(12) STANDARD PATENT (11) Application No. **AU 2017319728 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Depot systems comprising glatiramer acetate

(51) International Patent Classification(s)
A61K 38/10 (2006.01) ***A61K 9/50*** (2006.01)
A61K 9/16 (2006.01) ***A61K 38/16*** (2006.01)

(21) Application No: **2017319728** (22) Date of Filing: **2017.08.09**

(87) WIPO No: **WO18/042415**

(30) Priority Data

(31) Number	(32) Date	(33) Country
62/381,598	**2016.08.31**	**US**

(43) Publication Date: **2018.03.08**
(44) Accepted Journal Date: **2024.09.05**

(71) Applicant(s)
Mapi Pharma Ltd

(72) Inventor(s)
Marom, Ehud;Bleich Kimelman, Nadav;Danon, Uri;Rubnov, Shai

(74) Agent / Attorney
Pipers Intellectual Property, PO Box 5298 Imagetext House 3 Owens Road, Auckland, Auckland, 1141, NZ

(56) Related Art
ANONYMOUS: 8 August 2014 (2014-08-08), XP055679718, Retrieved from the Internet [retrieved on 20200325]
WO 2018/002930 A1

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
08 March 2018 (08.03.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/042415 A1

(51) International Patent Classification:
A61K 38/10 (2006.01) *A61K 9/16* (2006.01)
A61K 38/16 (2006.01) *A61K 9/50* (2006.01)

(21) International Application Number:
PCT/IL2017/050882

(22) International Filing Date:
09 August 2017 (09.08.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/381,598 31 August 2016 (31.08.2016) US

(71) Applicant: **MAPI PHARMA LTD** [IL/IL]; 16 Einstein Street, Weizmann Science Park, P.O. Box 4113, 74140 Ness Ziona (IL).

(72) Inventors: **MAROM, Ehud**; 7 Aluf Kalman Magen Street, 6107079 Tel Aviv (IL). **BLEICH KIMELMAN, Nadav**; 30/6 HaRav Berlin Street, 9250319 Jerusalem (IL). **DANON, Uri**; 8 Mavo Ganigar Street, 6935978 Tel Aviv (IL). **RUBNOV, Shai**; 37 Gruzenberg Street, 6581141 Tel Aviv (IL).

(74) Agent: **WEBB, Cynthia** et al.; WEBB & CO., P.O. BOX 2189, 7612101 REHOVOT (IL).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

Published:
— *with international search report (Art. 21(3))*

WO 2018/042415 A1

(54) Title: DEPOT SYSTEMS COMPRISING GLATIRAMER ACETATE

(57) Abstract: The present invention provides compositions and methods of use thereof for treating or ameliorating multiple sclerosis (MS) by administering a depot formulation comprising 40 mg glatiramer acetate intramuscularly to the MS patient.

DEPOT SYSTEMS COMPRISING GLATIRAMER ACETATE

FIELD OF THE INVENTION

The present invention relates to a long acting dosage form of glatiramer acetate and other pharmacologically acceptable salts of glatiramer. Particularly, the present invention relates to depot formulations and other implantable systems for prolonged release of glatiramer acetate.

BACKGROUND OF THE INVENTION

Multiple Sclerosis (MS) is a chronic, debilitating autoimmune disease of the central nervous system (CNS). MS disease activity can be monitored by magnetic resonance imaging (MRI) of the brain, accumulation of disability, as well as rate and severity of relapses. There are several forms of multiple sclerosis, including Relapsing-Remitting Multiple Sclerosis (RRMS). Patients suffering from RRMS experience sporadic exacerbations or relapses, as well as periods of remission.

Multiple therapies have been approved for relapsing remitting forms of multiple sclerosis (MS), including COPAXONE® (glatiramer acetate), TYSABRI® (natalizumab), PLEGRIDY® (peginterferon beta-1a) and ZINBRYTA® (daclizumab). Various measures of therapeutic efficacy have been reported for these therapies. An important treatment goal is No Evidence of Disease Activity (NEDA) has become a new outcome measure (Rotstein et al., JAMA Neurol., 2015, Vol. 72(2):152-158).

Glatiramer acetate

Copolymer-1, also known as glatiramer acetate (GA), marketed under the tradename COPAXONE®, comprises the acetate salts of a mixture of synthetic polypeptides containing L-glutamic acid, L-alanine, L-tyrosine and L-lysine. The average molar fractions of the amino acids are 0.141, 0.427, 0.095 and 0.338, respectively, and the average molecular weight of copolymer-1 is between 5,000 and 9,000 Daltons. Chemically, glatiramer acetate is designated L-glutamic acid polymer with L-alanine, L-lysine and L-tyrosine, acetate (salt). Its structural formula is: $(Glu, Ala, Lys, Tyr)_x \cdot xCH_3COOH$; $(C_5H_9NO_4 \cdot C_3H_7NO_2 \cdot C_6H_{14}N_2O_2 \cdot C_9H_{11}N0_3)_x \cdot xC_2H_4O_2$ [CAS - 147245-92-9]. COPAXONE® is a clear, colorless to slightly yellow, sterile, non-pyrogenic solution for subcutaneous injection. Each milliliter contains 20 mg or 40 mg of glatiramer acetate and 40 mg of mannitol. The pH range of the solution is approximately 5.5 to 7.0 (COPAXONE®, Full Prescribing Information, 2009). COPAXONE® is indicated for reduction of the frequency of relapses in patients with

RRMS, including patients who have experienced a first clinical episode and have MRI features consistent with multiple sclerosis (COPAXONE®, glatiramer acetate injection, Full Prescribing Information, 2016).

Mechanism of Action

The mechanism of action for glatiramer acetate is unknown, although some important immunological properties of this copolymer have emerged. Administration of copolymer- 1 shifts the population of T cells from pro-inflammatory Th1 cells to regulatory Th2 cells that suppress the inflammatory response (FDA Copaxone® label). Given its resemblance to myelin basic protein, copolymer- 1 may also act as a decoy, diverting an autoimmune response against myelin. The integrity of the blood-brain barrier, however, is not appreciably affected by copolymer- 1, at least not in the early stages of treatment.

Copolymer-1 is a non-autoantigen which has been demonstrated to suppress experimental allergic encephalomyelitis (EAE) induced by various encephalitogenic agents including mouse spinal cord homogenate (MSCH) which includes all myelin antigens, such as myelin basic protein (MBP) (Sela et al., Bull Inst. Pasteur (1990) 88 303-314), proteolipid protein (PLP) (Teitelbaum et al., J. Neuroimmunol. (1996) 64 209-217) and myelin oligodendrocyte glycoprotein (MOG) (Ben-Nun et al., J. Neurol. (1996) 243 (Suppl. 1) S14-S22) in a variety of species. EAE is an accepted model for multiple sclerosis.

In phase III clinical trials, daily subcutaneous injections of copolymer-1 were found to slow the progression of disability and reduce the relapse rate in exacerbating-remitting multiple sclerosis (Johnson KP, Neurology (1995), 1, 65-70). Copolymer-1 therapy is currently limited to daily or thrice weekly subcutaneous administration. Treatment with copolymer-1 by ingestion or inhalation is disclosed in US 6,214,791, but these routes of administration have not been shown to attain clinical efficacy in human patients.

Approved doses

COPAXONE® has been approved since 1996 for treating relapsing-remitting multiple sclerosis (RRMS) in a dose of 20 mg administered by daily subcutaneous injections. Since 2014, COPAXONE® has also been approved in a dose of 40 mg administered by three injections per week, performed at least 48 hours apart. Compared to daily administration of COPAXONE® in the 20 mg dose, the latter dose and regime reduce the yearly number of injections by about 200, while maintaining the same efficacy.

Side effects

Currently, some approved treatments of multiple sclerosis involve self-injection of the active substance. Frequently observed injection-site problems include irritation, hypersensitivity, inflammation, pain and even necrosis (in the case of interferon 1β treatment) and consequent problems in patient compliance.

Side effects generally include a lump at the injection site (injection site reaction), aches, fever, and chills. These side effects are generally mild in nature. Occasionally a reaction occurs minutes after injection in which there is flushing, shortness in breath, anxiety and rapid heartbeat. These side effects subside within thirty minutes. Over time, a visible dent at the injection site due to the local destruction of fat tissue, known as lipoatrophy, may develop. Therefore, an alternative method of administration is desirable.

Several serious side effects have been reported for glatiramer acetate, according to the FDA's prescribing label, these include serious side effects to the body's cardiovascular system, digestive system (including liver), hemic and lymphatic system, musculoskeletal system, nervous system, respiratory system, special senses (in particular the eyes), urogenital system; also reported have been metabolic and nutritional disorders; however a link between glatiramer acetate and these adverse effects has not been definitively established (FDA COPAXONE® label).

Depot systems

The parenteral route by intravenous (IV), intramuscular (IM), or subcutaneous (SC) injection is the most common and effective form of delivery for small as well as large molecular weight drugs. However, pain, discomfort and inconvenience due to needle sticks makes this mode of drug delivery the least preferred by patients. Therefore, any drug delivery technology that can at a minimum reduce the total number of injections is preferred. Such reductions in frequency of drug dosing in practice may be achieved through the use of injectable depot formulations that are capable of releasing drugs in a slow but predictable manner and consequently improve compliance. For many small molecular weight drugs, depending on the dose, it may be possible to reduce the injection frequency from daily to once or twice monthly or even longer (6 months) by employing depot systems. For large molecular weight drugs and in particular for protein or polypeptide drugs there has been little success in development of depot formulations.

Microparticles, implants and gels are the most common forms of biodegradable polymeric devices used in practice for prolonging the release of drugs in the body. Microparticles are

suspended in an aqueous media right before injection and one can load as much as 40% solids in suspensions. Implant/rod formulations are delivered to SC/IM tissue with the aid of special needles in the dry state without the need for an aqueous media. This feature of rods/implants allows for higher masses of formulation, as well as drug content to be delivered. Further, in the rods/implants, the initial burst problems are minimized due to much smaller area in implants compared to the microparticles. Besides biodegradable systems, there are non-biodegradable implants and infusion pumps that can be worn outside the body. Non-biodegradable implants require a doctor not only for implanting the device into the SC/IM tissue but also to remove them after the drug release period.

Injectable compositions containing microparticle preparations are particularly susceptible to problems. Microparticle suspensions may contain as much as 40% solids as compared with 0.5-5% solids in other types of injectable suspensions. Further, microparticles used in injectable depot products, range in size up to about 250μm (average, 60-100μm), as compared with a particle size of less than 5μm recommended for IM or SC administration. The higher concentrations of solids, as well as the larger solid particle size require larger size of needle (around 18-21 gauge) for injection. Overall, despite the infrequent uses of larger and uncomfortable needles, patients still prefer the considerably less frequently administered dosage forms over everyday drug injections with a smaller needle.

Biodegradable polyesters of poly(lactic acid) (PLA) and copolymers of lactide and glycolide referred to as poly(lactic-*co*-glycolic acid) or poly(lactide-co-glycolide) (PLGA) are the most common polymers used in biodegradable dosage forms. PLA is hydrophobic molecule and PLGA degrades faster than PLA because of the presence of more hydrophilic glycolide groups. These biocompatible polymers undergo random, non-enzymatic, hydrolytic cleavage of the ester linkages to form lactic acid and glycolic acid, which are normal metabolic compounds in the body. Resorbable sutures, clips and implants are the earliest applications of these polymers. Southern Research Institute developed the first synthetic, resorbable suture (DEXON®) in 1970. The first patent describing the use of PLGA polymers in a sustained release dosage form appeared in 1973 (US 3,773,919). According to some embodiments, the biodegradable polymer is selected from PLGA, PLA, PGA and any combination thereof. Each possibility represents a separate embodiment of the invention.

Today, PLGA polymers are commercially available from multiple suppliers. Besides PLGA and PLA, natural cellulosic polymers such as starch, starch derivatives, dextran and non-PLGA synthetic polymers are also being explored as biodegradable polymers in such systems.

WO 2011/080733 to some of the inventors describes long-acting parenteral pharmaceutical compositions comprising glatiramer. Khan et al., (Ann. Neurol. 2013, Vol. 73, pages 705–713) describes a study to assess the efficacy and safety of 40 mg glatiramer acetate administered 3 times weekly in patients with relapsing–remitting multiple sclerosis (RRMS). US Patents 8,232,250, 8,399,413, 8,969,302, US 9,155,776 and 9,402,874 describe methods of alleviating symptoms of relapsing-remitting multiple sclerosis by three injections of 40 mg GA over a period of seven days.

