate responder, the human subject is thereafter administered a multiple sclerosis drug which is not glatiramer acetate.

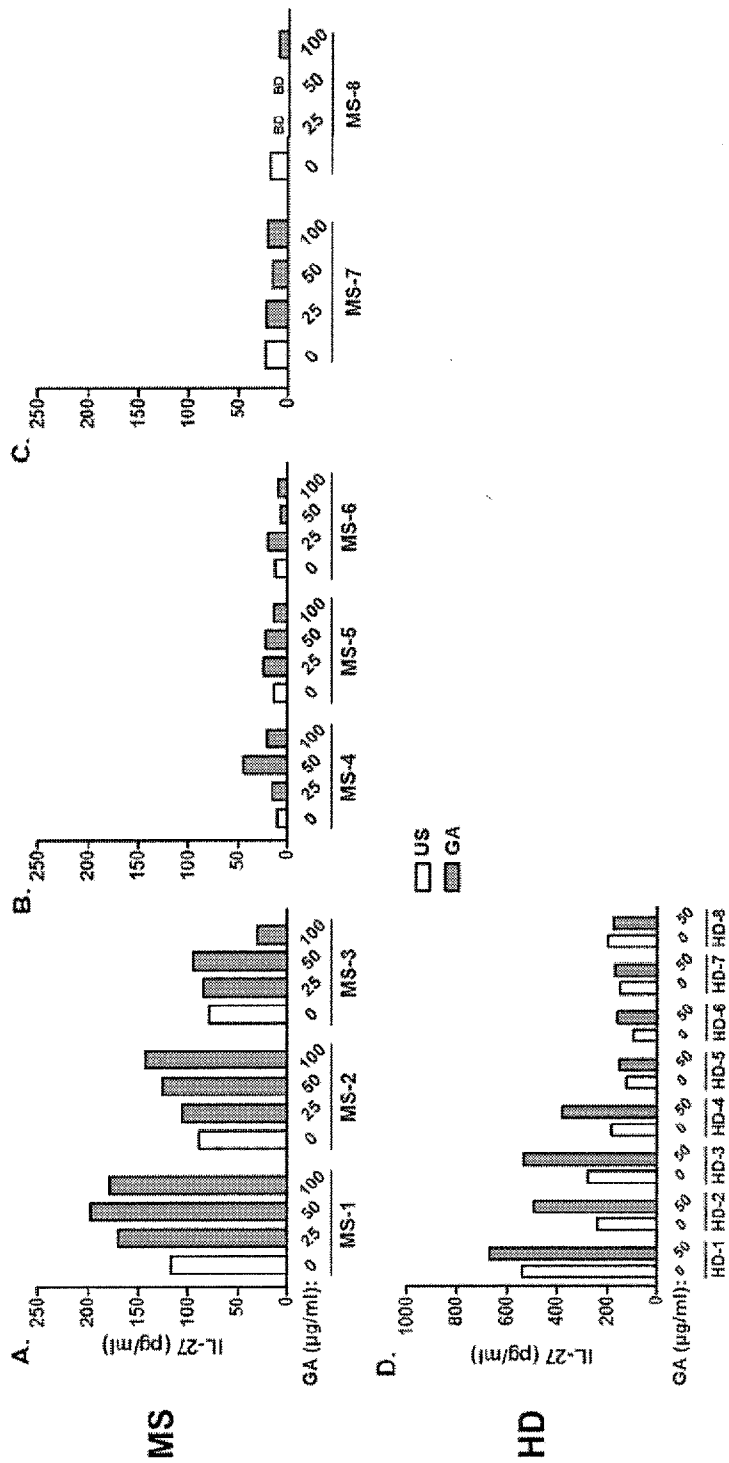


Fig. 1A-1D

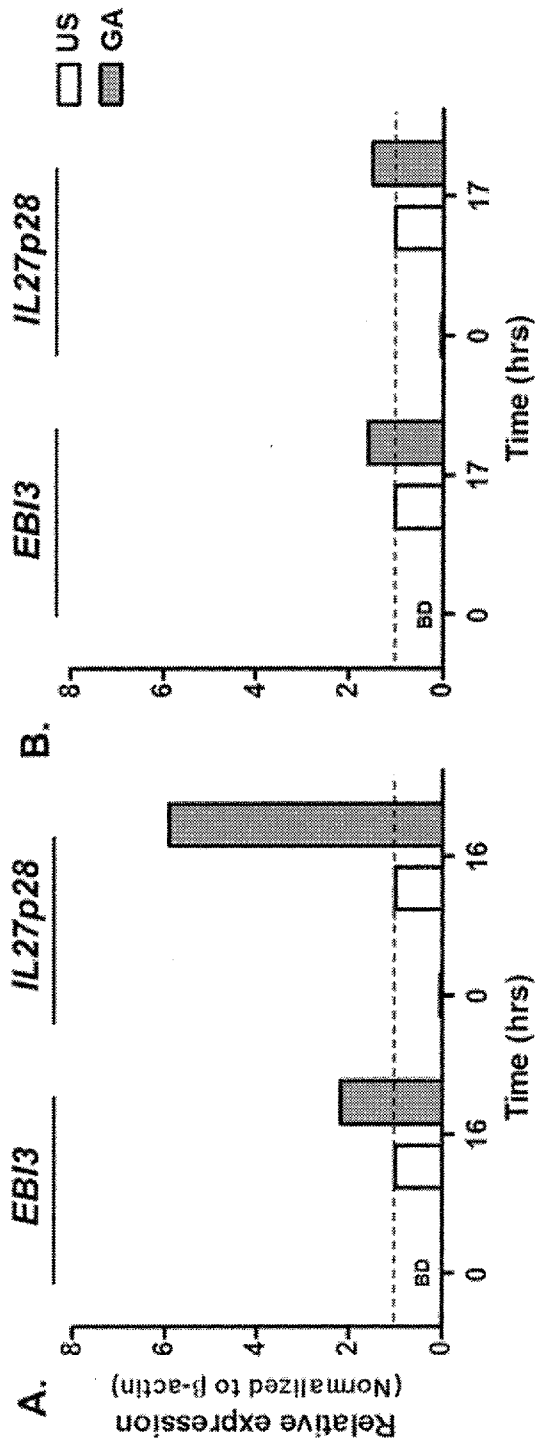


Fig. 2A-2B

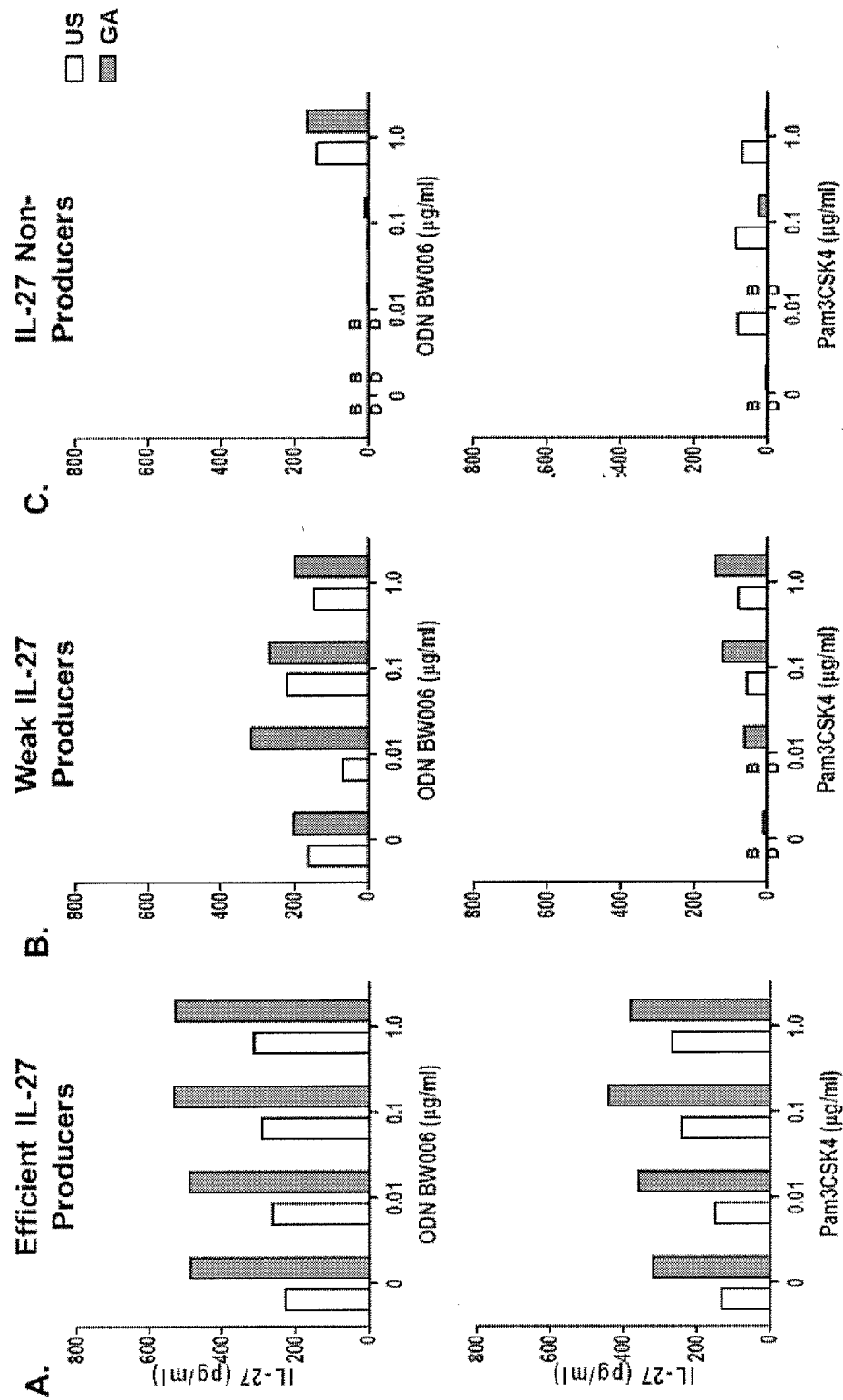


Fig. 3A-3C

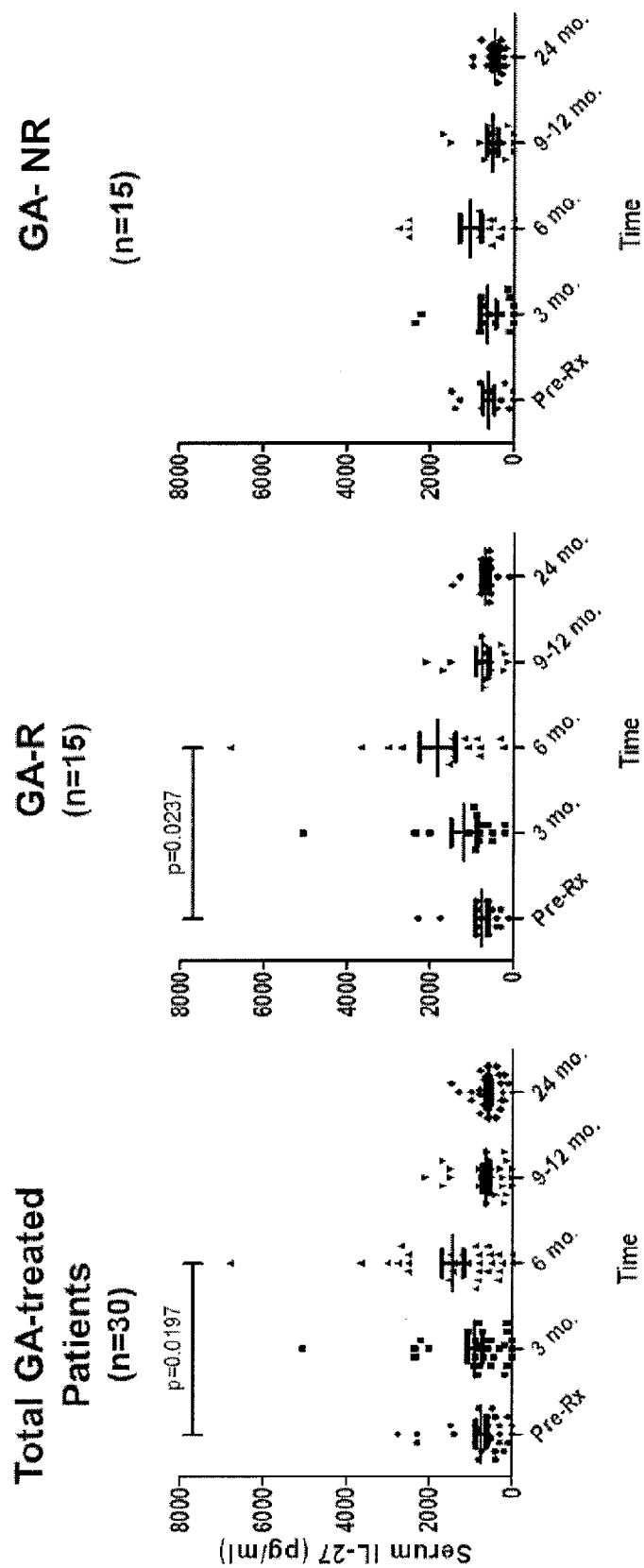


Figure 4

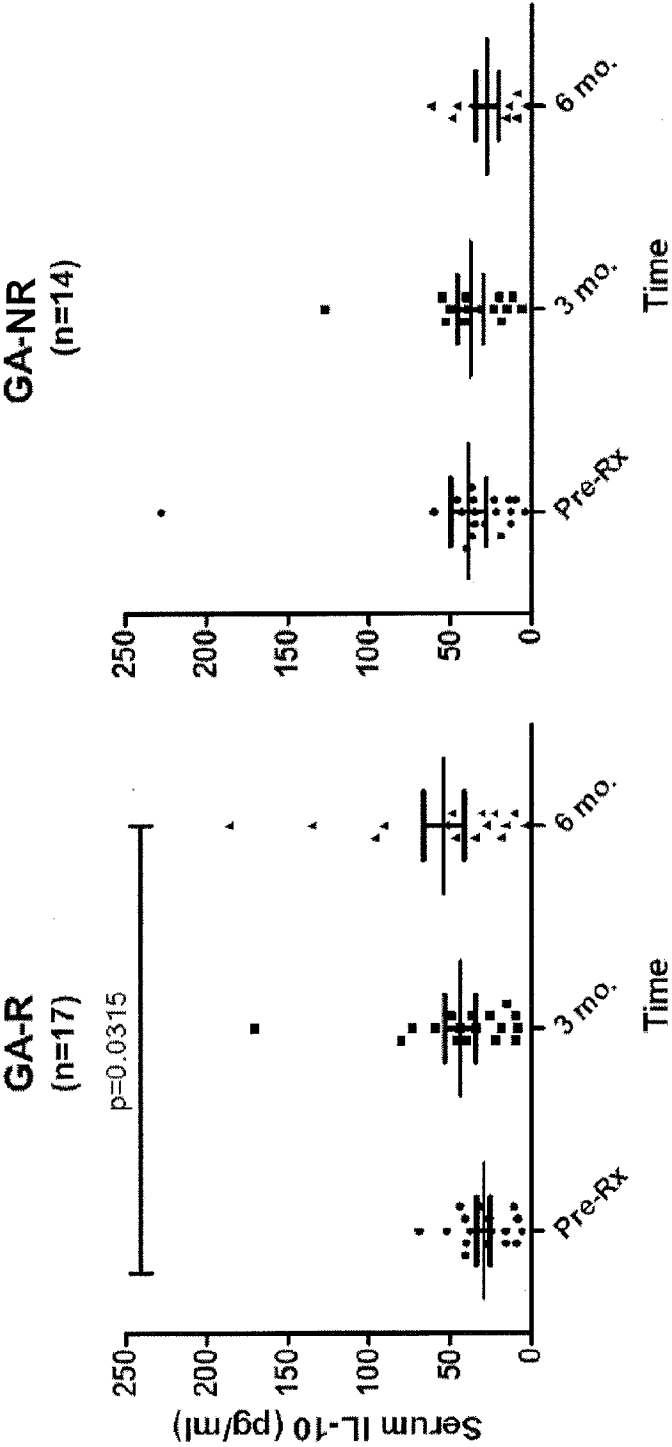


Figure 5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/065175

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/03; C07K 4/00; G01N 33/50 (2017.01)

CPC - G01N 33/5008; G01N 33/6869; G01N 2333/54; A61K 31/785; A61K 38/03; C07K 4/00 (2017.01)

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)

See Search History document

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

USPC - 424/184.1; 435/6.13; 514/567; 530/300 (keyword delimited)

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2014/0322158 A1 (TEVA PHARMACEUTICAL INDUSTRIES LTD) 30 October 2014 (30.10.2014) entire document	1-4
Y	WO 2015/112904 A1 (UNIVERSITY OF FLORIDA RESEARCH FOUNDATION INC) 30 July 2015 (30.07.2015) entire document	1-4

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* 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

23 January 2017

Date of mailing of the international search report

16 FEB 2017

Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/065175

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.: 5-31
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.