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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 June 2010 (10.06.2010)

PCT

(10) International Publication Number
WO 2010/065819 A1

(51) International Patent Classification:
C07K 16/28 (2006.01) *A61K 39/395* (2006.01)

(21) International Application Number:
PCT/US2009/066715

(22) International Filing Date:
4 December 2009 (04.12.2009)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/120,121 5 December 2008 (05.12.2008) US

(71) Applicant *(for all designated States except US)*: **ALS THERAPY DEVELOPMENT INSTITUTE** [US/US]; 215 First Street, Cambridge, Massachusetts 02142 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PERRIN, Steven** [US/US]; 100 Northern Blvd., Newbury, Massachusetts 01951 (US). **VIEIRA, Fernando** [US/US]; 29 West Cedar St., Boston, Massachusetts 02108 (US). **GILL, Alan** [US/US]; 6 Heather Drive, Reading, Massachusetts 01867 (US). **LINCECUM, John** [US/US]; 19 Kenton Road, Jamaica Plain, Massachusetts 02130 (US).

(74) Agent: **BOTT, Cynthia M.**; Honigman, Miller, Schwartz and Cohn LLP, 350 East Michigan Avenue, Suite 300, Kalamazoo, Michigan 49007-3800 (US).

(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, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, 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, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (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, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

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

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(54) **Title**: METHOD FOR THE TREATMENT OF NEURODEGENERATIVE DISEASES

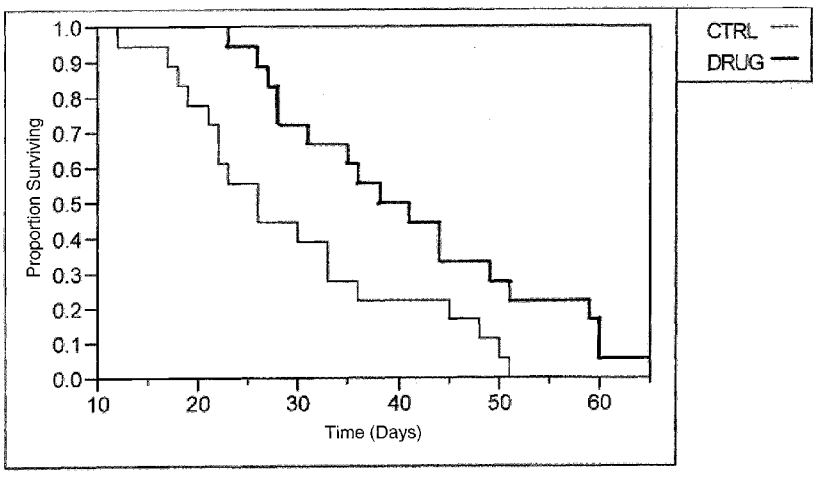


FIG. 5

(57) **Abstract**: Disclosed are methods for treating neurodegenerative diseases such as Amyotrophic Lateral Sclerosis, Alzheimer's Disease, Parkinson's Disease, Myasthenia Gravis, Multifocal Motor Neuropathy, Primary Lateral Sclerosis, Spinal Muscular Atrophy, Kennedy's Disease, and Spinocerebellar Ataxia, by administration of a compound that blocks the interaction of CD40 and CD40L.

WO 2010/065819 A1

METHOD FOR THE TREATMENT OF NEURODEGENERATIVE DISEASES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U. S. Provisional Application Serial No. 61/120,121 filed December 5, 2008. The entire contents of U. S. Provisional Application Serial No. 61/120,121 is incorporated herein by reference.

TECHNICAL FIELD OF THE INVENTION

[0002] The present invention relates to methods for treating neurodegenerative diseases such as Amyotrophic Lateral Sclerosis, Alzheimer's Disease Parkinson's Disease, Myasthenia Gravis, Multifocal Motor Neuropathy, Primary Lateral Sclerosis, Spinal Muscular Atrophy, Kennedy's Disease, and Spinocerebellar Ataxia.

BACKGROUND

[0003] Amyotrophic lateral sclerosis (ALS), sometimes called Lou Gehrig's Disease, is a progressive, fatal, neurological disorder characterized by muscle fiber atrophy resulting from the degeneration of motor neurons in the spinal column and brain. ALS affects approximately 30,000 US citizens with only about 10% of the cases being classified as the familial form of ALS. In a subset of familial patients with mutations in the metabolic enzyme superoxide dismutase 1 (*SOD1*), the pathological progression may be attributed to an unknown gain of function associated with a mutant form of the enzyme (*SOD1* dependant) (Rosen, 1993). However in the majority of ALS cases the *SOD1* gene contains no mutations, the

activity of the *SOD1* enzyme is normal, and the mechanism of disease pathology is unknown (*SOD1* independent). Therefore the remaining 90% of ALS cases are classified as sporadic cases with no well characterized genetic component or causal agent.

[0004] Because the cause of the sporadically occurring form of the disease is unknown, researchers have turned to transgenic strategies to create laboratory models of the disease. Identification role of the *SOD1* gene has led to the generation of transgenic rodent models of ALS. A transgenic mouse strain carrying 23 copies of the human $SOD1^{G93A}$ transgene (the "G93A mouse") is the most widely used murine model of ALS and is accepted as a standard model for ALS therapeutic studies (the "G93A mouse") (*see* Tu PH et. al.(1996) *Proc Natl Acad Sci U S A* 93:3155-3160 and Gurney ME (1997) *J Neuro Sci* 152 Suppl 1: S67-S73)

[0005] Although ALS is characterized by loss of motor neurons in the spinal cord resulting in muscle atrophy, the disease also manifests itself with changes in axon transport, protein aggregation, excitotoxicity, astrocytosis, mitochondrial dysfunction, microglial activation, and synaptic remodeling. Microglial activation, astrocytosis and the presence of infiltrating inflammatory cells from the periphery has been well described. There is accumulation of IgG immunoreactive deposits in the spinal cord of ALS patients, infiltration of lymphocytes, dendritic cells, monocytes, and macrophages into the spinal cord in ALS. Although the role of infiltrating immune cells is poorly understood, recent work would suggest that infiltrating T cell populations are neuroprotective and not cytotoxic. Although ALS has an immune component mediated by activation of microglia and astrocytes it is not considered to

be an autoimmune disorder. Unlike diseases such as rheumatoid arthritis or systemic lupus erythematosus in which involvement of specific immune modulatory pathways (e.g., the costimulatory pathway) has been described, involvement of such pathways has not been described for ALS.

[0006] Currently physicians have limited choices for treating ALS. At this time, riluzole is the only drug that has been approved by the FDA for treatment of ALS. In clinical trials, riluzole has shown only a slight benefit in modestly increasing survival time. Thus there is an urgent need for effective therapies for ALS.

BRIEF SUMMARY

[0007] According to the present disclosure are methods of treating a patient with a neurodegenerative or neuromuscular disorder by administering a therapeutically effective amount of a compound that blocks the interaction of CD40 and CD40L. Also according to the present disclosure are methods of treating a patient with a neurodegenerative or neuromuscular disorder by administering anti-CD40L antibodies or, anti-CD40 antibodies or, small molecules.

[0008] Disorders which may be treated according to the present disclosure include, but are not limited to, Alzheimer's Disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis, Myasthenia Gravis, Multifocal Motor Neuropathy, Primary Lateral Sclerosis, Spinal Muscular Atrophy, Kennedy's Disease, or Spinocerebellar Ataxia.

[0009] In some methods of the present disclosure, the patient with a neurodegenerative or neuromuscular disorder is treated with a compound that blocks the interaction of CD40 and CD40L in combination with a compound that blocks the interaction between CD28 and CD86 or between CD28 and CD80.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] Figures 1 A-P depict the temporal changes in RNA expression patterns of genes associated with induction of the co-stimulatory pathway in the spinal cord and gastrocnemius muscle of the G93A mouse model.

[0011] Figure 2 shows the results of an analysis of macrophage of cell infiltration in gastrocnemius muscle from non transgenic and G93A mice at days 60 and 110.

[0012] Figure 3A shows MR1 concentration over time using a linear concentration (Y) axis.

[0013] Figure 3B shows MR1 concentration over time using a log concentration (Y) axis.

[0014] Figure 4 is a graph of the mean daily body weight measurements starting at day 40 to attainment of peak body weight for the control and the treatment group.

[0015] Figure 5 depicts the mean daily body weight measurements from peak body weight to death for the control and treatment group.

[0016] Figure 6 depicts the time to disease onset based on the time to progress to a neurological score of 2 from the start of the study.

[0017] Figure 7 is a graph representing the median age (X-axis) each group was at a given neurological score 0 to 4 as well as the number of days that each group was stable at a given score (Y-axis)

[0018] Figure 8 shows a Kaplan Meier Survival Plot for the control and treatment group as calculated by a Cox proportional hazard model.

[0019] Figure 9A is a graph of the mean daily body weight measurements starting at day 40 to attainment of peak body weight for the control group and the MR1 treated group.

[0020] Figure 9B is a graph depicting the mean daily body weight measurements from peak body weight to death for the control group and the MR1 treated group.

[0021] Figure 9C shows the time to disease onset based on the time to progress to a neurological score of 2 in the control group and the MR1 treated group.

[0022] Figure 9D shows the mean daily body weight measurements from peak body weight to death for the control and the MR1 treated group.

DETAILED DESCRIPTION

I. Introduction

[0023] The present disclosure describes methods of treating a patient with a neurodegenerative or neuromuscular disorder by administering a therapeutically effective amount of a compound that blocks the interaction of CD40 and CD40L. The disclosure also describes methods of treating patients by co-administering a compound that blocks the interaction of CD40 and CD40L with a compound that blocks the interactions blocks the interaction between CD28 and CD86 or between CD28 and CD80.

II. Abbreviations and definitions

[0024] The following abbreviations are used herein: amyotrophic lateral sclerosis (ALS); super oxide dismutases-1 (*SOD1*); T cell receptor (TCR); major histocompatibility complex (MHC) antigen presenting cell (APC); Phosphate buffered saline (PBS), complementarity determining regions (CDR). "IP" means intraperitoneally and "IV" means intravenously.

[0025] MR1 is a hamster monoclonal antibody that binds to mouse CD40 ligand. "Wild type" as used herein means a non-transgenic mouse. As used herein "small molecule" means a compound having a molecular weight of less than 2000 Daltons. "Treatment" or "treating" as used herein includes prophylactic and therapeutic treatment. The phrase "therapeutically effective amount" means an amount of a compound, or a pharmaceutically acceptable salt thereof, sufficient to inhibit, or allow an improvement in the disorder or condition being treated with administered alone or in conjunction with another pharmaceutical agent or treatment in a particular subject or subject population. "ALS-TDI" is an abbreviation for ALS Therapy Development Institute. "h*SOD1*G93A preclinical mouse model", "h*SOD1*G93A mouse model", "G93A preclinical mouse model" and "G93A mouse model" have the same meaning as used herein. "h*SOD1*G93A mouse" and "G93A mouse" have the same meaning as used herein.