To date, no long acting dosage forms of glatiramer acetate were tested for safety and efficacy in humans afflicted with RRMS, and are therefore not commercially available for treating humans. This is an unmet medical need, as these formulations would be extremely beneficial to many patients.

SUMMARY OF THE INVENTION

The present invention provides compositions and methods for treating relapsing remitting multiple sclerosis, comprising a single parenteral administration or implantation of a composition in the form of a depot formulation providing a monthly dose of 40 mg of pharmaceutically acceptable salts of glatiramer, in particular glatiramer acetate (GA).

It is now disclosed for the first time that the long acting pharmaceutical compositions and depot formulations according to the principles of the present invention provide equal or superior therapeutic efficacy to the commercially available daily or thrice weekly injectable dosage forms, i.e. COPAXONE®. It has further been surprisingly found that a single administration of a depot of 40 mg glatiramer acetate according to the principles of the present invention every 4 weeks is at least as therapeutically effective as daily injections of 20 mg glatiramer acetate or thrice weekly administration of 40 mg glatiramer acetate, thus lowering the need for GA administrations (injections) by a factor of 28 and 12, respectively. Importantly, the therapeutic efficiency is comparable despite the fact that the total administered dosage of GA is decreased by a factor of 14 and 12, respectively. Treating MS patients by methods disclosed in the present invention requires fewer injections with lower dosages of GA, compared to previously available therapies with glatiramer acetate.

It is further disclosed herein for the first time that methods according to the present invention had substantially superior results after one year of treatment in achieving No Evidence of Disease Activity (NEDA) compared to reported one year NEDA results for other MS drugs, such as TYSABRI® (natalizumab), PLEGRIDY® (pegylated interferon beta-1a) and ZINBRYTA®

(daclizumab). Accordingly, treatment with compositions according to the principles of invention improve the likelihood of No Evidence of Disease Activity in comparison with known therapies.

The present invention provides, in one aspect, a method of alleviating at least one symptom of relapsing-remitting multiple sclerosis (RRMS) in a human patient suffering from RRMS or a human patient who has experienced a first clinical episode and is determined to be at high risk of developing clinically definite multiple sclerosis, comprising administering to the patient a therapeutically effective regimen of a single intramuscular injection of a depot formulation comprising 40 mg dose of glatiramer acetate (GA) every 2 to 6 weeks, the regimen being sufficient to alleviate the at least one symptom of the patient, wherein the symptom is selected from the group consisting of the frequency of relapses, the number of enhancing lesions or the number of new lesions images of brain MRI, and the Expanded Disability Status Scale (EDSS) score of the patient.

In certain embodiments, the depot formulation is administered once every 3 to 5 weeks. In certain embodiments, the depot formulation is administered once every 3 weeks. In certain embodiments, the depot formulation is administered once every 4 weeks. In certain embodiments, the depot formulation is administered once every 5 weeks.

In certain embodiments, alleviating a symptom comprises reducing the frequency of relapses.

In certain embodiments, alleviating a symptom comprises reducing the number or volume of enhancing lesions or the number of new lesions in the brain. In certain embodiments, alleviating a symptom comprises reducing brain atrophy, reducing the number or volume of Gd-enhancing lesions, reducing the number or volume of T_1-weighted enhancing lesions, or reducing the number of new T_2-weighted lesions, in the patient. In certain embodiments, alleviating a symptom comprises reducing the mean cumulative number of Gd-enhancing lesions, reducing the mean number of new T2 lesions, reducing the total volume of T2 lesions, or reducing the cumulative number of enhancing lesions on T1-weighted images in the brain of the patient. In certain embodiments, alleviating a symptom comprises reducing the number of new hypo-intense lesions on enhanced T1 scans in the patient or reducing the total volume of hypo-intense lesions on enhanced T1 scans in the patient.

In certain embodiments, alleviating a symptom comprises reducing the EDSS score of the patient. In certain embodiments, alleviating a symptom comprises reducing the EDSS score of the patient by at least 0.5 score.

In certain embodiments, alleviating a symptom comprises reducing a level of disability, as measured by the work productivity and activities impairment-General Health (WPAI-GH) questionnaire, or by EuroQoL (EQ-5D) questionnaire, in the patient. In certain embodiments, alleviating a symptom comprises reducing a change in Ambulation Index in the patient.

In certain embodiments of the methods described above, the method alleviates the symptom at least as effectively as daily subcutaneous administrations of 20 mg GA or thrice weekly subcutaneous injections of 40 mg GA.

In certain embodiments, the depot formulation is administered intramuscularly by the patient. In certain embodiments, the depot formulation is injected into the deltoid muscle.

In certain embodiments, the patient has not received GA therapy prior to initiation of the regimen. In certain embodiments, the patient has received GA therapy prior to initiation of the regimen. In certain embodiments, the patient has at least 1 cerebral lesion detectable by an MRI scan and wherein the lesion is associated with brain tissue inflammation, myelin sheath damage or axonal damage. In certain embodiments, the lesion is a demyelinating white matter lesion visible on brain MRI and wherein the white matter lesion is at least 3 mm in diameter. In certain embodiments, the patient has experienced a first clinical episode and wherein the first clinical episode includes a clinical episode of 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, muscle pains, facial pain, trigeminal neuralgia, stabbing sharp pains, burning tingling pain, slowing of speech, slurring of words, changes 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, loss of short term memory, loss of concentration, or loss of judgment or reasoning.

In certain embodiments, the frequency of an immediate post injection reaction, the severity of an immediate post injection reaction or the frequency of an injection site reaction is reduced relative to daily subcutaneous administration of 20 mg GA or a thrice weekly subcutaneous injection of 40 mg GA.

In certain embodiments, the depot formulation further comprises a pharmaceutically acceptable biodegradable carrier selected from the group consisting of poly(lactic-co-glycolic acid)

(PLGA), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and any combination thereof. In certain embodiments, the biodegradable carrier is PLGA.

In certain embodiments, the depot formulation is in the form of microparticles comprising an internal aqueous phase comprising the GA, a water immiscible polymeric phase comprising a biodegradable or non-biodegradable polymer, and an external aqueous phase.

In certain embodiments, the depot formulation is in the form of microparticles prepared by a water-in oil-in water (w/o/w) double emulsification process. In certain embodiments, the internal aqueous phase comprises the GA. In certain embodiments, the water immiscible polymeric phase comprises PLGA.

In certain embodiments, the external water phase comprises a surfactant selected from poly(vinyl alcohol) (PVA), polysorbate, polyethylene oxide-polypropylene oxide block copolymers and cellulose esters. In certain embodiments, the surfactant is PVA.

In certain embodiments, the depot formulation is administered in 2 mL of water for injection (WFI). In certain embodiments, the depot formulation comprises 10% to 40% solids. In certain embodiments, the depot formulation comprises 20% to 30% solids. In certain embodiments, the depot formulation comprises 25% solids.

In certain embodiments, the weight ratio between the GA and the pharmaceutically acceptable biodegradable carrier is between 1:1 to 1:100. In certain embodiments, the weight ratio between the GA and the pharmaceutically acceptable biodegradable carrier is between 1:5 to 1:25.

In certain embodiments, the depot formulation provides prolonged release or prolonged action of glatiramer in a subject as compared to a substantially similar dose of an immediate release formulation of glatiramer. In certain embodiments, about 80% of the glatiramer is released from the depot formulation within 22 days in PBS at 37ºC under continuous agitation. In certain embodiments, about 20% of the glatiramer is released from the depot formulation within 5 days in PBS at 37ºC under continuous agitation.

The present invention further provides, in another aspect, a method of increasing the tolerability of GA treatment in a human patient suffering from relapsing-remitting multiple sclerosis, comprising reducing the frequency of subcutaneous injections from daily subcutaneous injections of a 20 mg dose of GA or three subcutaneous injections of a 40 mg dose of GA over a period of seven days with at least one day between every injection, to a therapeutically effective

regimen of a single intramuscular injection of a depot formulation of a 40 mg dose of GA every 2 to 6 weeks, so as to thereby increase the tolerability of GA treatment in the patient.

In certain embodiments, the depot formulation is administered once every 3 to 5 weeks. In certain embodiments, the depot formulation is administered once every 4 weeks.

In certain embodiments, increasing the tolerability of GA treatment in the patient suffering from relapsing-remitting multiple sclerosis comprises reducing the frequency of injection. In certain embodiments, increasing the tolerability of GA treatment in the patient suffering from relapsing-remitting multiple sclerosis comprises reducing the frequency of an injection site reaction.

The present invention further provides, in another aspect, a method of reducing the frequency of relapses in a human patient suffering from relapsing-remitting multiple sclerosis or a human patient who has experienced a first clinical episode and is determined to be at high risk of developing clinically definite multiple sclerosis, comprising administering to the patient a therapeutically effective regimen of a single injection of a depot formulation of a 40 mg dose of GA every 2 to 6 weeks, the regimen being sufficient to reduce frequency of relapses in the patient.

The present invention further provides, in another aspect, a method of preventing or slowing progression of relapsing-remitting multiple sclerosis (RRMS) in a human patient suffering from RRMS, comprising administering to the human patient a therapeutically effective regimen of a single intramuscular injection of a depot formulation comprising 40 mg dose of glatiramer acetate (GA) every 2 to 6 weeks.

In certain embodiments, the regimen is sufficient to ameliorate (a) the frequency of relapses, (b) the number or volume of enhancing lesions, (c) the number of new lesions, or (d) the Expanded Disability Status Scale (EDSS) score of the patient. In certain embodiments, the regimen is sufficient to ameliorate the frequency of relapses, the number or volume of enhancing lesions, the number of new lesions, and the Expanded Disability Status Scale (EDSS) score of the patient.

The present invention further provides, in another aspect, a method of preventing disease activity in a human patient suffering from relapsing-remitting multiple sclerosis, comprising administering to the patient a therapeutically effective regimen of a single injection of a depot formulation of a 40 mg dose of GA every 2 to 6 weeks, the regimen being sufficient to prevent

relapses, 12-week confirmed disability progression (CDP), new lesion formation and enhancement of existing lesions in the patient.

The present invention further provides, in another aspect, a kit, comprising a first container comprising GA encapsulated with a poly(lactic-*co*-glycolic acid) (PLGA), and a second separate container comprising a pharmaceutically acceptable diluent for injection.

In certain embodiments, the kit comprises 40 mg GA and 2-10 ml WFI. In certain embodiments, mixing the content of the first container with 2 ml of diluent provides 40 mg GA per 2 ml of suspension. In certain embodiments, the kit is for use in a method of alleviating at least one symptom of relapsing-remitting multiple sclerosis (RRMS), increasing the tolerability of GA treatment, preventing or slowing progression of RRMS, or preventing RRMS activity.

Further embodiments and the full scope of applicability of the present invention will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the mean clinical score results for saline control, COPAXONE® (2 mg GA, days 0-8) and GA Depot (4 mg GA, day 0) in C57BL/6 mice groups. * $P<0.05$ for all treatment groups compared with untreated control, Single Factor ANOVA followed by one-tailed T Test assuming unequal variance. N = 20 / group, +/- standard error.

Figure 2 shows the mean body weight results for saline control, COPAXONE® (2 mg GA, days 0-8) and GA Depot (4 mg GA, day 0) in C57BL/6 mice groups. * $P<0.05$ for all treatment groups compared with untreated control, ** $P<0.05$ for COPAXONE® group compared with untreated control, Single Factor ANOVA followed by one-tailed T Test assuming unequal variance. N = 20 / group, +/- standard error.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides long acting depot formulations of glatiramer acetate, which afford equal or superior therapeutic efficacy compared to the daily or thrice weekly injections of COPAXONE®, and thus result in improved patient compliance. In addition to providing equal or

superior therapeutic efficacy, the long acting injections or implants of the present invention reduce the adverse side effects resulting from frequent injections.

The invention is based on the surprising finding that the current standard treatment for MS patients, i.e. the daily administration of 20 mg GA by injection, is unnecessarily aggressive in terms of the total drug used and the frequency of invasive administration. Specifically, it has been surprisingly found that 40 mg of GA, administered once every 28 days, where sufficient to achieve beneficial clinical efficacy, which is at least comparable with the beneficial clinical efficacy of GA administered every day over a period of 28 days for a total dose of 560 mg. The present invention thus provides a method of treating MS with a 14-fold decrease in the administered dose and a 28-fold decrease in the frequency of administration, compared to the original and still ongoing standard of care.

The present invention is further superior over the more recent COPAXONE® therapy regimen, which includes a thrice weekly administration of 40 mg GA. In this case, treating RRMS according to the present invention provides a 12-fold reduction in the total administered dose and in the frequency of GA administration.