III. In vivo evaluation of compounds

[0026] Compounds used in the methods of the present disclosure may be evaluated for their efficacy using the G93A mouse model , [see Tu PH et al. Proc Natl Acad Sci USA 1996; 93:3155-60 and Gurney ME. et al. J Neurol Sci 1997;152(Suppl 1):S67-73]. This model was constructed by inserting 23 copies of

the human *SOD1* gene, which contains a glycine to alanine mutation at position 93, into the mouse genome. These mice accurately recapitulate most major points of pathology of the human sporadic and familial forms of the disease making this model the best currently available option for testing disease altering interventions.

[0027] There is no discernable phenotypic expression of the abnormality in the G93A mouse at birth. Visible disease signs do not express until about 90 days of age, whereupon it experiences a progressive loss of hindlimb function resulting in complete paralysis and death around 134 days of age. While muscle wasting is caused by the death or dysfunction of motor neurons, the death of these cells is associated with and partially caused by interactions with surrounding cells including microglia and astrocytes. A pronounced astrocytosis first appears around 80 days of age while a neuroinflammation, mediated mainly by microglia, appears around 100 days of age and expands until death.

[0028] While the human disease may begin in any motor region, the mouse disease reliably affects the cervical and lumbar regions first. In the G93A mouse, motor neuron numbers have declined significantly by the time of visible symptom onset, at approximately day 85, and reach upwards of 50% loss at death. Abnormalities in the cytoskeleton, neurofilaments, axonal transport, golgi, endoplasmic reticulum, mitochondria, apoptotic machinery, proteasome, and cytosolic protein handling are observed in neurons during the course of the disease. While the human disease may begin in any motor region, the mouse disease reliably affects the lumbar and sacral regions first. In the G93A mouse, motor neuron numbers have declined significantly by the time of visible symptom onset, at approximately day 85, and reach upwards of 50% loss at death. Abnormalities in the cytoskeleton, neurofilaments, axonal transport, golgi, endoplasmic reticulum,

mitochondria, apoptotic machinery, proteasome, and cytosolic protein handling are observed in neurons during the course of the disease.

[0029] To date, there have been at least 50 publications describing therapeutic agents that extend the lifespan of this mouse. However, no therapeutic agent other than riluzole has show corresponding clinical efficacy. The ALS Therapy Development Institute has described optimized therapeutic drug screening in the G93A mouse model which controls for noise variables [Scott S. et al. *Amyotrophic Lateral Sclerosis* 2008; 9: 4-15 which is hereby incorporated by reference]. Scott et al. describe a minimum study design for the G93A mouse model which addresses and manages the noise caused by the inherent confounding biological variables. In validating this study design, nine compounds that had previously been reported to be efficacious on in the preclinical model and subsequently failed in human clinical trials, were evaluated, most at varying doses, Several of these molecules are anti-inflammatory molecules that inhibit tumor necrosis factor signaling (TNF) and microglial activation including Celebrex®, minocycline, thalidomide, and creatine. Celebrex® was reported to improve lifespan in the G93A model by 19% (24 days) yet a high powered study failed to detect any change in survival (1.8 days, 0.52%), (Scott et al.). Similar results were obtained by for minocycline (previously reported;15.8% improvement; high powered study, -0.60%), creatine (previously reported 17.8%; high powered study 0.67%), and thalidomide (previously reported 16% improvement in survival; high powered study -1.9%).

IV. Identification of candidate compounds

[0030] As described in detail in Example 1, genome wide expression profiling analysis was performed for wildtype mice and G93A mice at various time points

during disease progression. Genes identified as differentially expressed between the two groups were analyzed and the resulting data were used to focus the selection of drugs to be screened. Among the genes that were differentially expressed include genes involved in the immune response and cell adhesion including CD86, CD44, ICAM, ITGAM, ITGA ITGAX, ITGB2, H2-K1 (MHC II), H2-AB1(MHC II), H2-D1 (MHCII), and H2-Eb1 (MHC II). These data show that inflammatory signatures increase during disease progression and are consistent with the involvement of co-stimulatory pathway. The co-stimulatory pathway involves interactions between cell types via CD28/CD80 or CD28/CD86 or CD40/CD40L interactions some of which were identified in the gene expression analysis.

[0031] The co-stimulatory pathway involves, along with other interactions, the binding of CD40 on B cells to CD40L (also known as CD154, gp39, T-BAM, 5c8 antigen, CD40CR and TRAP) on T cells. Human CD40 is expressed on mature B cells, as well as macrophages, dendritic cells, fibroblasts and activated endothelial cells. It is thought that blockade of the CD40:CD40L binding promotes the development of Type I T-helper cell responses.

[0032] Using compounds that block these interactions and inhibit the co-stimulatory signals, a significant body of work has demonstrated the immunomodulatory effects of blocking one or more of CD40L, CD80 or CD86 in preclinical models of transplantation and autoimmunity. Blocking CD40L function with blocking antibodies or adenoviral expression of CD40L-Ig improves allograft transplant by 30 to 90 days. Similar studies blocking CD80/CD86 on APCs with CTLA4-Ig or adenoviral expression of CTLA4-Ig transiently improves allograft transplant survival. Transplant rejection in these models is transient and graft rejection ensues over time. Longer term repression of transplant rejection can be

accomplished by blocking both the co-stimulatory pathway with CTLA4-Ig and blocking CD40L activation of APCs with anti-CD40L antibodies.

[0033] Blocking antibodies to CD40L or genetic deletion of CD40L in mice has demonstrated that CD40L ameliorates disease progression, survival, and surrogate markers of disease in preclinical models of experimental allergic encephalomyelitis (EAE) a model of multiple sclerosis, collagen induced arthritis, and systemic lupus. Blockade of CD40:CD40L binding appears to reduce the ability of macrophages to produce nitric oxide, which mediates many of the macrophages' pro-inflammatory activities.

[0034] It appears from such studies that blocking CD40:CD40L interactions and/or blocking of CD28:CD80 or CD28:CD86 interactions can modulate immune responses.

[0035] Immunohistochemical data (Example 2) was shown to correlate well with the gene expression data and these data identify macrophages as an antigen presenting cell infiltrating skeletal muscle during disease progression in the G93A mouse.

[0036] Because the genetic expression data indicated the involvement of the co-stimulator pathway, the efficacy of MRI was evaluated in the G93A model. MR1 binds to CD40L thus blocking its interaction of CD40 which participates in the co-stimulatory pathway involved in an immune response. It has been reported in the literature that MR1 is efficacious for the treatment of rheumatoid arthritis and graft versus host disease both of which have a strong immunologic component. Rheumatoid arthritis is an autoimmune disease and graft versus host disease arises when the host's body mounts a vigorous immune response against the graft tissue.

[0037] Although ALS has an immune component mediated by activation of microglia and astrocytes it is not considered to be an autoimmune disorder. Several anti-inflammatory drugs failed to show efficacy in preclinical or clinical testing including TNFα-inhibitors, Celebrex®, minocycline, and thalidomide. It was thus unexpected to find that MR1, showed efficacy in the G93A ALS model.

[0038] Methods according to the present disclosure include methods of treating a patient with a neurodegenerative and/or a neuromuscular disorder by administering to the patient a compound that blocks the interaction of CD40L and CD40 and/or blocks the interaction of CD28and CD80 and/or blocks the interaction of CD28 and CD86. One embodiment is a method of treating a patient having Alzheimer's Disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis, Myasthenia Gravis, Multifocal Motor Neuropathy, Primary Lateral Sclerosis, Spinal Muscular Atrophy, Kennedy's Disease, and Spinocerebellar Ataxia by administering to the patient a compound that blocks the interaction of CD40L and CD40. In another embodiment the method of treating a patient is by administering an anti-CD40L antibody.

[0039] Therapeutic compounds useful for the methods of the invention include any compound that blocks the interaction of CD40 with CD40L. For example a number of animal studies describe agents capable of interrupting CD40:CD40L binding (see for example US2005158314 and US7173046 which are hereby incorporated by reference.) And for example, numerous anti-CD40L antibodies have been produced and characterized. (see, e.g., U.S. Pat. No. 5,876,950) to Bristol-Myers Squibb, which is hereby incorporated by reference). Anti-CD40L antibodies useful in the methods of the present disclosure include, but are not limited to, MR1, a hamster monoclonal antibody available from Taconic (Hudson, NY) and BD

Biosciences (San Jose, CA); 5c8, a humanized antibody described in U.S. Pat. No. 5,474,771 (which is hereby incorporated by reference); a hamster human chimeric antibody, IDEC 131/E6040 is a humanized monoclonal antibody comprising human gamma-1 heavy chains and human kappa-light chains with CDRs of murine monoclonal antibody clone 24-31, commercially available from Ancell (catalog X 353-020, Bayport, Minn.); ABI 793;, Sgn-40; ImxM90 (Immunex); ImxM91 (Immunex); ImxM92 (Immunex); and an anti-CD40L mAb commercially available from Genzyme (Cambridge, Mass., catalog No. 80-3703-01). Also commercially available is an anti-CD40L mAb from PharMingen (San Diego, Catalog #33580D). Embodiments according to the disclosure include methods of treating a patient with a neurodegenerative or neuromuscular disorder, comprises administering a therapeutically effective amount of an anti-CD40L antibody. One embodiment is a method of treating a patient with a neurodegenerative or neuromuscular disorder, comprising administering a therapeutically effective amount of an anti-CD40L antibody selected from MR1, 5c8, IDEC 131/E6040, clone 24-31, ABI 793, ImxM90, ImxM91, ImxM92, or Sgn-40. In one embodiment the antibody is 5c8. In another embodiment the antibody is MR1.

[0040] In some embodiments the neurodegenerative or neuromuscular disorder is Alzheimer's Disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis, Myasthenia Gravis, Multifocal Motor Neuropathy, Primary Lateral Sclerosis, Spinal Muscular Atrophy, Kennedy's Disease, or Spinocerebellar Ataxia. One embodiment is a method of treating a patient with Amyotrophic Lateral Sclerosis comprising administering a therapeutically effective amount of an anti-CD40L antibody. In a particular embodiment the anti-CD40L antibody is MR1. In another particular embodiment the anti-CD40L antibody is 5c8.

[0041] In another embodiment the method of treatment comprises administering a therapeutically effective amount of an anti-CD40 antibody. In some embodiments the anti-CD40L compounds are Fab fragments, $F(ab')_2$, F(ab'), single chain antibodies, polypeptides, fusion constructs of polypeptides and the like. In some embodiments the compounds are small molecule compounds that are capable of blocking the CD40:CD40L interaction. In other embodiments these compounds include BIO3417, or any of the compounds disclosed in United States Patent Number 7,173,046, having the ability to block the CD40:CD40L interaction.

[0042] The compounds that block the CD40:CD40L interaction may be administered in combination with other compounds. Thus, another embodiment is a method of treating a patient with a neurodegenerative or neuromuscular disorder comprising administering a compound that blocks the interaction of CD40L and CD40 in combination with a compound that blocks the interaction between CD80 and CD28. Another embodiment is a method of treating a patient by administering a compound that blocks the interaction of CD40L and CD40 in combination with a compound that blocks the interaction between CD86 and CD28. In one embodiment, the compound that blocks the interaction of CD80 and CD28 is galiximab, or H1f1 & h3d1, or 16C10, or 7C10. In one embodiment the compound that blocks the interaction between CD86 and CD28 is a CTLA4-Ig protein conjugate such as abetacept or belatacept. Embodiments according to the present disclosure also include a method of treating a patient with a neurodegenerative or neuromuscular disorder comprising administering a compound that blocks the interaction of CD40L and CD40 in combination with a compound that blocks the interaction between CD80 and CD28 or administering a therapeutically effective amount of a compound that blocks the interaction of CD40L and CD40 in

combination with a compound that blocks the interaction between CD86 and CD28, wherein the neurodegenerative or neuromuscular disorder is Alzheimer's Disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis, Myasthenia Gravis, Multifocal Motor Neuropathy, Primary Lateral Sclerosis, Spinal Muscular Atrophy, Kennedy's Disease, or Spinocerebellar Ataxia. In certain embodiments the compound that blocks the interaction of CD40L and CD40 is MR1 and the compound that blocks the interaction between CD28 and CD86 or between CD28 and CD80 is abatacept, galiximab or belatacept. In other embodiments, the compound that blocks the interaction of CD40L and CD40 is 5c8. Another embodiment is a method of treating a patient with Amyotrophic Lateral Sclerosis comprising administering a therapeutically effective amount of MR1 in combination with abatacept or belatacept. Another embodiment is a method of treating a patient with Amyotrophic Lateral Sclerosis comprising administering a therapeutically effective amount of 5c8 in combination with abatacept or belatacept.

V. Pharmaceutical compositions and methods of administration

[0043] To treat any of the foregoing disorders, pharmaceutical compositions for use in accordance with the methods of the present disclosure may be formulated in a conventional manner using one or more physiologically acceptable carriers. Pharmaceutically acceptable carriers are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Accordingly, there are a wide variety of suitable formulations of the compounds useful in the methods of the present disclosure (see, e.g., Remington: The Science and Practice of Pharmacy, 20th ed., Gennaro et al. Eds., Lippincott Williams and Wilkins, 2000).

[0044] Formulations suitable for oral administration include, for example, solid, semi-solid and liquid systems such as tablets; soft or hard capsules containing multi- or nano-particulates, liquids, or powders; lozenges (including liquid-filled); chews; gels; fast dispersing dosage forms; films; ovules; sprays; and buccal/mucoadhesive patches.

[0045] Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and nonaqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives.

[0046] According to the present disclosure the compounds can be administered by any suitable means, which can vary, depending on the type of disorder being treated and on the nature of the compound itself. For example the compounds may be administered orally, parenterally or topically. For proteins such as antibodies, administration routes preferably include parenteral, e.g., intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous. Preferably, the parenteral dosing is given by injection, most preferably intravenous, intramuscular or subcutaneous injection. The amount to be administered will depend on a variety of factors such as the clinical symptoms, weight of the individual, and whether other drugs are administered. It should be appreciated that determination of proper dosage forms, dosage amounts, and routes of administration is within the level of ordinary skill in the pharmaceutical and medical arts, and is described below.

VI. Examples

Example 1

Characterization of the molecular mechanisms of neurodegeneration in the G93A mouse model of ALS.

[0047] In order to identify molecular pathways amenable to therapeutic development, changes in gene expression patterns were characterized during disease progression in the G93A mouse model. Whole genome transcriptional profiling was studied using Affymetrix GeneChip® Mouse Expression set 430vII MOE430vII genechips. A longitudinal study design was employed comparing G93A skeletal muscle and spinal cord to non-transgenic littermates. In the G93A mouse model symptom onset is first seen as tail paralysis starting at approximately day 75 with progressive paralysis in the hindlimbs, then forelimbs, and finally diaphragm. The mean survival of the G93A animal colony is 134 days. The longitudinal study design collected calf muscle (gastrocnemius muscle) and spinal cord from G93A animals and wild type litter mates at days 30, 50, 60, 80, 90, 100, 110, and 120 (day 0 is date of birth). For each time point, tissues were collected from 5 wild type and 5 G93A animals and processed independently for a total of 160 tissues.

[0048] Animals were euthanized according to IACUC protocols at the appropriate time points described above. Tissues were immediately harvested and snap frozen in liquid nitrogen. Frozen tissues were stored at -80 degrees Celsius. Total RNA was isolated simultaneously from the tissues using the Qiagen RNaEasy kit as described by the manufacturer. The isolated total RNA was amplified using standard T7 linear amplification incorporating a biotinylated nucleotide using the Ambion Message AMPII T7 in vitro transcription kit. Labeled probe was fragmented and hybridized to Affymetrix GeneChip® Mouse Expression set 430vII according to

the manufacturer's protocol. Genechips were washed in Affymetrix GeneChip® Fluids Station 450 to remove non-hybridized probe. Genechips were scanned in an Affymetrix GeneChip® 3000 7G scanner.

[0049] All computational processing and modeling was performed using the R development language version 2.6 from Bioconductor. The spinal cord and gastrocnemius data sets were analyzed independently. Affymetrix CEL files were used for all data preprocessing. All the CEL files in each data set were quality controlled using the Bioconductor vignettes SIMPLE AFFY, AFFY and AFFY PLM. The statistical changes in gene expression between G93A and wild type tissues at a given time point were assessed using the LIMMA package. The bayes model was used to determine the significance of expression changes between groups.

[0050] Genes that were found to be differentially expressed between the wild type and G93A animals include genes that are involved in the immune response and cell adhesion including CD86, CD44, ICAM, ITGAM, ITGA ITGAX, ITGB2, H2-K1 (MHC II), H2-AB1(MHC II), H2-D1 (MHCII), and H2-Eb1 (MHC II). The log2 normalized expression profiles of these genes from wild type and G93A mice spinal cord and gastrocnemius at 30, 50, 60, 80, 90, 100, 110 and 120 days are shown in Figure 1. Figures 1A and 1B show the differential expression of CD89 in spinal cord and gastrocnemius respectively. Figures 1C and 1D show the differential expression of CD44 in spinal cord and gastrocnemius respectively. Figures 1E and 1F show the differential expression of ICAM in spinal cord and gastrocnemius respectively. Figures 1G and 1H show the differential expression of ITGAM (CD11b) in spinal cord and gastrocnemius respectively. Figures 1I and 1J show the differential expression of ITGAX in spinal cord and gastrocnemius respectively. Figures 1K and 1L show the differential expression of ITGB2 in spinal cord and gastrocnemius respectively.

Figure 1M shows the differential expression of H2-K1 (MHC II) in spinal cord. Figure 1N shows the differential expression of H2-AB1 (MHCII) in gastrocnemius. Figure 1O shows the differential expression of H2-D1(MHC II) in spinal cord. Figure 1 P shows the differential expression of H2-Eb1 (MHC II) in gastrocnemius.

[0051] These data show that inflammatory signatures increase during disease progression. These gene expression changes reflect the activation of antigen presenting cells such as dendritic cells, macrophages, and B cells. Blocking the interaction may ameliorate immune responses that exacerbate disease progression in ALS. The co-stimulatory pathway can be inhibited by blocking CD-28/CD80 or CD-28/CD86 or CD40/CD40L interactions.

Example 2

Characterization of antigen presenting cells in skeletal muscle and the peripheral nervous system.

[0052] In order to determine the presence and localization of potential antigen presenting cells in gastrocnemius muscle from G93A and wild type animals, immunohistochemistry was performed on gastrocnemius tissues that were harvested from G93A and wildtype mice, at day 110. Immediately after harvesting, the tissues were embedded in OCT. Frozen sections were H&E stained and hybridized with antibodies to myelin (anti-S100b antibody) or antibodies to hematopoietic cell lineages including T cells (anti-CD3 antibody), B cells (CD45R pan B cell antibody), and macrophages (anti-CD11b antibody). At day 110 there was infiltration of CD11b positive macrophages and the macrophages appear to be localized to the axons of nerves innervating the skeletal muscle. The localization of macrophages is not

dispersed across the entire muscle suggesting that the inflammation is not due to muscle atrophy or muscle fiber remodeling.

[0053] In order to confirm that identity of the monocyte lineage cells in the skeletal muscle at day 110 and to clarify the relevance of these cells compared to non-transgenic animals, additional immunohistochemistry was performed with a panel of antibodies specific for the macrophage lineage. An anti-S100b antibody was utilized to label the myelin associated axons innervating the skeletal muscle. All of the macrophage specific antibodies (anti-CD11b antibody, anti-CD86 antibody and anti-MAC1antibody) localized macrophages to the axons of nerves innervating the skeletal muscle of G93A mice with no macrophages present on the axons of wildtype animals. The localization of macrophages was specific for nerves innervating the muscle with no macrophages present on myofibers in the muscle.

[0054] The gene expression data suggest that the genes associated with the co-stimulatory pathway are temporally increasing during disease progression both in spinal cord as well as in skeletal muscle (Figure 1). In order to characterize the timing of macrophage infiltration into skeletal muscle immunohistochemistry was performed on gastrocnemius sections from days 60, 80, and 100 in G93A mice. There was no evidence of macrophage infiltration and localization to axons at day 60. Macrophages were evident at day 80 and localized to the axons innervating the muscle as described previously for skeletal muscle at day 110. The number of macrophages increased between day 80 and day 100 and accumulation of macrophages was specific to the axons innervating the skeletal muscle.

[0055] In order to quantitate the increase in macrophage infiltration, representative sections from 5 G93A and 5 wild type animals were hybridized with anti-CD86 antibody and the number of macrophages per 10,000 square microns

were counted. The number of macrophages in the wild type animals was the same at the 60, 80 and 110 day time points. As can be seen in Figure 2 macrophages are accumulating in skeletal muscle temporally between days 80 and 100 and there are very few macrophages present in wild type skeletal muscle.

[0056] In summary, the immunohistochemical data correlates very well with the gene expression data and identify macrophages as the antigen presenting cell infiltrating skeletal muscle during disease progression in the G93A mouse model. An unexpected finding is that the macrophage infiltration appears to be specifically targeted to the axons innervating the skeletal muscle as localized by labeling with antibodies to myelin and macrophages.

Example 3

Pharmacokinetic analysis of MR1 in G93A tissues

[0057] Tissue levels of MR1 directed against murine CD40L were determined using a matrix matched, non-competitive enzyme linked immunoabsorbent assay (ELISA) in the sandwich format. Seven point standard curves were included on each plate. Standards were prepared using purified MR1 spiked into PBS diluent solution. PBS diluent solution was matrix matched with the normal mouse tissue at the equivalent dilution of the unknown samples to correct for any non-specific effects resulting from tissue lysates.

[0058] 84 plasma samples were taken for pharmacokinetic analysis over a period of two weeks after dosing (10 mg/kg, IP) in both female and male G93A mice.

[0059] The elimination half-life was similar in females (23 d) and males (22 d) and similar to the half-life for a typical mouse IgG2-based antibody in the mouse. No signs of anti-hamster antibody response were seen.

[0060] Females show a somewhat smaller volume of distribution for MR1 than males, and thus show higher plasma levels when given the same 10 mg/kg dose. Males show a faster clearance and higher volume of distribution. Thus, to attain similar plasma levels, males would require a higher dose. Figure 3A shows MR1 concentration over time using a linear concentration (Y) axis. Figure 3B shows MR1 concentration over time using a log concentration (Y) axis.