The present invention provides, in one aspect, a method of alleviating at least one symptom of relapsing-remitting multiple sclerosis (RRMS) in a human patient suffering from RRMS or a human patient who has experienced a first clinical episode or a human patient who is determined to be at high risk of developing clinically definite multiple sclerosis, comprising administering to the patient a therapeutically effective regimen of a single injection of a long acting depot formulation comprising at least 20 mg dose of glatiramer or any pharmaceutically acceptable salt of glatiramer every 2 to 6 weeks, the regimen being sufficient to alleviate the at least one symptom of the patient, wherein the symptom is selected from the group consisting of the frequency of relapses, the number of enhancing lesions or the number of new lesions images of brain MRI, and the Expanded Disability Status Scale (EDSS) score of the patient.

The present invention provides, in one aspect, a method of alleviating at least one symptom of relapsing-remitting multiple sclerosis (RRMS) in a human patient suffering from RRMS or a human patient who has experienced a first clinical episode or a human patient who is determined to be at high risk of developing clinically definite multiple sclerosis, comprising administering to the patient a therapeutically effective regimen of a single injection of a long acting depot formulation comprising at least 20 mg dose of glatiramer or any pharmaceutically acceptable salt of glatiramer every 2 to 6 weeks, the regimen being sufficient to alleviate the at least one

symptom of the patient, wherein the symptom is selected from the group consisting of the frequency of relapses, the number of enhancing lesions or the number of new lesions images of brain MRI, and the Expanded Disability Status Scale (EDSS) score of the patient.

In certain embodiments, the depot formulation is administered once every 3 to 5 weeks. In certain embodiments, the depot formulation is administered once every 4 weeks.

The term "long acting" as used herein refers to a composition which provides prolonged, sustained or extended release of the glatiramer salt to the general systemic circulation of a subject or to local sites of action in a subject. This term may further refer to a composition which provides prolonged, sustained or extended duration of action (pharmacodynamics) of the glatiramer salt in a subject. Depending on the expected duration of action required, each depot or implantable device of the present invention will typically contain between about 40 and 520 mg of the active ingredient, designed to be released once over a period of 4 weeks to 13 times over a period of a year, every four weeks.

The term "treating" as used herein refers to suppression or alleviation of at least one symptom after the onset of multiple sclerosis. Common symptoms after the onset of multiple sclerosis include, but are not limited to, reduced or loss of vision, stumbling and uneven gait, slurred speech, as well as urinary frequency and incontinence. In addition, multiple sclerosis can cause mood changes and depression, muscle spasms and severe paralysis.

The phrase “alleviating at least one symptom of RRMS” as used herein refers to any beneficial change in the severity of a symptom or in the frequency of a symptom of RRMS. This phrase further includes preventing or delaying the progress of RRMS symptoms compared to untreated RRMS patients or compared to RRMS patients treated by other therapies, drugs or regimens.

The phrase “a human patient who is determined to be at high risk of developing clinically definite multiple sclerosis” as used herein refers to patient(s) which are defined to be in at least one risk group to develop RRMS. RRMS risk groups are long known, and are reviewed e.g. by McKay et al. (Biomed. Res. Int., 2015, Vol. 2015, Article ID 817238). In certain embodiments, a human patient who is determined to be at high risk of developing clinically definite multiple sclerosis has MRI features consistent with multiple sclerosis. In certain embodiments, a human patient who is determined to be at high risk of developing clinically definite multiple sclerosis has experienced a first clinical episode and has MRI features consistent with multiple sclerosis.

The term "multiple sclerosis" as used herein refers to an auto-immune disease of the central nervous system which is accompanied by one or more of the symptoms described hereinabove.

The term "glatiramer acetate" as used herein refers to a compound formerly known as Copolymer 1 that is sold under the trade name COPAXONE® and includes the acetate salts of synthetic polypeptides, containing four naturally occurring amino acids: L-glutamic acid, L-alanine, L-tyrosine, and L-lysine with an average molar fraction of 0.141, 0.427, 0.095, and 0.338, respectively. The average molecular weight of glatiramer acetate in COPAXONE® is 4,700-11,000 Daltons (FDA COPAXONE® label) and the number of amino acid ranges between about 15 to about 100 amino acids. The term also refers to chemical derivatives and analogues of the compound. Typically the compound is prepared and characterized as specified in any one of US Patent Nos. 5,981,589; 6,054,430; 6,342,476; 6,362,161; 6,620,847; and 6,939,539.

According to some embodiments, the glatiramer acetate comprises the acetate salt of L-alanine, L-glutamic acid, L-lysine, and L-tyrosine in the molar ratios of about 0.14 glutamic acid, about 0.43 alanine, about 0.10 tyrosine and about 0.33 lysine. According to other embodiments, the glatiramer acetate or other pharmaceutically acceptable salt of glatiramer comprises about 15 to about 100 amino acids. According to certain embodiments, the implantable depot is suitable for subcutaneous or intramuscular implantation. According to alternative embodiments, the long acting parenteral pharmaceutical composition comprises a pharmaceutically acceptable biodegradable or non-biodegradable carrier for glatiramer salts. According to some embodiments, the carrier is selected from PLGA, PLA, PGA, polycaprolactone, polyhydroxybutyrate, polyorthoesters, polyalkaneanhydrides, gelatin, collagen, oxidized cellulose, and polyphosphazene. According to some embodiments, the carrier is selected from PLGA, PLA, PGA and any combination thereof. Each possibility represents a separate embodiment of the invention.

In certain embodiments, the at least one symptom alleviated is the frequency of relapses and the number of enhancing lesions or the number of new lesions images of brain MRI.

In certain embodiments, the at least one symptom alleviated is the number of enhancing lesions or the number of new lesions images of brain MRI and the Expanded Disability Status Scale (EDSS) score of the patient.

In certain embodiments, the at least one symptom alleviated is the frequency of relapses and the Expanded Disability Status Scale (EDSS) score of the patient.

In certain embodiments, the at least one symptom alleviated the frequency of relapses, the number of enhancing lesions or the number of new lesions images of brain MRI, and the Expanded Disability Status Scale (EDSS) score of the patient.

The copolymers can be made by any procedure available to one of skill in the art. For example, the copolymers can be made under condensation conditions using the desired molar ratio of amino acids in solution, or by solid phase synthetic procedures. Condensation conditions include the proper temperature, pH, and solvent conditions for condensing the carboxyl group of one amino acid with the amino group of another amino acid to form a peptide bond. Condensing agents, for example, dicyclohexylcarbodiimide, can be used to facilitate the formation of the peptide bond.

In some embodiments, the composition may comprise any other pharmaceutically acceptable salt of glatiramer including, but not limited to, sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, hydrochloride, hydrobromide, hydroiodide, acetate, nitrate, propionate, decanoate, caprylate, acrylate, formate, isobutyrate, caprate, heptanoate, propiolate, oxalate, malonate, succinate, tocopheryl succinate, suberate, sebacate, fumarate, maleate, butyne-l,4-dioate, hexyne-1 ,6-dioate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, phthalate, terephthalate, sulfonate, xylenesulfonate, phenylacetate, phenylpropionate, phenylbutyrate, citrate, lactate, β-hydroxybutyrate, glycollate, tartrate, methanesulfonate, propanesulfonate, naphthalene-2-sulfonate, p-toluenesulfonate, mandelate and the like salts. Each possibility represents a separate embodiment of the invention.

In certain embodiments, the dosage forms include, but are not limited to, biodegradable injectable depot systems such as, PLGA based injectable depot systems; non-PLGA based injectable depot systems, and injectable biodegradable gels or dispersions. Each possibility represents a separate embodiment of the invention. The term "biodegradable" as used herein refers to a component which erodes or degrades at its surfaces over time due, at least in part, to contact with substances found in the surrounding tissue fluids, or by cellular action. In particular, the biodegradable component is a polymer such as, but not limited to, lactic acid-based polymers such as polylactides e.g. poly(D,L-lactide) i.e. PLA; glycolic acid-based polymers such as polyglycolides or poly(glycolic acid) (PGA) e.g. LACTEL®; poly(D,L-lactide-co-glycolide) i.e. PLGA, (RESOMER® RG-504, RESOMER® RG-502, RESOMER® RG-504H, RESOMER® RG-502H, RESOMER® RG-504S, RESOMER® RG-502S, LACTEL®); polycaprolactones such as Poly(e-caprolactone) i.e. PCL (LACTEL®); polyanhydrides; poly(sebacic acid) SA;

poly(ricenolic acid) RA; poly(fumaric acid), FA; poly(fatty acid dimmer), FAD; poly(terephthalic acid), TA; poly(isophthalic acid), IPA; poly(p-{carboxyphenoxy}methane), CPM; poly(p-{carboxyphenoxy} propane), CPP; poly(p-{carboxyphenoxy}hexane)s CPH; polyamines, polyurethanes, polyesteramides, polyoithoesters {CHDM: cis/trans-cyclohexyl dimethanol, HD:1,6-hexanediol. DETOU: (3,9-diethylidene-2,4,8,10- tetraoxaspiro undecane)}; polydioxanones; polyhydroxybutyrates; polyalkylene oxalates; polyamides; polyesteramides; polyurethanes; polyacetals; polyketals; polycarbonates; polyorthocarbonates; polysiloxanes; polyphosphazenes; succinates; hyaluronic acid; poly(malic acid); poly(amino acids); polyhydroxyvalerates; polyalkylene succinates; polyvinylpyrrolidone; polystyrene; synthetic cellulose esters; polyacrylic acids; polybutyric acid; triblock copolymers (PLGA-PEG-PLGA), triblock copolymers (PEG-PLGA-PEG), poly(N-isopropylacrylamide) (PNIPAAm), poly(ethylene oxide)- poly(propylene oxide)- poly(ethylene oxide) tri-block copolymers (PEO-PPO-PEO), poly valeric acid; polyethylene glycol; polyhydroxyalkylcellulose; chitin; chitosan; polyorthoesters and copolymers, terpolymers; lipids such as cholesterol, lecithin; poly(glutamic acid-co-ethyl glutamate) and the like, or mixtures thereof.

In some embodiments, the compositions of the present invention comprise a biodegradable polymer selected from, but not limited to, PLGA, PLA, PGA, polycaprolactone, polyhydroxybutyrate, polyorthoesters, polyalkaneanhydrides, gelatin, collagen, oxidized cellulose, polyphosphazene and the like. Each possibility represents a separate embodiment.

In certain embodiments, the biodegradable polymer is a lactic acid-based polymer, for example polylactide, or poly(D, L-lactide-co-glycolide) i.e. PLGA. The biodegradable polymer is present in an amount between about 10% to about 98% w/w of the composition. The lactic acid-based polymer has a monomer ratio of lactic acid to glycolic acid in the range of 100:0 to about 0:100, for example 100:0 to about 10:90 and has an average molecular weight of from about 1,000 to 200,000 Daltons. However, it is understood that the amount of biodegradable polymer is determined by parameters such as the duration of use and the like.

According to particular embodiments, the long acting pharmaceutical compositions of the present invention are in the form of microparticles prepared by a water-in oil-in water double emulsification process. In certain embodiments, the long acting pharmaceutical compositions of the present invention comprise an internal aqueous phase comprising a therapeutically effective amount of a pharmaceutically acceptable salt of glatiramer, a water immiscible polymeric phase comprising a carrier selected from a biodegradable and a non-biodegradable polymer, and an external aqueous phase. In other embodiments, the water immiscible polymeric phase comprises

a biodegradable polymer selected from PLGA, PLA and combinations thereof. Each possibility represents a separate embodiment of the invention. In additional embodiments, the external aqueous phase comprises a surfactant selected from poly(vinyl alcohol) (PVA), polysorbate, polyethylene oxide-polypropylene oxide block copolymers and cellulose esters. Each possibility represents a separate embodiment of the invention.

The compositions of the present invention may further comprise one or more pharmaceutically acceptable excipient(s) selected from, but not limited to, co-surfactants, solvents/co-solvents, water immiscible solvents, water, water miscible solvents, oily components, hydrophilic solvents, emulsifiers, preservatives, antioxidants, anti-foaming agents, stabilizers, buffering agents, pH adjusting agents, osmotic agents, channel forming agents, osmotic adjustment agents, or any other excipient known in the art. Suitable co-surfactants include, but are not limited to, polyethylene glycols, polyoxyethylene- polyoxypropylene block copolymers known as "poloxamer", polyglycerin fatty acid esters such as decaglyceryl monolaurate and decaglyceryl monomyristate, sorbitan fatty acid ester such as sorbitan monostearate, polyoxyethylene sorbitan fatty acid ester such as polyoxyethylene sorbitan monooleate (Tween), polyethylene glycol fatty acid ester such as polyoxyethylene monostearate, polyoxyethylene alkyl ether such as polyoxyethylene lauryl ether, polyoxyethylene castor oil and hardened castor oil such as polyoxyethylene hardened castor oil, and the like or mixtures thereof. Each possibility represents a separate embodiment of the invention. Suitable solvents/co-solvents include, but not limited to, alcohols, triacetin, dimethyl isosorbide, glycofurol, propylene carbonate, water, dimethyl acetamide, and the like or mixtures thereof. Each possibility represents a separate embodiment of the invention. Suitable anti-foaming agents include, but are not limited to, silicon emulsions or sorbitan sesquioleate. Suitable stabilizers to prevent or reduce the deterioration of the components in the compositions of the present invention include, but are not limited to, antioxidants such as glycine, a-tocopherol or ascorbate, BHA, BHT, and the like or mixtures thereof. Each possibility represents a separate embodiment of the invention. Suitable tonicity modifiers include, but are not limited to, mannitol, sodium chloride, and glucose. Each possibility represents a separate embodiment of the invention. Suitable buffering agents include, but are not limited to, acetates, phosphates, and citrates with suitable cations. Each possibility represents a separate embodiment of the invention.

The particle size of the "water-in oil-in water (w/o/w) double emulsion" can be determined by various parameters including, but not limited to, the amount of applied force at this step, the speed of mixing, surfactant type and concentration, etc. Suitable particle sizes range from about 1 to 100 μm.

The depot systems of the present invention encompass any forms known to a person of skill in the art. Suitable forms include, but are not limited to, biodegradable or non-biodegradable microspheres, implantable rods, implantable capsules, and implantable rings. Each possibility represents a separate embodiment of the invention. Further contemplated are prolonged release gel depot and erodible matrices. Each possibility represents a separate embodiment of the invention. Suitable implantable systems are described for example in US 2008/0063687. Implantable rods can be prepared as is known in the art using suitable micro-extruders.

In some embodiments, the depot formulations of the present invention include, but are not limited to, suspensions of glatiramer acetate in water, oil or wax phase; poorly soluble polyelectrolyte complexes of glatiramer acetate; "in-situ" gel-forming matrices based on the combination of water-miscible solvent with glatiramer acetate; and biodegradable polymeric microparticles with incorporated glatiramer acetate. Each possibility represents a separate embodiment of the invention. In particular, the compositions of the present invention are in the form of injectable microparticles wherein the glatiramer acetate is entrapped in a biodegradable or non-biodegradable carrier. The microparticulate compositions of the present invention may comprise a water-in oil-in water double emulsion. Within the scope of the present invention is a microparticulate composition comprising an internal aqueous phase comprising glatiramer or any pharmaceutically acceptable salt thereof, an oil phase or water-immiscible phase comprising a biodegradable or non-biodegradable polymer and an external aqueous phase. The external aqueous phase may further comprise a surfactant, for example poly(vinyl alcohol) (PVA), polysorbate, polyethylene oxide-polypropylene oxide block copolymers or cellulose esters. The terms "oil phase" and "water-immiscible phase" may be used interchangeably herein.

According to some embodiments, the glatiramer acetate comprises the acetate salt of L-alanine, L-glutamic acid, L-lysine, and L-tyrosine in the molar ratios of about 0.14 glutamic acid, about 0.43 alanine, about 0.10 tyrosine and about 0.33 lysine.

According to other embodiments, the glatiramer acetate or other pharmaceutically acceptable salt of glatiramer comprises about 15 to about 100 amino acids.

In certain embodiments, the depot formulation is administered once every 2 weeks. In certain embodiments, the depot formulation is administered once every 3 weeks. In certain embodiments, the depot formulation is administered once every 4 weeks. In certain embodiments, the depot formulation is administered once every 5 weeks. In certain embodiments, the depot formulation is administered once every 6 weeks.

According to some embodiments, the long acting pharmaceutical composition is suitable for a dosing schedule from once weekly to once in every 6 months. According to particular embodiments, the composition is suitable for dosing from once every 2 weeks to once monthly. According to some embodiments, the long acting compositions comprise a dose between 20 to 750 mg of glatiramer acetate per injection. According to some embodiments, the long acting compositions comprise a dose between 20 to 160 mg of glatiramer acetate per injection. According to some embodiments, the long acting compositions comprise a dose between 30 to 50 mg of glatiramer acetate per injection.

In certain embodiments, alleviating a symptom comprises reducing the frequency of relapses. In certain embodiments, alleviating a symptom comprises reducing the frequency of relapses in the patient by 30% or more compared to the frequency of relapses measured in a period of 12 months before starting the regimen. In certain embodiments, alleviating a symptom comprises increasing the time to a confirmed relapse in the patient compared to the time between relapses measured in a period of 12 months before starting the regimen. In certain embodiments, alleviating a symptom comprises reducing the total number of confirmed relapses in the patient measured in a period of 12 months during the regimen compared to a period of 12 months before starting the regimen.

In certain embodiments, alleviating a symptom comprises reducing the number or volume of enhancing lesions in the brain. In certain embodiments, alleviating a symptom comprises reducing the number of new lesions in the brain. In certain embodiments, alleviating a symptom comprises reducing the number of enhancing lesions images of brain MRI, measured in a period of 12 months. In certain embodiments, alleviating a symptom comprises reducing the number of new lesions images of brain MRI, measured in a period of 12 months. In certain embodiments, alleviating a symptom comprises reducing brain atrophy, reducing the number or volume of Gd-enhancing lesions, reducing the number or volume of T_1-weighted enhancing lesions, or reducing the number of new T_2-weighted lesions, in the patient. In certain embodiments, alleviating a symptom comprises reducing the mean cumulative number of Gd-enhancing lesions, reducing the mean number of new T2 lesions, reducing the total volume of T2 lesions, or reducing the cumulative number of enhancing lesions on T1-weighted images in the brain of the patient. In certain embodiments, alleviating a symptom comprises reducing the number of new hypointense lesions on enhanced T1 scans in the patient or reducing the total volume of hypointense lesions on enhanced T1 scans in the patient. Each possibility represents a separate embodiment of the invention. In another embodiment, alleviating a symptom comprises reducing the progression of MRI-monitored disease activity in the patient.

The Kurtzke Expanded Disability Status Scale (EDSS) is a method of quantifying disability in multiple sclerosis. The EDSS quantifies disability in eight Functional Systems (FS) and allows neurologists to assign a Functional System Score (FSS) in each of these. In certain embodiments, alleviating a symptom comprises reducing the EDSS score of the patient. In certain embodiments, alleviating a symptom comprises reducing the EDSS score of the patient, measured in a period of 12 months. In certain embodiments, alleviating a symptom comprises reducing the EDSS score of the patient by at least 0.5. In certain embodiments, alleviating a symptom comprises reducing the EDSS score of the patient by at least 1. In certain embodiments, alleviating a symptom comprises reducing the EDSS score of the patient from a score in the range of 5.0-9.5 to a score in the range of 0.0-4.5.

In certain embodiments, alleviating a symptom comprises reducing a level of disability, as measured by the work productivity and activities impairment-General Health (WPAI-GH) questionnaire, or as measured by EuroQoL (EQ5D) questionnaire, in the patient. In certain embodiments, alleviating a symptom comprises reducing a change in Ambulation Index in the patient.

In another embodiment, alleviating a symptom comprises reducing the level of disability as measured by EDSS Score in the patient. In another embodiment, alleviating a symptom comprises reducing the change in EDSS Score in the patient.

In certain embodiments of the methods described above, the method alleviates the symptom at least as effectively as daily subcutaneous administrations of 20 mg GA or thrice weekly subcutaneous injections of 40 mg GA. According to the principles of the present invention, the long acting pharmaceutical compositions of the present invention provide equal or superior therapeutic efficacy to the commercially available daily injectable dosage forms, with reduced incidence of side effects and with reduced severity of side effects at the local and/or systemic level. In some embodiments, the compositions of the present invention provide prolonged release or prolonged action of glatiramer in a subject as compared to a substantially similar dose of an immediate release formulation of glatiramer acetate.

In certain embodiments, the depot formulation is self-administered intramuscularly by the patient. In certain embodiments, the depot formulation is injected into the deltoid muscle.

In certain embodiments, the patient has not received GA therapy prior to initiation of the regimen. In certain embodiments, the patient has received GA therapy prior to initiation of the regimen.

Encompassed by the present invention is a combination therapy of glatiramer acetate or any other pharmaceutically acceptable salt of glatiramer with at least one other active agent. Active agents within the scope of the present invention include, but are not limited to interferons, e.g. pegylated or non-pegylated α-interferons, or β-interferons, e.g. interferon β-la or interferon β-lb, or τ-interferons; immuno-suppressants with optionally antiproliferative/antineoplastic activity, e.g. mitoxantrone, methotrexate, azathioprine, cyclophosphamide, or steroids, e.g. methylprednisolone, prednisone or dexamethasone, or steroid-secreting agents, e.g. ACTH; adenosine deaminase inhibitors, e.g. cladribine; IV immunoglobulin G (e.g. as disclosed in Neurology, 1998, May 50(5): 1273-81) monoclonal antibodies to various T-cell surface markers, e.g. natalizumab (ANTEGREN®) or alemtuzumab; TH2 promoting cytokines, e.g. IL-4, IL-10, or compounds which inhibit expression of TH1 promoting cytokines, e.g. phosphodiesterase inhibitors, e.g. pentoxifylline; antispasticity agents including baclofen, diazepam, piracetam, dantrolene, lamotrigine, rifluzole, tizanidine, clonidine, beta blockers, cyproheptadine, orphenadrine or cannabinoids; AMPA glutamate receptor antagonists, e.g. 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline, [1,2,3,4,-tetrahydro-7-morpholin-yl-2,3-dioxo-6-(trifluoromethyl)quinoxalin-i-yl]methylphosphonate, 1-(4-aminophenyl)-4-methyl-7,8-methylene-dioxy-5H-2,3-benzodiazepine, or (-)l-(4-aminophenyl)-4-methyl-7,8-methylene-dioxy-4,5-dihydro-3-methylcarbarnoyl-2,3-benzodiazepine; inhibitors of VCAM-1 expression or antagonists of its ligand, e.g. antagonists of the α4β1 integrin VLA-4 and/or α-4-β-7 integrins, e.g. natalizumab (ANTEGREN®); anti-macrophage migration inhibitory factor (Anti-MIF); xii) Cathepsin S inhibitors; xiii) mTor inhibitors. Each possibility represents a separate embodiment of the invention. An example of one other active agent is FTY720 (2-amino-2-[2-(4-octylphenyl)ethyl] propane-1,3-diol; fingolimod) belonging to the class of immuno-suppressants.

In another embodiment, prior to administration the patient has at least 1 cerebral lesion detectable by an MRI scan and suggestive of multiple sclerosis. In yet another embodiment, the lesion is associated with brain tissue inflammation, myelin sheath damage or axonal damage. In an additional embodiment, the lesion is a demyelinating white matter lesion visible on brain MRI. In a further embodiment, the white matter lesions are at least 3 mm in diameter. In certain embodiments, the patient has at least 1 cerebral lesion detectable by an MRI scan and wherein the lesion is associated with brain tissue inflammation, myelin sheath damage or axonal damage. In certain embodiments, the lesion is a demyelinating white matter lesion visible on brain MRI and the white matter lesion is at least 3 mm in diameter. In certain embodiments, the patient has experienced a first clinical episode and the first clinical episode includes a clinical episode of 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, muscle pains, facial pain, trigeminal neuralgia, stabbing sharp pains, burning tingling pain, slowing of speech, slurring of words, changes 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, loss of short term memory, loss of concentration, or loss of judgment or reasoning. Each possibility represents a separate embodiment of the invention.

In certain embodiments, the frequency of an immediate post injection reaction is reduced relative to daily subcutaneous administration of 20 mg GA or a thrice weekly subcutaneous injection of 40 mg GA. In certain embodiments, the severity of an immediate post injection reaction is reduced relative to daily subcutaneous administration of 20 mg GA or a thrice weekly subcutaneous injection of 40 mg GA. In certain embodiments, the frequency of an injection site reaction is reduced relative to daily subcutaneous administration of 20 mg GA or a thrice weekly subcutaneous injection of 40 mg GA.

In certain embodiments, the depot formulation is administered in 2 mL of water for injection (WFI), or a buffer containing a suspending agent (e.g. carboxymethylcellulose, CMC), a buffering agent (e.g. citrate salts) and a tonicity agent (e.g. NaCl). In certain embodiments, the depot formulation comprises 20% to 30% solids. As used herein, the term "water for injection" means sterile, purified water that meets regulatory standards for particulates, dissolved solids, organics, inorganics, microbial and endotoxin contaminants.