Example 4

MR1 delays disease onset, slows disease progression and prolongs survival in the G93A mouse model of ALS.

[0061] 36 female G93A mice were litter matched and randomized into two study groups. 18 G93A mice were placed into the MRI-treatment group and the other 18 G93A mice were placed into the control group. Study days are based on days from birth.

[0062] A single injection of 56ug of MR1 was administered intraperitoneally (IP) on day 50. Subsequent to the bolus injection a weekly maintenance injection of 18ug of MR1 was administered by IP injection. Doses were prepared in vehicle (phosphate buffered saline (PBS, pH 7.3)), to a total volume 200µl. Control animals where administered 200µl PBS. Beginning at day 54, animals were monitored daily throughout the course of the study and daily body weight measurements as well as neurological score were measured.

[0063] Neurological scores for both hind legs were assessed daily for each mouse from 50 days of age. The neurological score employed a scale of 0 to 4. (Scott et al., ALS Journal Jan 2008). Briefly animals assigned a score of 0 had full extension of hind legs away from lateral midline when mouse is suspended by its tail,

and mouse can hold this for 2 seconds, suspended 2-3 times. Animals score a 1 when they display collapse or partial collapse of leg extension towards lateral midline (weakness) or trembling of hind legs during tail suspension. Animals score a 2 when the toes curl under at least twice during walking of 12 inches, or any part of foot is dragging along cage bottom/table. Animals score a 3 when they have rigid paralysis or minimal joint movement, or a foot not being used for forward motion. Animals score a 4 when they cannot right itself within 30 seconds from either side. If one hind leg is scored as 2, food pellets are left on bedding. If both hind legs are scored as 2, Nutra-Gel® (Bio-Serve #S4798) is provided as food in addition to food pellets on bedding and a long sipper tube is placed on the water bottle.

[0064] Date and cause of death were recorded for each mouse. For humane reasons, animals are closely monitored and sacrificed as moribund prior to actual death using criteria for severe moribundity. To determine duration of survival reliably and humanely, the moribund state, defined as the inability of mice to right themselves 30 seconds after being placed on a side (a neurological score of 4) was used. The moribund mice were scored as "dead", and were euthanized using carbon dioxide.

[0065] Standard procedure is to remove non ALS related deaths in both treatment and control groups prior to statistical analysis. In this case all animal deaths in either the control or treatment groups were attributed to ALS. Thus, no animals were censored due to non ALS related deaths.

[0066] Mutant *SOD1* transgenic animals display normal body weight (BW) characteristics as neonates and gain weight normally compared to non-transgenic animals into adulthood. Depending on the nature of the genetic mutation in the transgene and the number of copies of mutant transgene weight loss becomes

apparent in adult animals and continues until death. Analysis of weight loss in treatment and control groups can provide insight into putative treatment effects on disease onset and rate of progression. In order to assess the impact of MR1 treatment on body weight, two summary parameters are examined (1) Changes in BW from initiation of study to the attainment of peak body weight which may reflect an impact on disease onset (2) The changes in BW from peak body weight until death which may reflect an impact on disease progression.

[0067] Comparative MR1-treated and control group time-to-event curves for the time from day 40 to the attainment of peak body weight are shown in Figure 4. The median time to peak body weight for the control group was 50 days compared to the MR1 treated group which was 51 days. This difference was not significant when analyzed by Kaplan Meier using the log rank and Wilcoxon statistical models, Cox proportional hazard, or parametric statistical tests.

[0068] Comparative MR1-treated and control group time-to-event curves for the time from peak body weight to death are shown in Figure 5. Time from peak body weight to death was statistically significantly later by 15 days in MRI treated animals. Control animals have a 2.4 to 4.7-fold greater risk of dying sooner after attaining peak body weight than did MR1-treated animals. The median time from peak body weight to death in the control group was 26 days whereas in the MR1 treated group it was 41 days. Significance for each of the analyses in this example was calculated in several ways in order to better evaluate significance. The delay is statistically significant when analyzed using several approaches. (Kaplan Meier, log rank p=0.0110 and Wilcoxon, p=0.0069; Cox proportional Hazard model p=0.05151; parametric statistical model, p=0.0122). Based on the body weight data MR1 appears to have less of an impact on the onset of disease in the G93A mouse

model but has a dramatic effect in slowing down the rate of body weight loss from peak body weight until death.

[0069] The timing of disease onset was also characterized by analyzing the daily neurological scores of the saline treated and MR1 treated groups. At the start of the study (day 50), all animals have a neurological score of 0 with no observable symptoms or paralysis. Disease onset can be characterized by examining the progression in neurological score from a neurological score of 0 to a neurological score of 2 when animals are clearly dragging a hind limb. The time-to-event plot for the age at which MR1 and control group animals progressed to a neurological score of 2 and the number of days at a neurological score of 2 is shown in Figure 6. The median time at a neurological score of 2 for each group is plotted in Figure 7. The time to attain a score of 2 in the control group is 115 days and for the MR1 treated group is 122 days. Based on the neurological score data MR1 delays disease onset in the G93A mouse model by approximately 7 days and the delay is statistically significant when analyzed using several approaches. (Kaplan Meier, log rank ($p = 0.0378$) and Wilcoxon, ($p = 0.0591$); Cox proportional hazard model, ($p = 0.0521$); parametric analysis, ($p = 0.0582$)). Days spent at each neurological score level is plotted against the median age at that score level in Figure 7.

[0070] Treated animals survival time was later by 13 days than control animals. Comparative MR1-treated and control group time-to-event curves for the time from peak body weight to death are shown in Figure 8. The median survival time for the control group was128 days and the median survival for the MR1 treated group was 141 days. Control animals had a 2.8 to 3.2-fold greater risk of dying sooner than did MR1 treated animals. The delay is statistically significant when analyzed using several approaches. (Kaplan Meier, log rank ($p = 0.0040$) and

Wilcoxon test (p = 0.0109); Cox proportional hazard model (p = 0.0060); parametric analysis, (p = 0.0049)).

Example 5

Optimized dosing and meta analysis demonstrate that MR1 treatment delays disease onset and improves survival in SOD1G93 mice

[0071] 60 female and 36 male G93A mice were litter matched and randomly assigned to treatment or control groups. 30 of the female and 18 of the male mice were treated with MR1 starting at day 50. Study days are based on birth.

[0072] A single bolus injection of 5.22 mg/kg or 6.75 mg/kg of MR1 was administered intraperitoneally at day 50 in females or males respectively. Subsequent to the bolus injection, females received weekly injections of 1mg/kg of MR1 and males received weekly injections of 1.34 mg/kg of MR1 via IP injection. Doses were prepared in vehicle (phosphate buffered saline (PBS, pH 7.3)), to a total volume of 200µl. Control animals where administered 200µl PBS. Animals were monitored for body weight, neurological score, non-related ALS deaths, and criteria for euthanization as previously described.

[0073] Comparative MR1-treated and control group time-to-event curves for the time from day 40 to the attainment of peak body weight are shown in Figure 9A . The median time to peak body weight for the control group was 49 days compared to the MR1 treated group which was 53 days. This difference was not significant when analyzed by Kaplan Meier using the log rank and Wilcoxon statistical models, Cox proportional hazard, or parametric statistical tests.

[0074] Comparative MR1-treated and control group time-to-event curves for the time from peak body weight to death are shown in Figure 9B. Time from peak body weight to death was statistically significantly later by 6 days in MRI treated

animals. The median time from peak body weight to death in the control group was 29 days whereas in the MR1 treated group it was 35 days. Significance for each of the analyses in this example was calculated in several ways in order to better evaluate significance. The delay is statistically significant when analyzed using several statistical models: Kaplan Meier, log rank (p=0.0413) and Wilcoxon, (p=0.0732); and the Cox proportional Hazard model (p=0.0460). Based on the body weight data, MR1 appears to have less of an impact on the onset of disease in the G93A mouse model but has a dramatic effect in slowing down the rate of body weight loss from peak body weight until death.

[0075] The time-to-event plots for the age at which MR1 and control group animals progressed to a neurological score of 2 and the number of days at a neurological score of 2 is shown in Figure 9C. The time to attain a score of 2 in the control group was 113 days and for the MR1 treated group is 121 days. Based on the neurological score data, MR1 delays disease onset in the G93A mouse model by approximately 8 days and the delay is statistically significant when analyzed using several statistical models: Kaplan Meier, log rank (p = 0.0038) and Wilcoxon; (p = 0.0017); and the Cox proportional hazard model, (p = 0.0010).

[0076] Treated animals survival time was later by 9 days than control animals. The median survival time for the control group was124 days and the median survival for the MR1 treated group was 133 days, as shown in figure 9D. The delay is statistically significant when analyzed using several statistical models: Kaplan Meier, log rank (p = 0.0043) and Wilcoxon test (p = 0.0040); and the Cox proportional hazard model (p = 0.0030).

[0077] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes

in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

CLAIMS

1. A method of treating a patient with a neurodegenerative or neuromuscular disorder, comprising administering to the patient a therapeutically effective amount of a compound that blocks the interaction of CD40 and CD40L.

2. The method of claim 1 wherein the compound is an anti-CD40L antibody or an anti-CD40 antibody or a small molecule.

3. The method of claim 1 wherein the disorder is Alzheimer's Disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis, Myasthenia Gravis, Multifocal Motor Neuropathy, Primary Lateral Sclerosis, Spinal Muscular Atrophy, Kennedy's Disease, or Spinocerebellar Ataxia.

4. The method of claim 3 wherein the compound is an anti-CD40L antibody or an anti-CD40 antibody or a small molecule.

5. The method of claim 4 wherein the compound is an anti-CD40L antibody.

6. The method of claim 4 wherein the compound is a small molecule.

7. The method of claim 5 wherein the anti-CD40L antibody is MR1, 5c8, IDEC 131/E6040, clone 24-31, ABI 793, ImxM90, ImxM91, ImxM92, or Sgn-40.

8. The method of claim 5 wherein the anti-CD40L antibody is MR1.

9. The method of claim 5 wherein the anti-CD40L antibody is 5c8.

10. The method of claim 5 wherein the disorder is Amyotrophic Lateral Sclerosis.

11. The method of claim 10 wherein the compound is an anti-CD40L antibody.

12. The method of claim 10 wherein the anti-CD40L antibody is antibody is MR1, 5c8, IDEC 131/E6040, clone 24-31, ABI 793, ImxM90, ImxM91, ImxM92 or Sgn-40.

13. The method of claim 12 wherein the anti-CD40L antibody is MR1.

14. The method of claim 12 wherein the anti-CD40L antibody is 5c8.

15. A method of treating a patient with a neurodegenerative or neuromuscular disorder, comprising administering to the patient a therapeutically effective amount of a compound that blocks the interaction of CD40 and CD40L in combination with a compound that blocks the interaction between CD28 and CD86 or between CD28 and CD80.

16. The method of Claim 15 wherein the compound that blocks the interaction of CD40 and CD40L is an anti-CD40L antibody or an anti-CD40 antibody or a small molecule.

17. The method of claim 16 wherein the disorder is Alzheimer's Disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis, Myasthenia Gravis, Multifocal Motor Neuropathy, Primary Lateral Sclerosis, Spinal Muscular Atrophy, Kennedy's Disease, or Spinocerebellar Ataxia.

18. The method of claim 17 wherein the compound that blocks the interaction of CD40 and CD40L is an anti-CD40L antibody or an anti-CD40 antibody.

19. The method of claim 18 wherein the compound that blocks the interaction of CD40 and CD40L is MR1 or 5c8.

20. The method of claim 19 wherein the disorder is Amyotrophic Lateral Sclerosis.

21. The method of claim 18 wherein the compound that blocks the interaction between CD28 and CD86 or between CD28 and CD80 is a CTLA4-Ig fusion protein.

22. The method of claim 18 wherein the compound that blocks the interaction between CD28 and CD86 or between CD28 and CD80 is abatacept or belatacept or galiximab.

23. The method of claim 22 wherein the compound that blocks the interaction of CD40 and CD40L is MR1 or 5c8.

24. The method of claim 23 wherein the compound that blocks the interaction between CD28 and CD86 or between CD28 and CD80 is abatacept or belatacept.

FIG. 1A

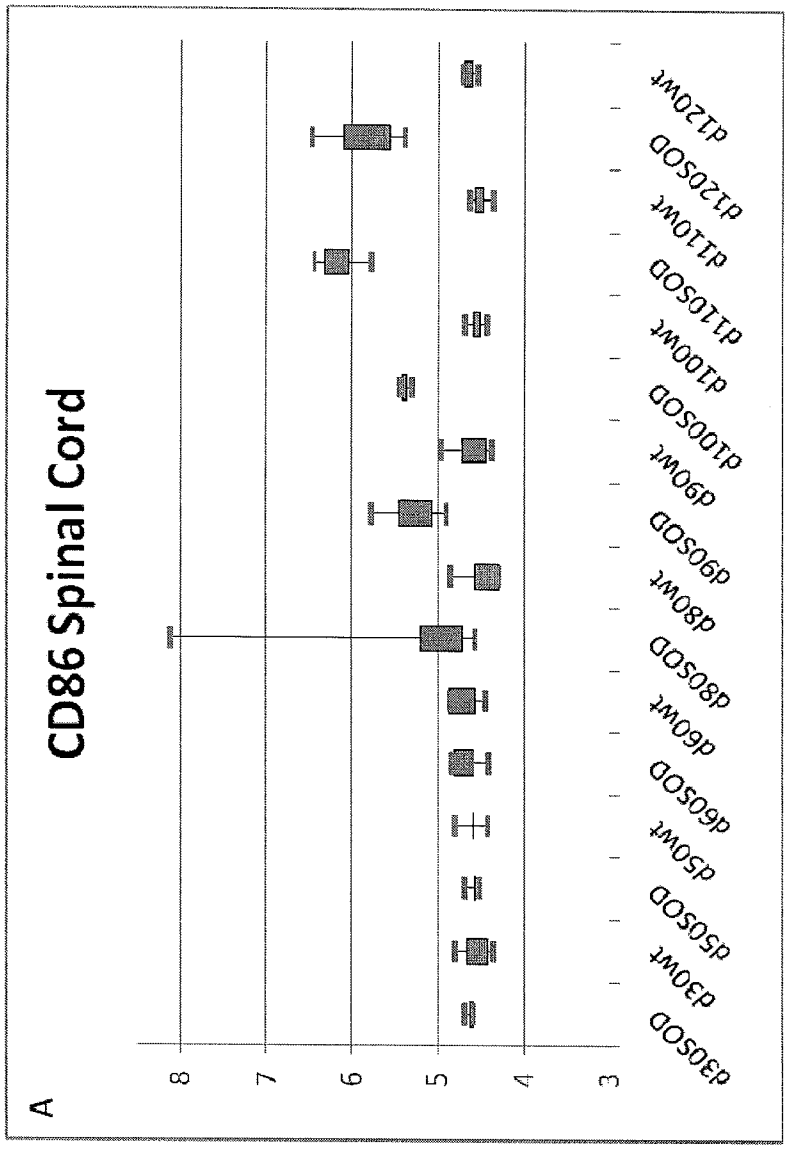

A
CD86 Spinal Cord
8
7
6
5
4
3
d30SOD
d30wt
d50SOD
d50wt
d60SOD
d60wt
d80SOD
d80wt
d90SOD
d90wt
d100SOD
d100wt
d120SOD
d120wt
d120SOD
d120wt

FIG. 1B

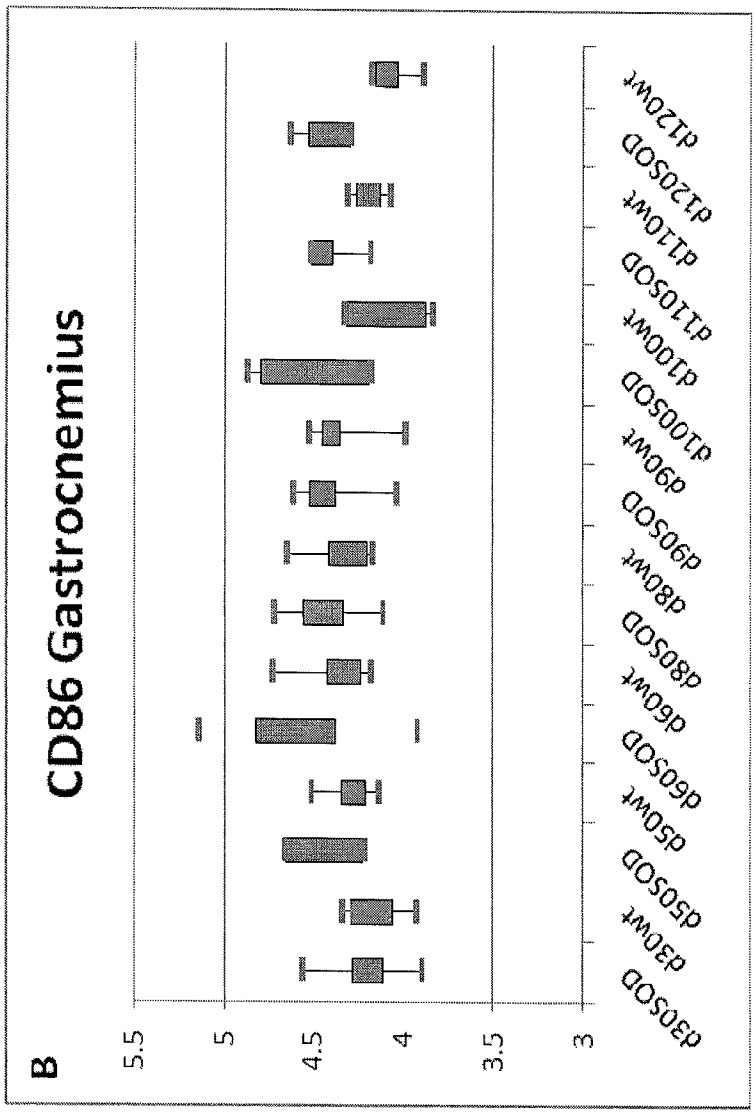

B
CD86 Gastrocnemius
5.5
5
4.5
4
3.5
3
d30SOD
d30wt
d50SOD
d50wt
d60SOD
d60wt
d80SOD
d80wt
d90SOD
d90wt
d100SOD
d100wt
d105OD
d110wt
d120SOD
d120wt

FIG. 1C

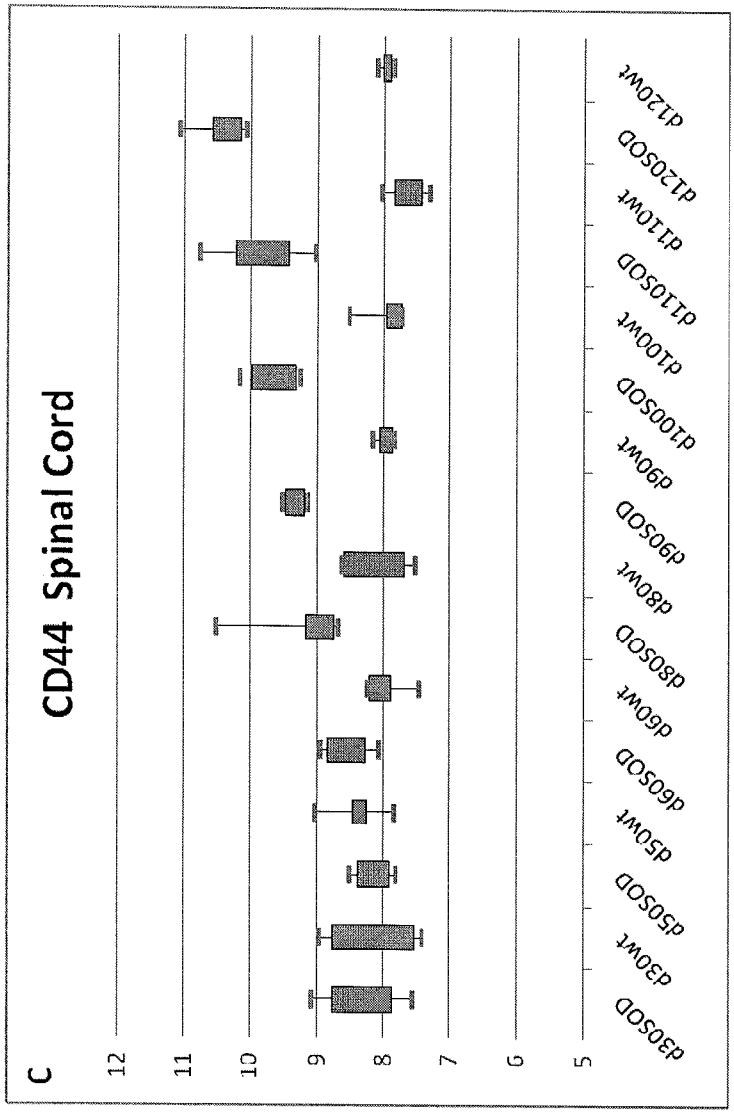

C
CD44 Spinal Cord
12
11
10
9
8
7
6
5
d30OD
d30wt
d50OD
d50wt
d60OD
d60wt
d80OD
d80wt
d90OD
d90wt
d100OD
d100wt
d110OD
d110wt
d120OD
d120wt