In certain embodiments, in PBS at 37ºC under continuous agitation, (i) up to 14% of the glatiramer is released from the depot formulation within 0 days, and/or (ii) up to 15% of the glatiramer is released from the depot formulation within 1 day, and/or (iii) up to 21% of the glatiramer is released from the depot formulation within 5 days, and/or (iv) up to 25% of the glatiramer is released from the depot formulation within 8 days, and/or (v) up to 34% of the glatiramer is released from the depot formulation within 13 days, and/or (vi) up to 43% of the glatiramer is released from the depot formulation within 15 days, and/or (vii) up to 80% of the glatiramer is released from the depot formulation within 22 days, and/or (viii) up to 96% of the glatiramer is released from the depot formulation within 27 days, and/or (ix) up to 99% of the glatiramer is released from the depot formulation within 32 days. Each possibility and each combination of possibilities represents a separate embodiment of the invention.

In certain embodiments, in PBS at 37ºC under continuous agitation, (i) up to 14% of the glatiramer is released from the depot formulation within 0 days, and (ii) up to 15% of the glatiramer is released from the depot formulation within 1 day, and (iii) up to 21% of the glatiramer is released from the depot formulation within 5 days, and (iv) up to 25% of the glatiramer is released from the depot formulation within 8 days, and (v) up to 34% of the glatiramer is released from the depot formulation within 13 days, and (vi) up to 43% of the glatiramer is released from the depot formulation within 15 days, and (vii) up to 80% of the glatiramer is released from the depot formulation within 22 days, and (viii) up to 96% of the glatiramer is released from the depot formulation within 27 days, and (ix) up to 99% of the glatiramer is released from the depot formulation within 32 days.

In certain embodiments, in PBS at 37ºC under continuous agitation, (i) about 14% of the glatiramer is released from the depot formulation within 0 days, and/or (ii) about 15% of the glatiramer is released from the depot formulation within 1 day, and/or (iii) about 21% of the glatiramer is released from the depot formulation within 5 days, and/or (iv) about 25% of the glatiramer is released from the depot formulation within 8 days, and/or (v) about 34% of the glatiramer is released from the depot formulation within 13 days, and/or (vi) about 43% of the glatiramer is released from the depot formulation within 15 days, and/or (vii) about 80% of the glatiramer is released from the depot formulation within 22 days, and/or (viii) about 96% of the glatiramer is released from the depot formulation within 27 days, and/or (ix) about 99% of the glatiramer is released from the depot formulation within 32 days. Each possibility and each combination of possibilities represents a separate embodiment of the invention.

In certain embodiments, in PBS at 37ºC under continuous agitation, (i) about 14% of the glatiramer is released from the depot formulation within 0 days, and (ii) about 15% of the glatiramer is released from the depot formulation within 1 day, and (iii) about 21% of the glatiramer is released from the depot formulation within 5 days, and (iv) about 25% of the glatiramer is released from the depot formulation within 8 days, and (v) about 34% of the glatiramer is released from the depot formulation within 13 days, and (vi) about 43% of the glatiramer is released from the depot formulation within 15 days, and (vii) about 80% of the glatiramer is released from the depot formulation within 22 days, and (viii) about 96% of the glatiramer is released from the depot formulation within 27 days, and (ix) about 99% of the glatiramer is released from the depot formulation within 32 days.

The present invention further provides, in another aspect, a method of increasing the tolerability of GA treatment in a human patient suffering from relapsing-remitting multiple sclerosis,

comprising reducing the frequency of subcutaneous injections from daily subcutaneous injections of a 20 mg dose of GA or three subcutaneous injections of a 40 mg dose of GA over a period of seven days with at least one day between every injection, to a therapeutically effective regimen of a single intramuscular injection of a depot formulation of a 40 mg dose of GA or any other pharmaceutically acceptable salt of glatiramer every 2 to 6 weeks, so as to thereby increase the tolerability of GA treatment in the patient.

In certain embodiments, the depot formulation is administered once every 3 to 5 weeks. In certain embodiments, the depot formulation is administered once every 4 weeks.

In certain embodiments, increasing the tolerability of GA treatment in the patient suffering from relapsing-remitting multiple sclerosis comprises reducing the frequency of injection. In certain embodiments, increasing the tolerability of GA treatment in the patient suffering from relapsing-remitting multiple sclerosis comprises reducing the frequency of an injection site reaction. In a further embodiment, the injection site reaction is erythema, hemorrhage, induration, inflammation, mass, pain, pruritus, urticaria, or welt that occurs immediately around the site of injection.

In another embodiment, increasing the tolerability of GA treatment in the human patient suffering from a relapsing form of multiple sclerosis comprises reducing the frequency of an immediate post injection reaction. In yet another embodiment, the immediate post injection reaction is palpitations, feeling hot, flushing, hot flushes, tachycardia, dyspnoea, chest discomfort, chest pain, non-cardiac chest , asthenia, back pain, bacterial infection, chills, cyst, face edema, fever, flu syndrome, infection, injection site erythema, injection site hemorrhage, injection site induration, injection site inflammation, injection site mass, injection site pain, injection site pruritus, injection site urticaria, injection site welt, neck pain, pain, migrane, syncope, tachycardia, vasodilatation, anorexia, diarrhea, gastroenteritis, gastrointestinal disorder, nausea, vomiting, ecchymosis, peripheral edema, arthralgia, agitation, anxiety, confusion, foot drop, hypertonia, nervousness, nystagmus, speech disorder, tremor, vertigo, bronchitis, dyspnea, laryngismus, rhinitis, erythema, herpes simplex, pruritus, rash, skin nodule, sweating, urticaria, ear pain, eye disorder, dysmenorrheal , urinary urgency, or vaginal moniliasis. In an embodiment the frequency of an immediate post injection reaction or the frequency of an injection site reaction is reduced relative to daily subcutaneous administration of 20 mg glatiramer acetate or thrice weekly subcutaneous administration of 40 mg glatiramer acetate.

The present invention further provides, in another aspect, a method of reducing the frequency of relapses in a human patient suffering from relapsing-remitting multiple sclerosis or a human patient who has experienced a first clinical episode and is determined to be at high risk of developing clinically definite multiple sclerosis, comprising administering to the patient a therapeutically effective regimen of a single injection of a depot formulation of a 40 mg dose of GA or any other pharmaceutically acceptable salt of glatiramer every 2 to 6 weeks, the regimen being sufficient to reduce frequency of relapses in the patient.

The present invention further provides, in another aspect, a method of preventing or slowing progression of relapsing-remitting multiple sclerosis (RRMS) in a human patient suffering from RRMS, comprising administering to the human patient a therapeutically effective regimen of a single intramuscular injection of a depot formulation comprising 40 mg dose of glatiramer acetate (GA) every 2 to 6 weeks.

In certain embodiments, the regimen is sufficient to ameliorate (a) the frequency of relapses, (b) the number or volume of enhancing lesions, (c) the number of new lesions, or (d) the Expanded Disability Status Scale (EDSS) score of the patient.

In certain embodiments, the regimen is sufficient to ameliorate the frequency of relapses, the number or volume of enhancing lesions, the number of new lesions, and the Expanded Disability Status Scale (EDSS) score of the patient.

The present invention further provides, in another aspect, a method of preventing disease activity in a human patient suffering from relapsing-remitting multiple sclerosis, comprising administering to the patient a therapeutically effective regimen of a single injection of a depot formulation of a 40 mg dose of GA every 2 to 6 weeks, the regimen being sufficient to prevent relapses, 12-week confirmed disability progression (CDP), new lesion formation and enhancement of existing lesions in the patient.

In certain embodiments, preventing RRMS activity comprises achieving No Evidence of Disease Activity (NEDA).

The present invention further provides, in another aspect, a kit, comprising a first container comprising GA or any other pharmaceutically acceptable salt of glatiramer encapsulated with a poly(lactic-*co*-glycolic acid) (PLGA), and a second separate container comprising a pharmaceutically acceptable diluent for injection.

In certain embodiments, the kit comprises 40 mg GA and 2 mL WFI. In certain embodiments, the kit further comprises a syringe and a needle. In certain embodiments, mixing the content of the first container and the second container provides 40 mg GA per 2 mL of diluent. In certain embodiments, the kit is for use in a method of alleviating at least one symptom of relapsing-remitting multiple sclerosis (RRMS), increasing the tolerability of GA treatment, preventing or slowing progression of RRMS, or preventing RRMS activity. In certain embodiments, the kit is for use in a method of alleviating at least one symptom of relapsing-remitting multiple sclerosis (RRMS), increasing the tolerability of GA treatment, preventing or slowing progression of RRMS, or preventing RRMS activity, the method comprising a single intramuscular injection of a depot formulation comprising 40 mg dose of glatiramer acetate (GA) every 2 to 6 weeks. In certain embodiments of the methods and kits provided above, GA Depots are administered in 21G or 20G needles.

In another embodiment, the therapeutically effective dose of glatiramer acetate is 40 mg. According to various embodiments of the present invention, the therapeutically effective amount of the glatiramer corresponds to ranges from about 1 mg to about 2 mg/day. Alternatively, such therapeutically effective amount of the glatiramer corresponds to about 1.5 mg/day. According to certain embodiments, the dosing regimen ranges from once a week, twice monthly (approximately once in every 2 weeks) to once monthly. Depending on the duration of action required, each depot or implantable device of the present invention will typically contain between about 20 and 750 mg of the active ingredient, designed to be released over a period ranging from a couple of weeks to a number of months.

In an embodiment, the pharmaceutical composition is in the form of a sterile solution. In another embodiment, the pharmaceutical composition further comprises mannitol. In yet another embodiment, the pharmaceutical composition has a pH in the range of 5.5 to 8.5. In an embodiment, the pharmaceutical composition has a pH in the range of 5.5 to 7.0.

The present invention provides, in one aspect, a depot formulation comprising 40 mg dose of glatiramer acetate (GA) for use in a method of alleviating at least one symptom of relapsing-remitting multiple sclerosis (RRMS) in a human patient suffering from RRMS or a human patient who has experienced a first clinical episode and is determined to be at high risk of developing clinically definite multiple sclerosis.

In certain embodiments, the depot formulation is administered to the patient in a therapeutically effective regimen of a single intramuscular injection of the depot formulation every 2 to 6 weeks,

the regimen being sufficient to alleviate the at least one symptom of the patient, wherein the symptom is selected from the group consisting of the frequency of relapses, the number of enhancing lesions or the number of new lesions images of brain MRI, and the Expanded Disability Status Scale (EDSS) score of the patient

According to any one of the above embodiments, the depot formulation is administered once every 3 to 5 weeks. According to any one of the above embodiments, the depot formulation is administered once every 4 weeks.

According to any one of the above embodiments, alleviating a symptom comprises reducing the frequency of relapses. According to any one of the above embodiments, alleviating a symptom comprises reducing the number or volume of enhancing lesions or the number of new lesions in the brain. According to any one of the above embodiments, alleviating a symptom comprises reducing brain atrophy, reducing the number or volume of Gd-enhancing lesions, reducing the number or volume of T1-weighted enhancing lesions, or reducing the number of new T2-weighted lesions, in the patient. According to any one of the above embodiments, alleviating a symptom comprises reducing the EDSS score of the patient.

According to any one of the above embodiments, the method alleviates the symptom at least as effectively as daily subcutaneous administrations of 20 mg GA or thrice weekly subcutaneous injections of 40 mg GA.

According to any one of the above embodiments, the patient has received GA therapy prior to initiation of the regimen.

According to any one of the above embodiments, the depot formulation further comprises a pharmaceutically acceptable biodegradable carrier selected from the group consisting of poly(lactic-co-glycolic acid) (PLGA), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and any combination thereof. According to any one of the above embodiments, the biodegradable carrier is PLGA.

According to any one of the above embodiments, depot formulation is in the form of microparticles comprising an internal aqueous phase comprising the GA, a water immiscible polymeric phase comprising a biodegradable or non-biodegradable polymer, and an external aqueous phase.

According to any one of the above embodiments, depot formulation is in the form of microparticles prepared by a water-in oil-in water (w/o/w) double emulsification process.

According to any one of the above embodiments, the internal aqueous phase comprises the GA.

According to any one of the above embodiments, the water immiscible polymeric phase comprises PLGA.

According to any one of the above embodiments, the external water phase comprises a surfactant selected from poly(vinyl alcohol) (PVA), polysorbate, polyethylene oxide-polypropylene oxide block copolymers and cellulose esters.

According to any one of the above embodiments, the depot formulation comprises 20% to 30% solids. According to any one of the above embodiments, the weight ratio between the GA and the pharmaceutically acceptable biodegradable carrier is between 1:1 to 1:100. According to any one of the above embodiments, the GA and the pharmaceutically acceptable biodegradable carrier is between 1:5 to 1:25. According to any one of the above embodiments, the GA and the pharmaceutically acceptable biodegradable carrier is about 11.

According to any one of the above embodiments, the depot formulation provides prolonged release or prolonged action of glatiramer in a subject as compared to a substantially similar dose of an immediate release formulation of glatiramer. According to any one of the above embodiments, about 80% of the glatiramer is released from the depot formulation within 22 days in PBS at 37ºC under continuous agitation. According to any one of the above embodiments, about 20% of the glatiramer is released from the depot formulation within 5 days in PBS at 37ºC under continuous agitation.

The present invention provides, in another aspect, a therapeutically effective regimen of a single intramuscular injection of a depot formulation of a 40 mg dose of GA every 2 to 6 weeks, for use in a method of increasing the tolerability of GA treatment in a human patient suffering from relapsing-remitting multiple sclerosis.

In certain embodiments, the method comprises reducing the frequency of subcutaneous injections from daily subcutaneous injections of a 20 mg dose of GA or three subcutaneous injections of a 40 mg dose of GA over a period of seven days with at least one day between every injection, to a therapeutically effective regimen of a single intramuscular injection of a depot formulation of a 40 mg dose of GA every 2 to 6 weeks

The present invention provides, in another aspect, a depot formulation comprising 40 mg dose of glatiramer acetate (GA), for use in a method of preventing or slowing progression of relapsing-remitting multiple sclerosis (RRMS) in a human patient suffering from RRMS.

In certain embodiments, the method comprises administering to the human patient a therapeutically effective regimen of a single intramuscular injection of the depot formulation every 2 to 6 weeks

According to any one of the above embodiments, the method is sufficient to ameliorate (a) the frequency of relapses, (b) the number or volume of enhancing lesions, (c) the number of new lesions, or (d) the Expanded Disability Status Scale (EDSS) score of the patient.

According to any one of the above embodiments, the method is sufficient to ameliorate the frequency of relapses, the number or volume of enhancing lesions, the number of new lesions, and the Expanded Disability Status Scale (EDSS) score of the patient.

The present invention provides, in another aspect, a depot formulation of a 40 mg dose of GA, for use in a method of preventing disease activity in a human patient suffering from relapsing-remitting multiple sclerosis.

In certain embodiments, the method comprises administering to the patient a therapeutically effective regimen of a single injection of the depot formulation every 2 to 6 weeks, the regimen being sufficient to prevent relapses, 12-week confirmed disability progression (CDP), new lesion formation and enhancement of existing lesions in the patient.

The present invention provides, in another aspect, a kit, comprising a first container comprising GA encapsulated with a poly(lactic-*co*-glycolic acid) (PLGA), and a second separate container comprising a pharmaceutically acceptable diluent for injection.

According to any one of the above embodiments, the kit comprises 40 mg GA and 2 mL water for injection (WFI).

According to any one of the above embodiments, the content of the first container and the second container provides 40 mg GA per 2 mL of diluent.

According to any one of the above embodiments, the kit is for use in a method of alleviating at least one symptom of relapsing-remitting multiple sclerosis (RRMS), increasing the tolerability of GA treatment, preventing or slowing progression of RRMS, or preventing RRMS activity.

The following examples are presented in order to more fully illustrate certain embodiments of the invention. They should in no way, however, be construed as limiting the broad scope of the invention. One skilled in the art can readily devise many variations and modifications of the principles disclosed herein without departing from the scope of the invention.

Examples

Example 1: Preparation method of Depot of 40 mg GA in-vitro.

Preparation Process: (1) External water phase preparation: Partially hydrolyzed poly(vinyl alcohol) (PVA) solution at a concentration of 2% w/w in sterile WFI was prepared in a reactor and filtered through a 0.22 μm membrane. (2) A solution of NaCl in sterile WFI was prepared and filtered through a 0.22 μm membrane into the reactor containing the PVA. (3) Organic phase preparation: Organic phase composed of dichloromethane and poly(lactide-co-glycolide) was prepared in a reactor and filtered through a 0.22 μm membrane. (4) Internal water phase preparation: A solution containing sterile WFI and glatiramer acetate was prepared and filtered through a 0.22 μm membrane. (5) Water-in-oil (w/o) emulsion preparation: Internal water phase was added to the organic phase and processed using IKA Ultra-Turrax T50 homogenizer equipped with a rotor stator dispersion device at 7,200 RPM for 10 minutes (high shear mixing). (6) Water-in-oil-in-water (w/o/w) emulsion preparation: Water in oil emulsion (w/o) prepared in step 5 was added to half of the external water phase during continuing mixing of the w/o emulsion. The w/o/w double emulsion was processed using IKA Ultra-Turrax UTS80 homogenizer with a rotor stator head at 2,900 RPM for 3 minutes from the end of w/o transfer into the external water phase. Following, another 30 liters of the external water phase was added to the emulsion (quench). (7) Solvent removal/evaporation: The w/o/w double emulsion formed in step (6) was mixed using the IKA UTS80 homogenizer at different speeds for 15-17 hours. Compressed air was bubbled at 0.5 Pa through the emulsion for 10-12 hours. Vacuum was applied for the portion of the process. (8) Separation and washing: The suspension was centrifuged at 5,300 RPM for 10 minutes. The supernatant was discarded and the pellet (sediment microparticles) is resuspended in 550 g WFI and mixed using a magnetic stirrer for 3 minutes. The resuspended microparticles were centrifuged at 2900 RPM for 10 minutes. (9) Lyophilization: The washed microparticles were resuspended in about 750 g sterile WFI and are kept at -20°C until lyophilization. Lyophilization was carried out using sterile lyoguard trays as follows: Freeze at -40°C, 24 hours. Primary drying at 0.2 hPa, -5°C, 48 hours. Secondary drying at 0.2 hPa, 10°C, 48 hours. The resulting composition includes GA and PLGA (50:50, molecular weight 7,000-17,000) in a 1:11.5 weight ratio.

Example 2: Release profile of GA from a PLGA Depot of 40 mg GA in-vitro.

The release of the incorporated glatiramer acetate was carried out in tightly closed 20 ml glass vials, using incubator at 37°C, equipped with a multi-point magnetic stirrer. Phosphate buffered

saline (PBS) with pH 7.4 was used as a release media. Table 1 summarizes the release profile of GA from a PLGA Depot.

Table 1.

Days	0	1	5	8	13	15	22	27	32
% GA released into medium	14	15	21	25	34	43	80	96	99

Example 3: Depot of 4 mg GA vs. commercially available GA (COPAXONE®) in-vivo.

Material and Methods

Animals: All animal studies were approved by the Israeli MOH Animal Care and Use Committee. C57BL/6 female mice, 7-9 weeks old were randomized into control or treatment groups with similar mean weight. Animals were given food and water ad-libitum throughout the experiment.

Induction of EAE: In order to induce EAE, an acceptable animal model of multiple sclerosis, an emulsion of MOG 35-55 (GL Biochem co. Ltd, Shanghai, China) in Modified Complete Freund's adjuvant (CFA) (Sigma-Aldrich, St. Louis, MO, USA) was prepared as follows: heat-killed M. tuberculosis Strain H37RA (Sigma) was added to CFA to a final concentration of 4mg/mL. Subsequently, 2mg/mL MOG 35-55 were emulsified with equal amount of modified CFA. EAE had been induced by injection of this emulsion subcutaneously (SC) on the shaved back of the mouse at one site, followed by an intraperitoneal injection of Bordetella pertussis toxin (Sigma) in PBS on Day 0 and 48 hours post MOG immunization. A 21G needle was used for injections in mice.

Measurements: Body weight was measured every two days from day 0 to day 28. EAE was assessed by clinical scoring of the mice once daily from Day 0 to Day 28 post immunization (table 2). For analysis, dead animals received clinical score of 5 and the weight recorded at the last measurement before animal death.

Table 2: EAE clinical Score.

Score	Clinical signs
0	Normal mouse; no overt signs of disease
1	Limp tail
2	Hind limb paralysis
3	Hind and front limb paralysis
4	Complete paralysis: sacrifice for humane reasons
5	Moribund state; Death by EAE

The following calculations were derived from clinical score raw data: mean maximum score is the mean of the highest scores noted for each mouse in a specific group up to indicated day of analysis; mean disease duration and mean day of onset were calculated as follows: Mean Disease Duration = Sum of (day of analysis – day of disease onset for each mouse) / (number of mice per group); Mean Day of Onset = (sum of day of disease onset of each mouse) / (number of mice per group). Area under the curve (AUC) of clinical score was calculated using Microsoft Excel and represents disease burden.

Glatiramer Acetate Depot (GA Depot): GA Depot was suspended in water for injection (WFI) and immediately injected intramuscularly (IM) at the indicated dose. Dose of GA Depot are given according to amount of active ingredient (i.e. GA Depot 4 mg contains 4 mg GA).

GA Binding Antibodies Analysis: At day 35 following disease induction, 5 animals from each treatment group were sacrificed. Blood samples were retrieved and serum was isolated and stored at -80° C (see Tables 3 and 5).

ELISA plates were prepared as following: flat bottom ELISA plates (Nunc) were coated with 100ul of 50 µg/ml GA in borate buffer (BB) 0.17M pH 8.0 overnight at 4° C. Wells were emptied and washed with phosphate buffer saline (PBS) containing 0.05% Tween 20 at room temperature. Following the washing 1% BSA was applied for 2 hours at room temperature (RT), for blocking of non-specific binding sites. Subsequently, wells were washed three times with wash solution.

ELISA test was performed as follows: 100 µl of sera samples were diluted 1:1,000 added to wells for 18 hours at 4°C (sera dilution was performed using PBS containing 1% BSA and 0.05% Tween 20), followed by three washes with phosphate buffer saline (PBS) containing 0.05% Tween 20 at room temperature. Subsequently, 100 µl 1:50,000 diluted alkaline phosphatase conjugated AffinityPure Goat anti-mouse IgG+IgM (H+L) (Jackson Laboratories) was added to

the wells and incubated for 2 hours at RT. The wells were washed again three times using wash solution and the color reaction was developed by adding 100µl of the substrate p-nitrophenyl phosphate (Jackson Laboratories) and incubation for 40-60 min at RT. The reaction was terminated with 30µl 3N NaOH. The absorbance at 405nm was then recorded using micro-ELISA reader (Dynatech). Each assay plate contained positive anti-GA serum samples and control of normal mouse serum (N = 5).

The results were expressed as Binding Index (BI) according to the following formula: Binding Index = mean optical density of tested serum/ mean optical density of control serum. The mean value for normal mice serum was 0.230 OD and the cut off values for Binding Index is 2.0 ± 1.0. Thus, values above 3.0 were considered as positive.

Experimental Design: Studies experimental design is specified in table 3.

Table 3: experimental design.

Group	Test Article, N = 20 / Group	Route	Dose	Days of Administration	Solvent
1	GA Depot	IM	4 mg	0, 1*	WFI, 0.2 mL
2	COPAXONE®	SC	2 mg	0-8, 9 in total	N/A
3	Untreated Control (saline)	SC	N/A	0-8, 9 in total	Saline, 0.1 ml

*Dose was administered using two injections on consecutive days since there is a maximal volume of injection that can be tolerated in a single injection in mice.

Statistical analysis: Data was analyzed using Microsoft Excel. Each data set was analyzed using single-factor analysis of variance (ANOVA) followed by one tailed student's T-test.

Results

GA Depot Dose Conversion.

Figure 1 shows mean clinical score results for saline control (black sphere marker), 2 mg COPAXONE® (black square marker) and GA Depot 4 mg (gray triangle marker) groups, as those groups represent the range of the proposed human dose (using an allometric 1:10 scale, as deducted from previous studies) of 0, 20 and 40 mg, respectively. *P<0.05 for all treatment groups compared with untreated control, Single Factor ANOVA followed by one-tailed T Test assuming unequal variance. N = 20 / group, +/- standard error.

Mean clinical score AUC (area under curve), mean day of onset and mean disease duration were significantly reduced in the GA Depot group and in the COPAXONE® group compared with untreated control (Table 4, $p<0.05$). No statistically significant difference was found between the GA Depot and COPAXONE® at any of the computed values (Table 4). At days 11-19, mean clinical score of the saline group was significantly higher than that of all other groups (Figure 1, $p<0.05$). At day 20, mean clinical score of the saline group was significantly higher than that of COPAXONE® and the GA Depot group (Figure 1, $p<0.05$). Body weight of the GA Depot and COPAXONE® treated groups was significantly higher than that of the untreated control at days 10 to 17 following immunization. At day 21, body weight of the COPAXONE® group was significantly higher than that of the untreated control group (Figure 2, $P<0.05$). *$P<0.05$ for all treatment groups compared with untreated control. ** $P<0.05$ for COPAXONE® group compared with untreated control. Single Factor ANOVA followed by one-tailed T Test assuming unequal variance. N = 20 / group, +/- standard error.