FIG. 1D

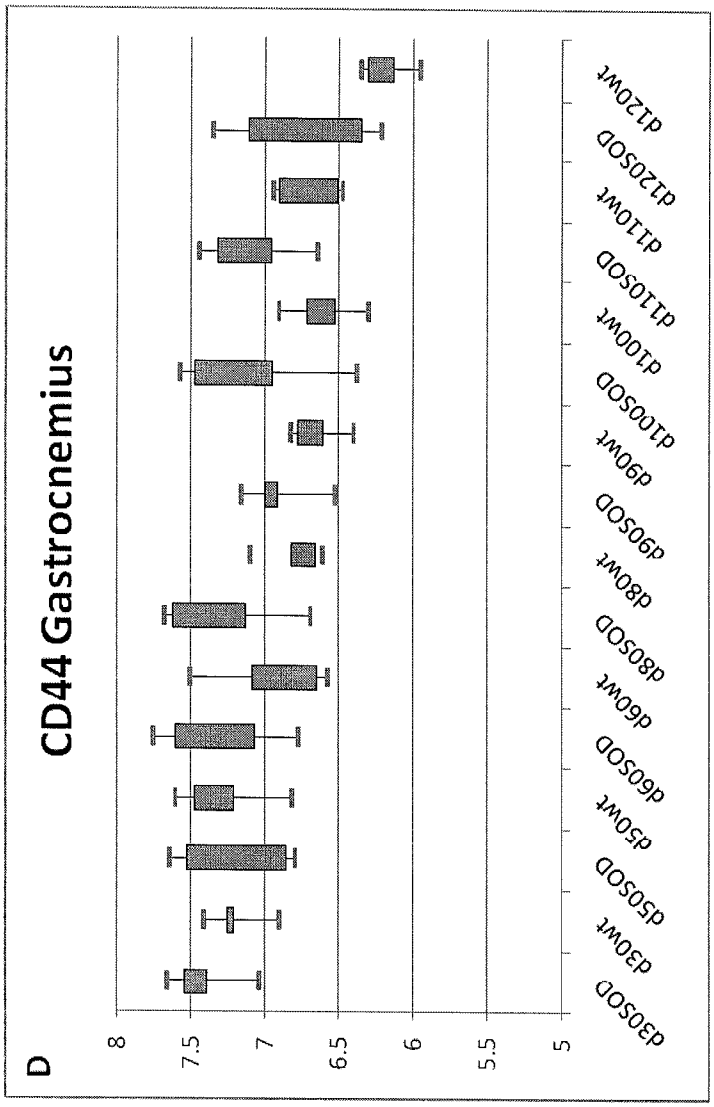

D
CD44 Gastrocnemius
8
7.5
7
6.5
6
5.5
5
d30SOD
d30wt
d50SOD
d50wt
d60SOD
d60wt
d80SOD
d80wt
d90SOD
d90wt
d100SOD
d100wt
d105OD
d110wt
d120SOD
d120wt

FIG. 1E

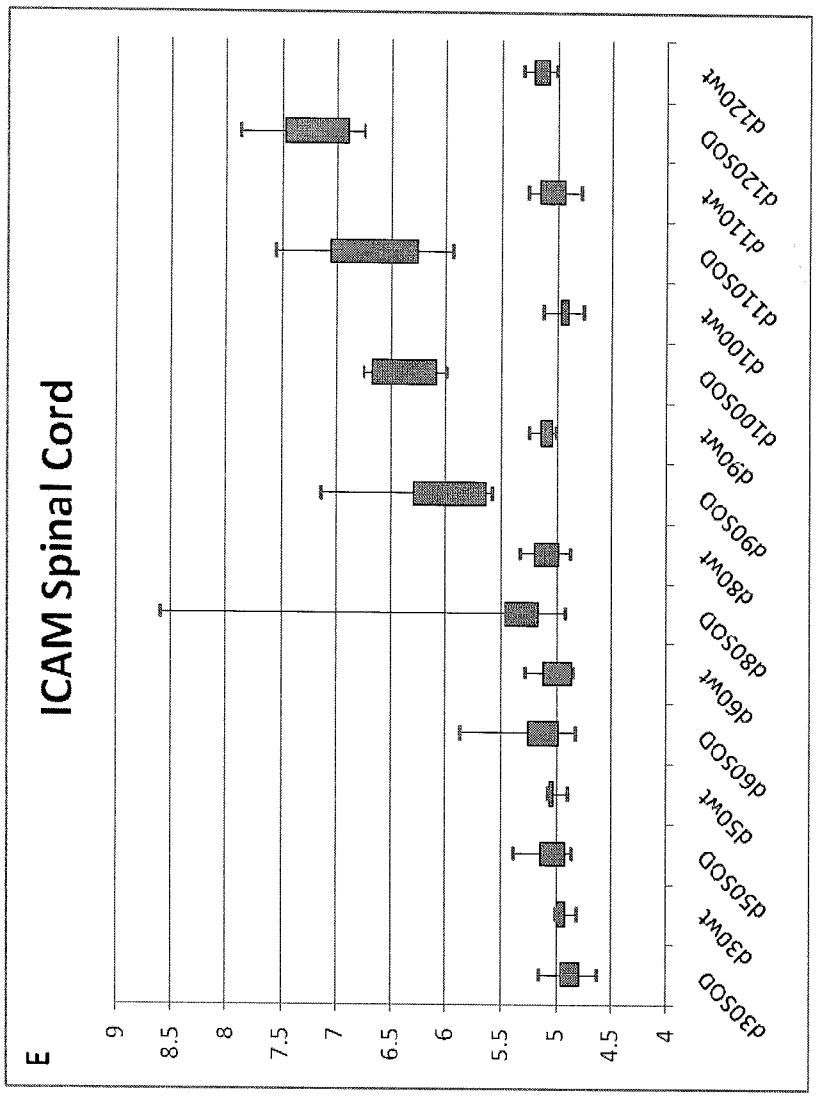

E
ICAM Spinal Cord
9
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
d30SOD
d30wt
d50SOD
d50wt
d60SOD
d60wt
d80SOD
d80wt
d90SOD
d90wt
d100SOD
d100wt
d110SOD
d110wt
d120SOD
d120wt

FIG. 1F

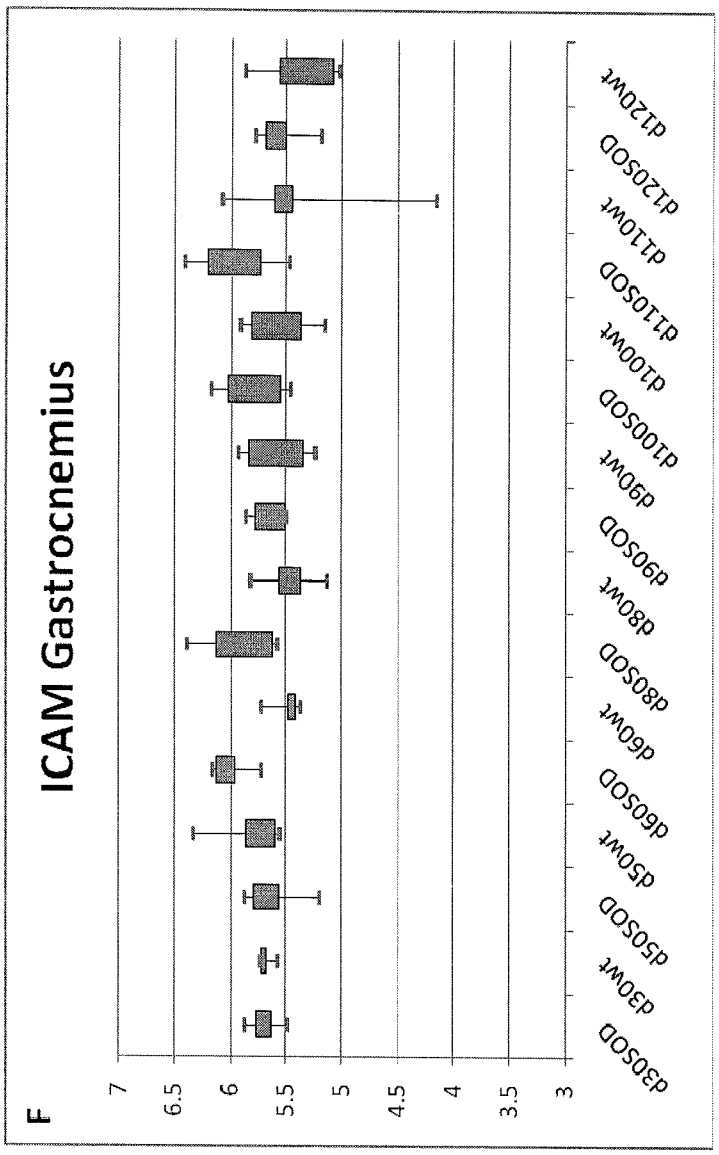

F
ICAM Gastrocnemius
7
6.5
6
5.5
5
4.5
4
3.5
3
d3OSOD
d30wt
d5OSOD
d50wt
d6OSOD
d60wt
d8OSOD
d80wt
d9OSOD
d90wt
d10OSOD
d100wt
d11OSOD
d110wt
d12OSOD
d120wt

FIG. 1G

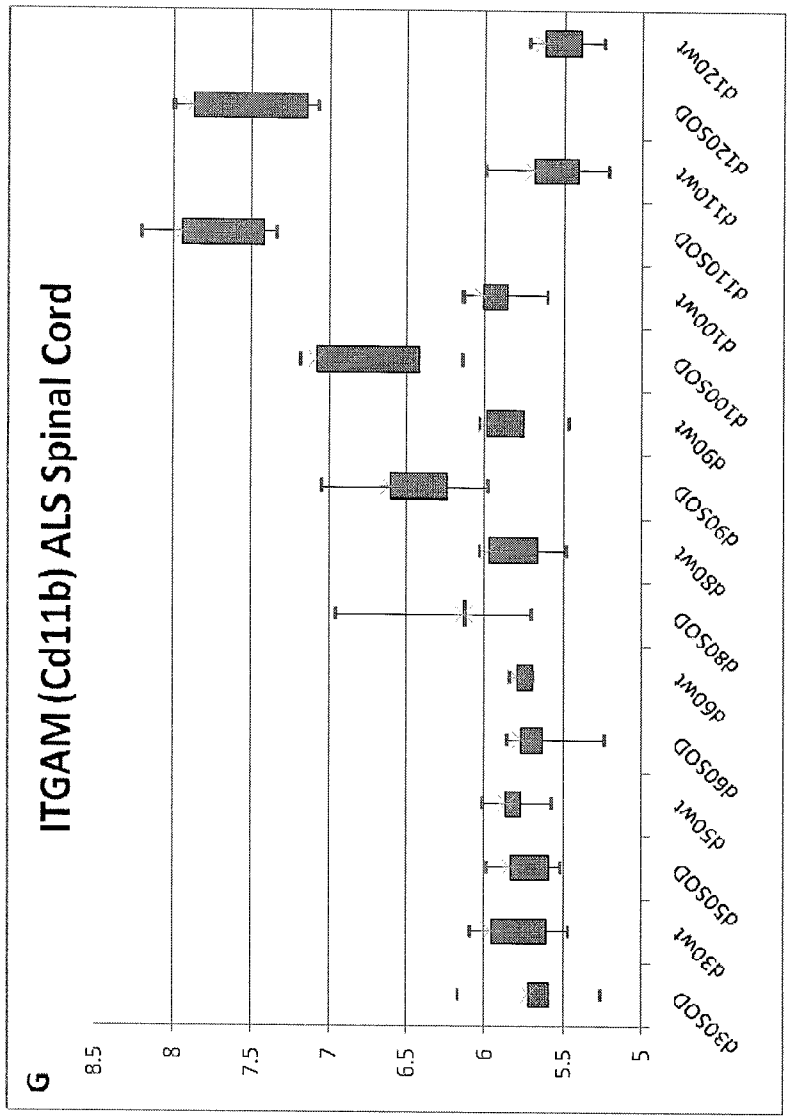

G
ITGAM (Cd11b) ALS Spinal Cord
8.5
8
7.5
7
6.5
6
5.5
5
d30SOD
d30wt
d50SOD
d50wt
d60SOD
d60wt
d80SOD
d80wt
d90SOD
d90wt
d100SOD
d100wt
d110SOD
d110wt
d120SOD
d120wt