Table 4: Calculated Values.

Groups	Maximum Mean Disease Score	Mean Disease Duration*	Mean Day of Onset*	AUC Clinical Score*	Survival Rate at Day 28
GA Depot 4 mg IM Day 0, 1	2.55±0.25	15.05±0.51	12.95±0.51	29.85±2.59	85%
COPAXONE® 2 mg SC, Days 0-8	2.25±0.22	15.65±0.23	12.35±0.23	26.83±2.49	90%
Control (saline, SC) D0-D8	3.15±0.24	17.80±0.09	10.20±0.09	42.89±3.22	80%

*$P<0.05$ for all treatment groups compared with untreated control, Single Factor ANOVA followed by one-tailed T Test assuming unequal variance. N = 20 / group, +/- standard error.

Immunological response in mice measured by antibodies to GA.

Serum was isolated from mice in MOG-EAE study at day 35 following disease induction. Mice were treated with either the GA Depot (at 4 mg once) or COPAXONE® (2 mg/day, days 0-8). Antibodies (Abs) titer was evaluated using an ELISA assay. Results are expressed as Binding Index (BI). N = 5. Data as presented in Table 5 demonstrate that mice exposed to COPAXONE® or to the GA Depot developed similar titer of total anti-GA antibodies while control mice that were treated with saline had no such antibodies. The antibodies titer was similar in all treatment groups, suggesting a similar immunological response.

Table 5: Binding Index.

	Saline control	GA Depot 4 mg	COPAXONE® 2 mg
Binding Index	1.71	9.48	11.14

The data presented, comparing the effect of a single administration of the 4 mg GA Depot across to the standard daily administration of 2 mg GA, show similar efficacy between the two dosage regimes. Specifically, the GA Depot showed a clear significant effect of delayed disease onset and amelioration of symptoms, at least as effective as noted for the COPAXONE® treated group (see Figure 1).

In addition, this experiment shows that intramuscular administration of the GA Depot induced a humoral response of anti-GA antibodies, at similar levels as with standard daily subcutaneous injection of GA (see Table 5). Therefore, the similar humoral responses to GA Depot as compared to standard GA injections might represent a similarity in the immunologic response to the GA Depot. This may therefore indicate also equivalent clinical immuno-modulatory therapeutic effects, as can be seen in this EAE study of which the AUC between COPAXONE® and GA Depot are not different in statistical significant manner (see Table 4). Therefore, the existence of anti-GA antibodies can serve as a biomarker to the therapeutic bio-availability of the drug, when with a new formulation and administered via a new route.

Overall, data supports that the efficacy of GA Depots at dose of 4 mg GA in MOG-induced EAE is at least comparable to that of COPAXONE® and that the immune response to both treatments is similar. Based on the results obtained with the 4 mg GA depot in the EAE animal model, it was predicted that a dose of 80 mg would be superior to 40 mg in humans.

Example 4: Depot of 40 mg and 80 mg GA in humans diagnosed with RRMS.

Brief Summary: A prospective 1-year, open-label, two arms, multicenter, phase IIa study, in which a GA Depot of 40 mg or 80 mg GA was administered as an intramuscular (IM) injection to subjects with RRMS at 4 week intervals for 52 weeks of treatment. The purpose of the study was to assess safety, tolerability, and efficacy of a monthly long-acting IM injection of 40 mg or 80 mg GA Depot in subjects with RRMS. The study included subjects switching from daily or thrice weekly administration of 20 mg or 40 mg respectively of COPAXONE® injections.

Detailed Description: Subjects with a diagnosis of relapsing remitting multiple sclerosis (RRMS) who were treated with daily or thrice weekly subcutaneous injections of 20 mg or 40 mg respectively of GA (COPAXONE®) during the previous 12 months. Study product was GA long-

acting injection (GA Depot) which is a combination of extended-release microspheres for injection and diluent (water for injection) for parenteral use. GA Depot was administered intramuscularly (IM). The study duration for an individual subject was 60 weeks, consisting of 4 weeks of screening evaluation (weeks -4 to 0), followed by a 52-week open-label treatment period, and a 4 weeks follow up period: through a total of 17 visits. Physical, vital signs and safety assessment at each visit. MRI at visit 1 (screenings), at week 24, and week 52 (end of treatment visit). Neurological and safety laboratory tests at screening visit and on visits in weeks 4, 12, 24, 36, and 52 (end of treatment). GA Depots were administered in 21G or 20G needles.

Population and Sample Size: 25 patients with RRMS, treated with COPAXONE® for at least 12 months prior to study enrollment. Mean age at time of signing of the informed consent form: 52.7 in both groups. Mean duration of MS: 16.5 years and 14.6 years in the 40 mg and 80 mg groups, respectively. Efficacy population: 12 patients on 80 mg, 13 patients on 40 mg.

Trial extension beyond core study: 13 patients that finalized the core study (13 injections) asked to continue treatment with GA Depot 40 mg (the longest treated patient received his 34th injection, thus being in the 2nd half of the 3rd year of his ongoing treatment).

Inclusion Criteria: Male or female subjects with a diagnosis of RRMS; diagnosis of multiple sclerosis (MS) consistent with the McDonald Criteria (revisions of 2010); treatment with 20 mg or 40 mg of GA (COPAXONE) during the previous 12 months with ongoing treatment at the Screening Visit; normal renal function; normal liver function; normal hemoglobin concentration; absence of any clinically significant medical, psychiatric or laboratory abnormalities; ability to provide written informed consent.

Exclusion Criteria: Any relevant medical, surgical, or psychiatric condition, laboratory value, or concomitant medication which, in the opinion of the investigator, makes the subject unsuitable for study entry or potentially unable to complete all aspects of the study; Severe anemia (hemoglobin < 10 g/dL); abnormal renal function (serum creatinine > 1.5xULN); pregnant or breastfeeding women; women capable of child bearing must have a negative urine pregnancy test at screening visit and use an adequate contraceptive method throughout the study. Women who are surgically sterile (hysterectomy or tubal ligation) or whose last menstruation was 12 months or more prior to the Screening Visit are considered to be of non-child-bearing potential. Acceptable forms of contraception include oral, implanted, or injected contraceptives; intrauterine devices in place for at least 3 months; estrogen patch; and adequate barrier methods in conjunction with spermicide. Abstinence is considered an acceptable contraceptive regimen;

history of any anaphylactic reaction and/or serious allergic reaction following a vaccination, a proven hypersensitivity to any component of the study drug, e.g. GA, poly(lactic-*co*-glycolic acid) (PLGA), poly(vinyl alcohol) (PVA); known or suspected history of drug or alcohol abuse; positive test for HIV, hepatitis, venereal disease research laboratory test (VDRL), or tuberculosis; participation in an investigational drug study within 30 days prior to start of this study; treatment with any kind of steroids during the last 30 days; confirmed relapse during the last 30 days.

Outcome Measures: Relapse rate detected during the study compared to relapse rate observed in the 12 months prior to study start; changes from baseline to end of treatment visit in the number of enhancing lesions and new lesions images of brain MRI; change from baseline to end of treatment visit of Expanded Disability Status Scale (EDSS) score.

Adverse events (AEs) included mainly mild injection site reactions (ISRs) and no unexpected AEs were reported. Statistically significantly fewer ISRs were reported with the 40 mg dose than with the 80 mg dose. No immediate post-injection reactions, as recorded with GA (Copaxone®), were detected.

Results

Endpoint #1: Relapse rate compared to relapse rate observed in the 12 months prior to study entry. During the study: 1 relapse (which did not include changes in EDSS) detected for the entire study population. Same subjects during one year prior to the trial, while treated with COPAXONE®: 2 relapses.

Endpoint #2: Brain MRI scans compared to baseline, change defined as either new lesions (by T2-FLAIR), change in enhancing lesions (by SPGR T1 GdE), or both.

Table 6: MRI Scans.

	Total	80 mg Group	40 mg Group
Cumulative change at months 6 and 12 or Last Observation Carried Forward (LOCF)	20/23 (87%) of patients w/no change vs. baseline	9/11 (81.8%) of patients w/no change vs. baseline*/♣♦	11/12 (91.7%) of patients w/no change vs. baseline**/***
12 months	12/13 (92.3%) of patients w/no change vs. baseline	4/5 (80%) of patients w/no change vs. baseline♣	8/8 (100%) of patients w/no change vs. baseline**
Early termination	7/9 (77.8%) of patients w/no change vs. baseline	5/6 (83.3%) of patients w/no change vs. baseline*♦	2/3 (66.7%) of patients w/no change vs. baseline***

* One 80 mg patient showed 7 new enhancing lesions at 6 months MRI scan; ** One 40 mg patient showed a reduction of enhancing lesions from 3 at baseline to 0 at 6 months; considered as "no change"; *** One 40 mg patient showed 1 new enhancing lesion at early termination MRI scan; ♣ One 80 mg patient showed 2 new enhancing lesions at 12 months MRI scan; ♦ One 80 mg patient diagnosed with breast cancer did not performed MRI scans at early termination.

Endpoint #3: EDSS Score.

Table 7: EDSS scores.

	40 mg Group			80 mg Group			All		
	N	**Mean***	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**
Screening	12	3.3	1.6	12	1.6	1.2	24	2.4	1.6
12 Months	8	3.0	1.5	5	1.5	1.7	13	2.4	1.7
Early Termination	4	3.4	1.4	6**	1.3	0.6	10	2.2	1.4

* Three out of 12 patients in the 40 mg group had a baseline EDSS score of 5-6; ** One patient had no EDSS data at Early Termination.

It is noteworthy that the data demonstrate that a very high proportion of the patients treated with the depot formulation of the invention for one year achieve NEDA, defined as No Evidence of Disease Activity; defined as the absence of all the followings: relapses, 12-week confirmed disability progression (CDP), new/enlarging T2 lesions and T1 gadolinium-enhancing lesions.

This proportion of patients achieving NEDA is high, especially in comparison to the NEDA values reported for other drugs approved for treatment of MS.

Table 8: NEDA.

	40 mg Group	**80 mg Group**	**All**
Per Protocol (PP) Population	8 (100%)	3 (60%)	11 (84.6%)
mITT* (LOCF**)	11 (91.7%)	8 (72.7%)	19 (82.6%)

* modified intention-to-treat (mITT) population who have data for the 3 NEDA parameters at Week 52 or at Early Termination or at Week 24. One patient was excluded due to no MRI assessment other than baseline. ** Last observation carried forward (LOCF).Conclusions:

Treatment with a single intramuscular injection of 40 mg GA depot was superior to a corresponding treatment with 80 mg GA in preventing formation of new lesions and enhancement of lesions (Table 6).

Treatment with a single intramuscular injection of 40 mg GA depot was comparable to a corresponding treatment with 80 mg GA in preventing an increased EDSS score (Table 7).

The treatment with depot compositions of GA provided superior results of NEDA at one year compared to TYSABRI® (47%), PLEGRIDY® (34%) and ZINBRYTA® (39%) (as reported by Rotstein et al., JAMA Neurol., 2015, Vol. 72(2):152-158).

While the present invention has been particularly described, persons skilled in the art will appreciate that many variations and modifications can be made. Therefore, the invention is not to be construed as restricted to the particularly described embodiments, and the scope and concept of the invention will be more readily understood by reference to the claims, which follow.

CLAIMS

1. A method of alleviating at least one symptom of relapsing-remitting multiple sclerosis (RRMS) in a human patient suffering from RRMS or a human patient who has experienced a first clinical episode and is determined to be at high risk of developing clinically definite multiple sclerosis, comprising administering to the patient a therapeutically effective regimen of a single intramuscular injection of a depot formulation comprising 40 mg dose of glatiramer acetate (GA) every 2 to 6 weeks, the regimen being sufficient to alleviate the at least one symptom of the patient, wherein the symptom is selected from the group consisting of the frequency of relapses, the number of enhancing lesions or the number of new lesions images of brain MRI, and the Expanded Disability Status Scale (EDSS) score of the patient.

2. The method of claim 1, wherein the depot formulation is administered once every 4 weeks.

3. The method of claim 1 or claim 2, wherein alleviating a symptom comprises reducing the frequency of relapses, or wherein alleviating a symptom comprises reducing the number or volume of enhancing lesions or the number of new lesions in the brain, preferably wherein alleviating a symptom comprises reducing brain atrophy, reducing the number or volume of Gd-enhancing lesions, reducing the number or volume of T_1-weighted enhancing lesions, or reducing the number of new T_2-weighted lesions, in the patient, or wherein alleviating a symptom comprises reducing the EDSS score of the patient, preferably wherein the method alleviates the symptom at least as effectively as daily subcutaneous administrations of 20 mg GA or thrice weekly subcutaneous injections of 40 mg GA.

4. The method of claim 1, wherein the patient has received GA therapy prior to initiation of the regimen.

5. The method of any one of the preceding claims, wherein the depot formulation further comprises a pharmaceutically acceptable biodegradable carrier selected from the group consisting of poly(lactic-*co*-glycolic acid) (PLGA), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and any combination thereof.

6. The method of claim 5, wherein the biodegradable carrier is PLGA.

7. The method of any one of the preceding claims, wherein the depot formulation is in the form of microparticles comprising an internal aqueous phase comprising the GA, a water immiscible polymeric phase comprising a biodegradable or non-biodegradable polymer, and an external aqueous phase.

8. The method of any one of the preceding claims, wherein the depot formulation is in the form of microparticles prepared by a water-in oil-in water (w/o/w) double emulsification process.

9. The method of claim 8, wherein the internal aqueous phase comprises the GA.

10. The method of any one of claims 7 to 9, wherein the water immiscible polymeric phase comprises PLGA.

11. The method of any one of claims 7 to 10, wherein the external aqueous phase comprises a surfactant selected from poly(vinyl alcohol) (PVA), polysorbate, polyethylene oxide-polypropylene oxide block copolymers and cellulose esters.

12. The method of any one of the preceding claims, wherein the depot formulation comprises 20% to 30% solids.

13. The method of any one of claims 5 to 12, wherein the weight ratio between the GA and the pharmaceutically acceptable biodegradable carrier is between 1:1 to 1:100, preferably wherein the weight ratio between the GA and the pharmaceutically acceptable biodegradable carrier is between 1:5 to 1:25.

14. The method of any one of the preceding claims, wherein the depot formulation provides prolonged release or prolonged action of glatiramer in a subject as compared to a substantially similar dose of an immediate release formulation of glatiramer, and wherein:

about 80% of the glatiramer is released from the depot formulation within 22 days in PBS at 37ºC under continuous agitation, or

about 20% of the glatiramer is released from the depot formulation within 5 days in PBS at 37ºC under continuous agitation.

15. A method of increasing the tolerability of GA treatment in a human patient suffering from relapsing-remitting multiple sclerosis, comprising reducing the frequency of subcutaneous injections from daily subcutaneous injections of a 20 mg dose of GA or three subcutaneous injections of a 40 mg dose of GA over a period of seven days with at least one day between every injection, to a therapeutically effective regimen of a single intramuscular injection of a depot formulation of a 40 mg dose of GA every 2 to 6 weeks, so as to thereby increase the tolerability of GA treatment in the patient.

16. A method of preventing or slowing progression of relapsing-remitting multiple sclerosis (RRMS) in a human patient suffering from RRMS, comprising administering to the human patient a therapeutically effective regimen of a single intramuscular injection of a depot formulation comprising 40 mg dose of glatiramer acetate (GA) every 2 to 6 weeks.

17. The method of claim 16, wherein the regimen is sufficient to ameliorate (a) the frequency of relapses, (b) the number or volume of enhancing lesions, (c) the number of new lesions, or (d) the Expanded Disability Status Scale (EDSS) score of the patient, preferably wherein the regimen is sufficient to ameliorate the frequency of relapses, the number or volume of enhancing lesions, the number of new lesions, and the Expanded Disability Status Scale (EDSS) score of the patient.

18. A method of preventing disease activity in a human patient suffering from relapsing-remitting multiple sclerosis, comprising administering to the patient a therapeutically effective regimen of a single injection of a depot formulation of a 40 mg dose of GA every 2 to 6 weeks, the regimen being sufficient to prevent relapses, 12-week confirmed disability progression (CDP), new lesion formation and enhancement of existing lesions in the patient.

19. Use of a kit, comprising a first container comprising GA encapsulated with a poly(lactic-*co*-glycolic acid) (PLGA), and a second separate container comprising a pharmaceutically acceptable diluent for injection, in the manufacture of a medicament for alleviating at least one symptom of relapsing-remitting multiple sclerosis (RRMS), increasing the tolerability of GA treatment, preventing or slowing progression of

RRMS, or preventing RRMS activity, wherein mixing the content of the first container and the second container provides 40 mg GA per 2 mL of diluent.

20. The use of claim 19, wherein the kit comprises 40 mg GA and water for injection (WFI).

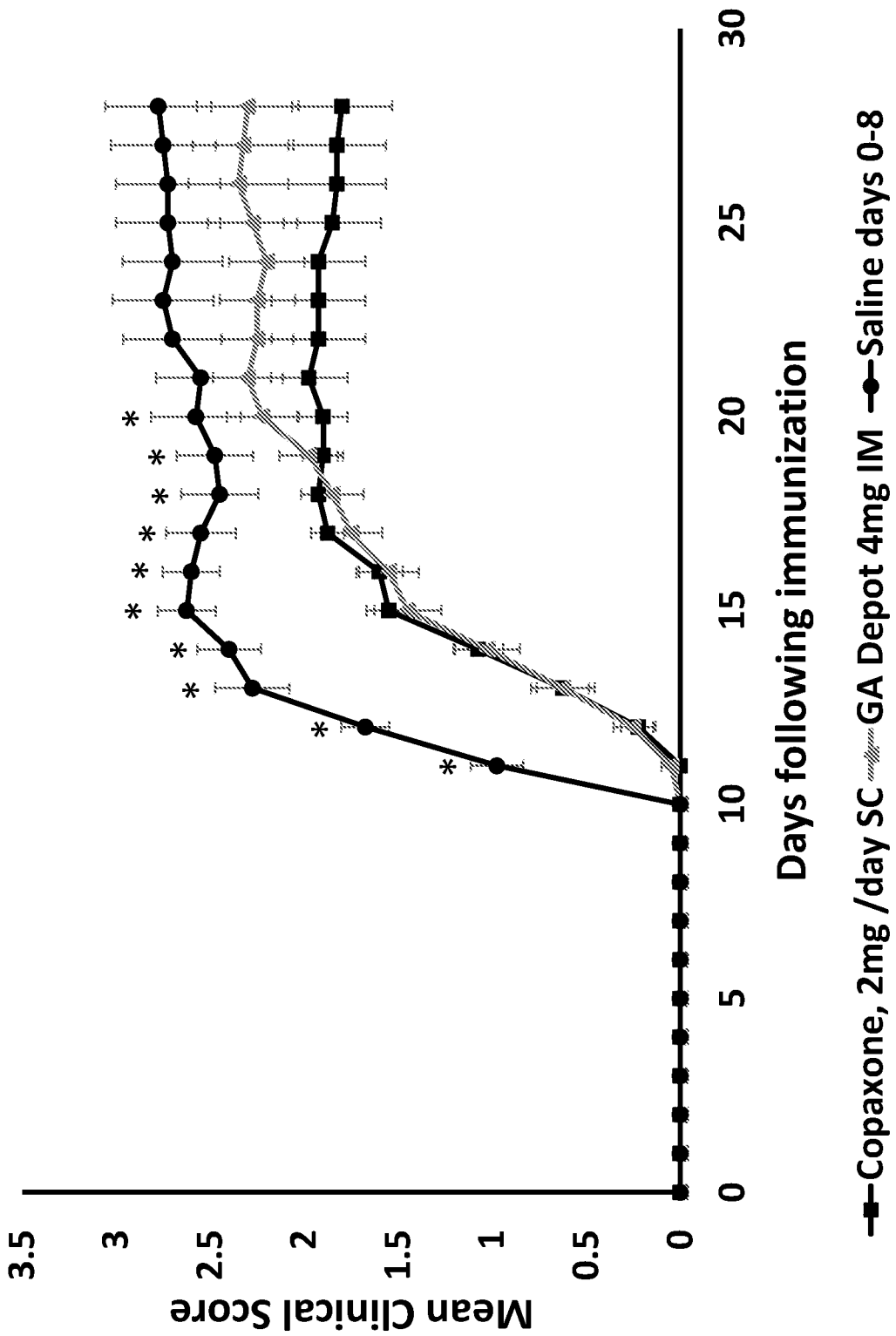
Mean Clinical Score
3.5
3
2.5
2
1.5
1
0.5
0
0
5
10
15
20
25
30
Days following immunization
Copaxone, 2mg /day SC
GA Depot 4mg IM
Saline days 0-8

FIGURE 1

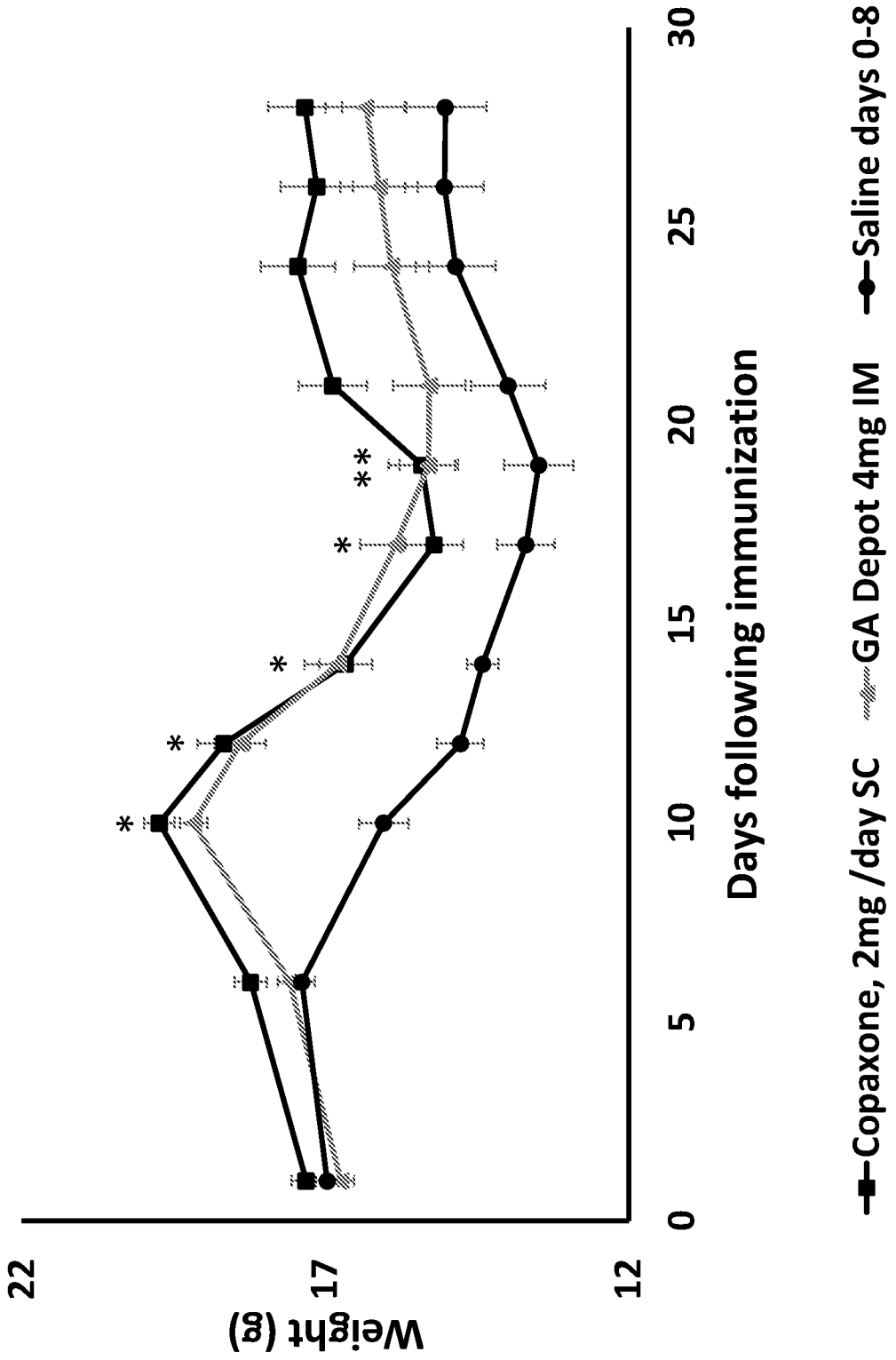
Weight (g)
22
17
12
0
5
10
15
20
25
30
Days following immunization
Copaxone, 2mg /day SC
GA Depot 4mg IM
Saline days 0-8
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FIGURE 2