FIG. 1H

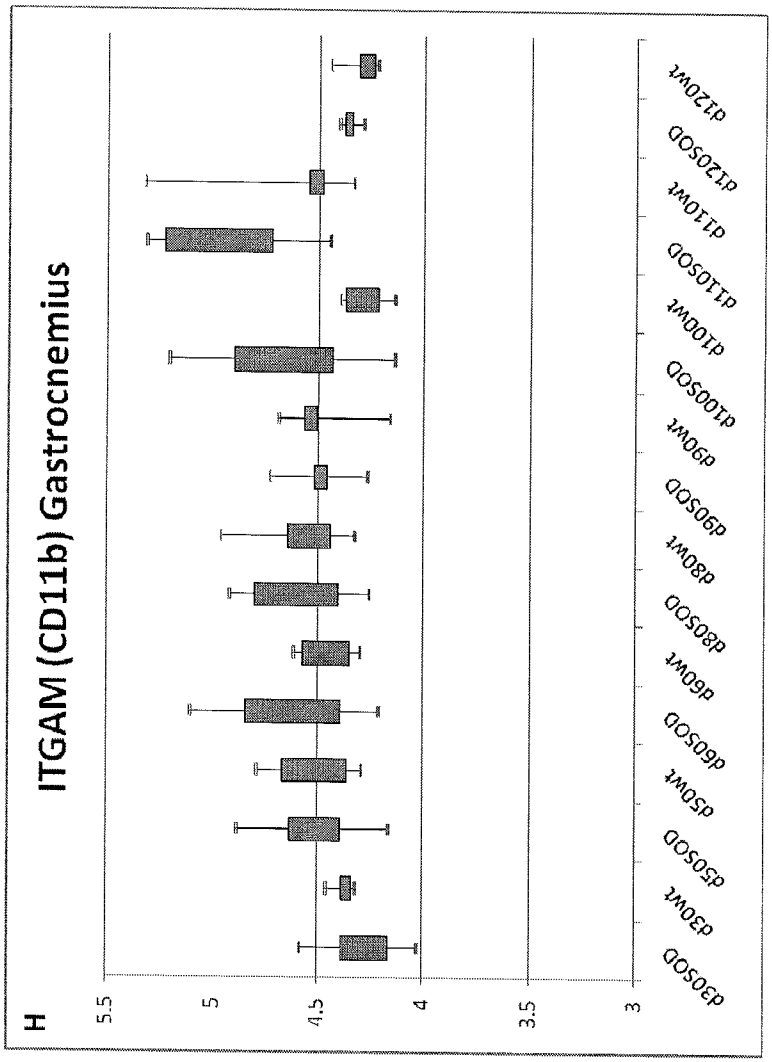

H
ITGAM (CD11b) Gastrocnemius
5.5
5
4.5
4
3.5
3
d30SOD
d30wt
d50SOD
d50wt
d60SOD
d60wt
d80SOD
d80wt
d90SOD
d90wt
d100SOD
d100wt
d110SOD
d110wt
d120SOD
d120wt

FIG. 1I

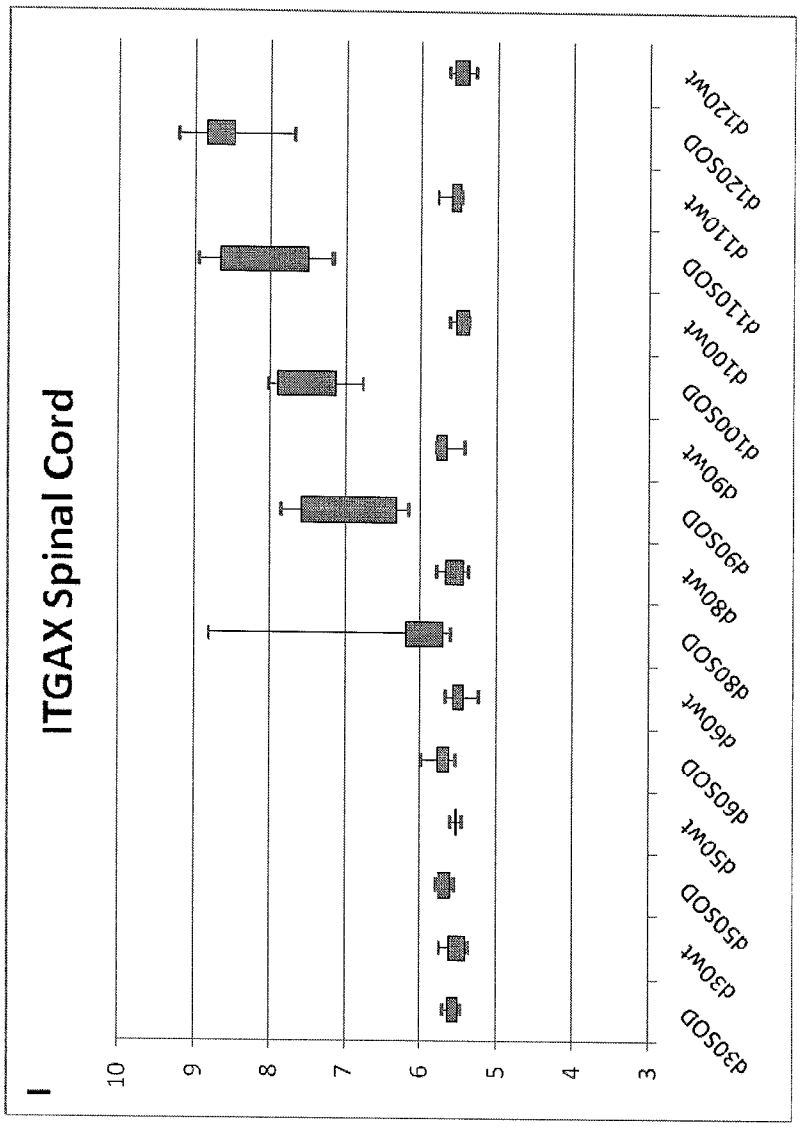

ITGAX Spinal Cord
I
10
9
8
7
6
5
4
3
d30SOD
d30wt
d50SOD
d50wt
d60SOD
d60wt
d80SOD
d80wt
d90SOD
d90wt
d100SOD
d100wt
d110SOD
d110wt
d120SOD
d120wt

FIG. 1J

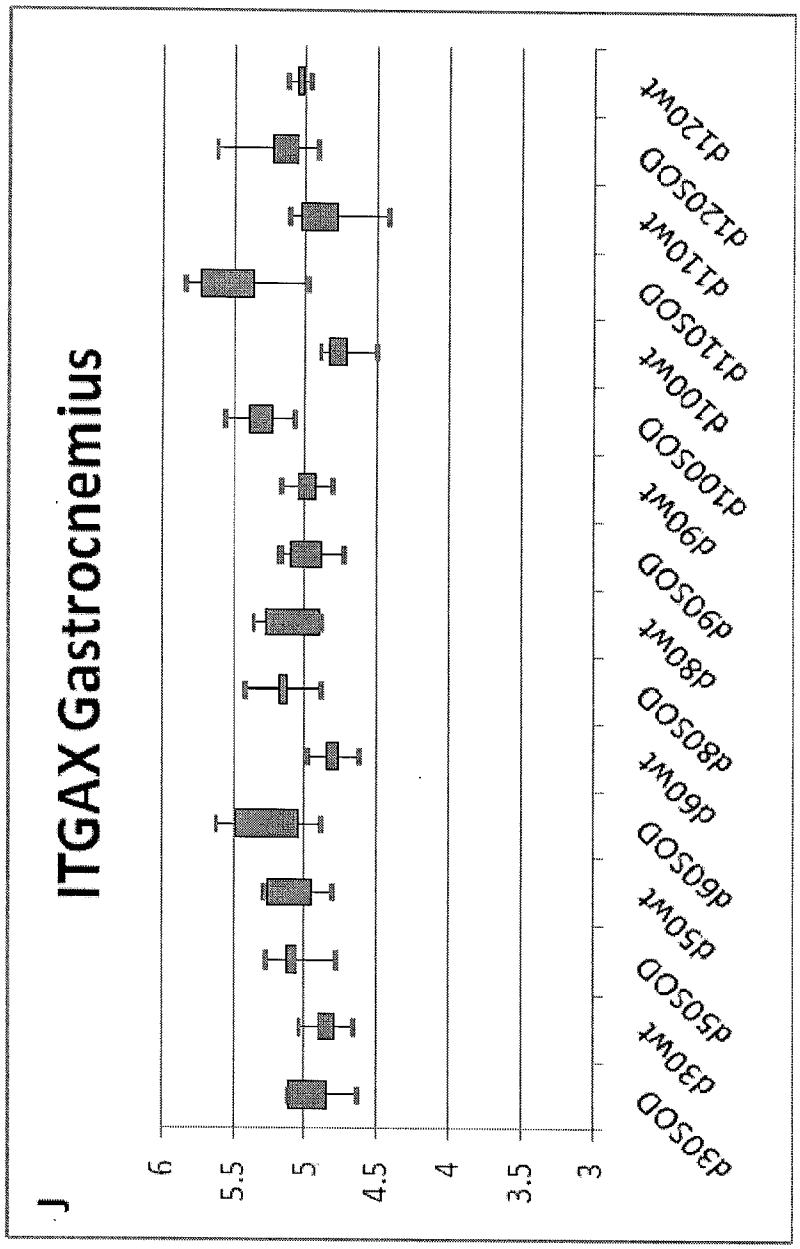

J
ITGAX Gastrocnemius
6
5.5
5
4.5
4
3.5
3
d30SOD
d30wt
d50SOD
d50wt
d60SOD
d60wt
d80SOD
d80wt
d90SOD
d90wt
d100SOD
d100wt
d110SOD
d110wt
d120SOD
d120wt

FIG. 1K

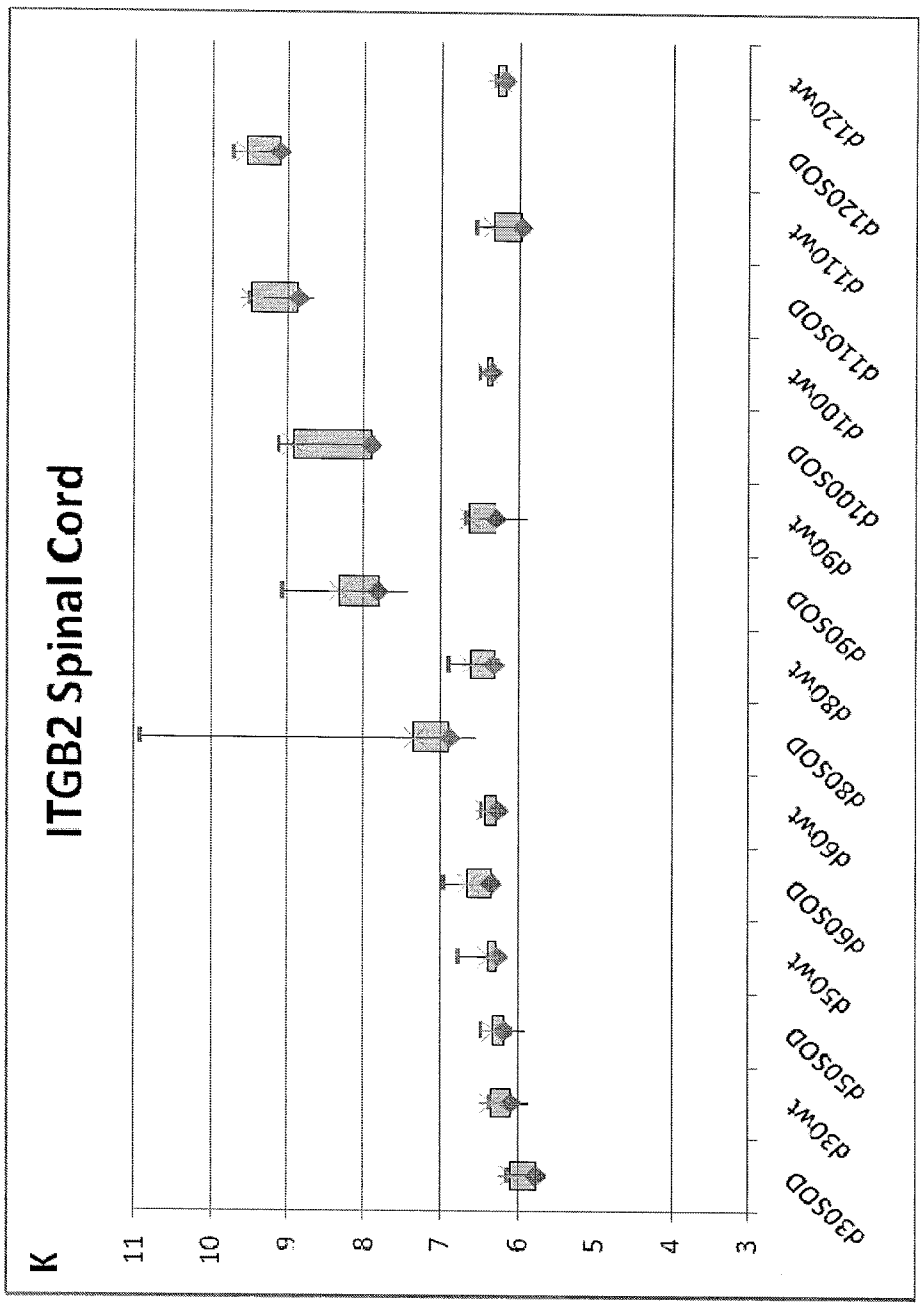

K
ITGB2 Spinal Cord
11
10
9
8
7
6
5
4
3
d30SOD
d30wt
d50SOD
d50wt
d60SOD
d60wt
d80SOD
d80wt
d90SOD
d90wt
d100SOD
d100wt
d110SOD
d110wt
d120SOD
d120wt

FIG. 1L

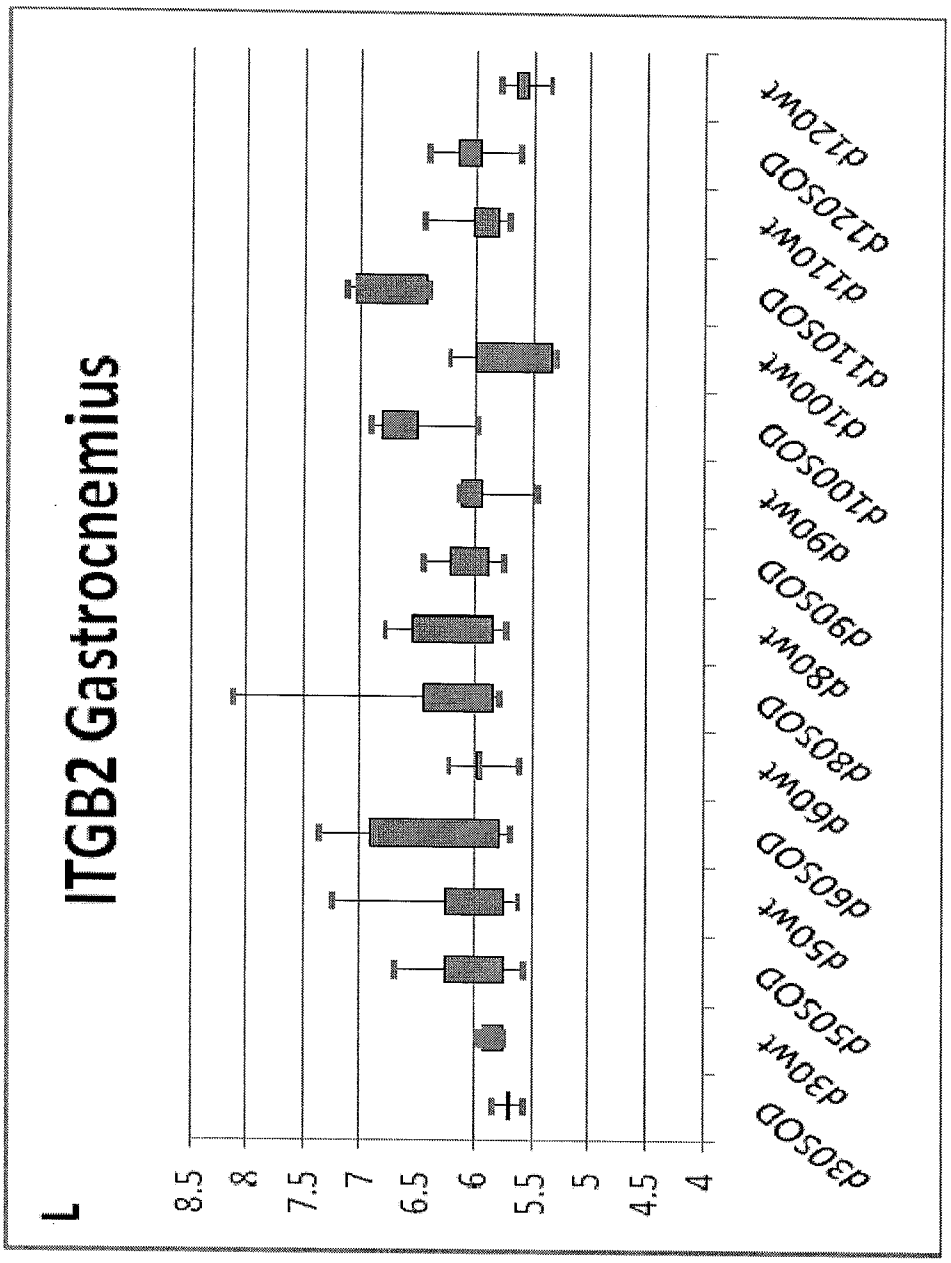

L
ITGB2 Gastrocnemius
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
d30SOD
d30wt
d50SOD
d50wt
d60SOD
d60wt
d80SOD
d80wt
d90SOD
d90wt
d100SOD
d100wt
d110SOD
d110wt
d120SOD
d120wt

FIG. 1M

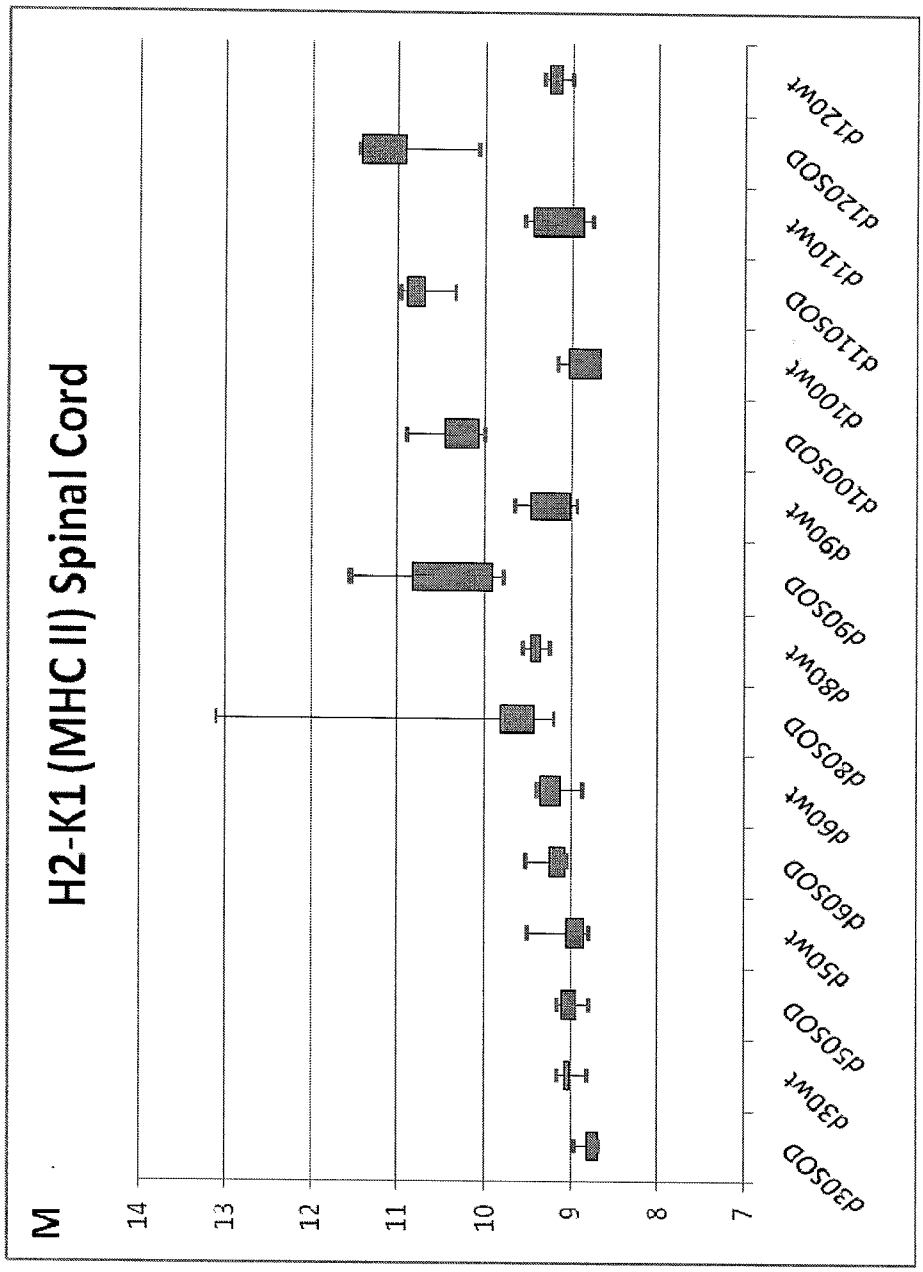

M
H2-K1 (MHC II) Spinal Cord
14
13
12
11
10
9
8
7
d120wt
d120SOD
d110wt
d110SOD
d100wt
d100SOD
d90wt
d90SOD
d80wt
d80SOD
d60wt
d60SOD
d50wt
d50SOD
d30wt
d30SOD

FIG. 1N

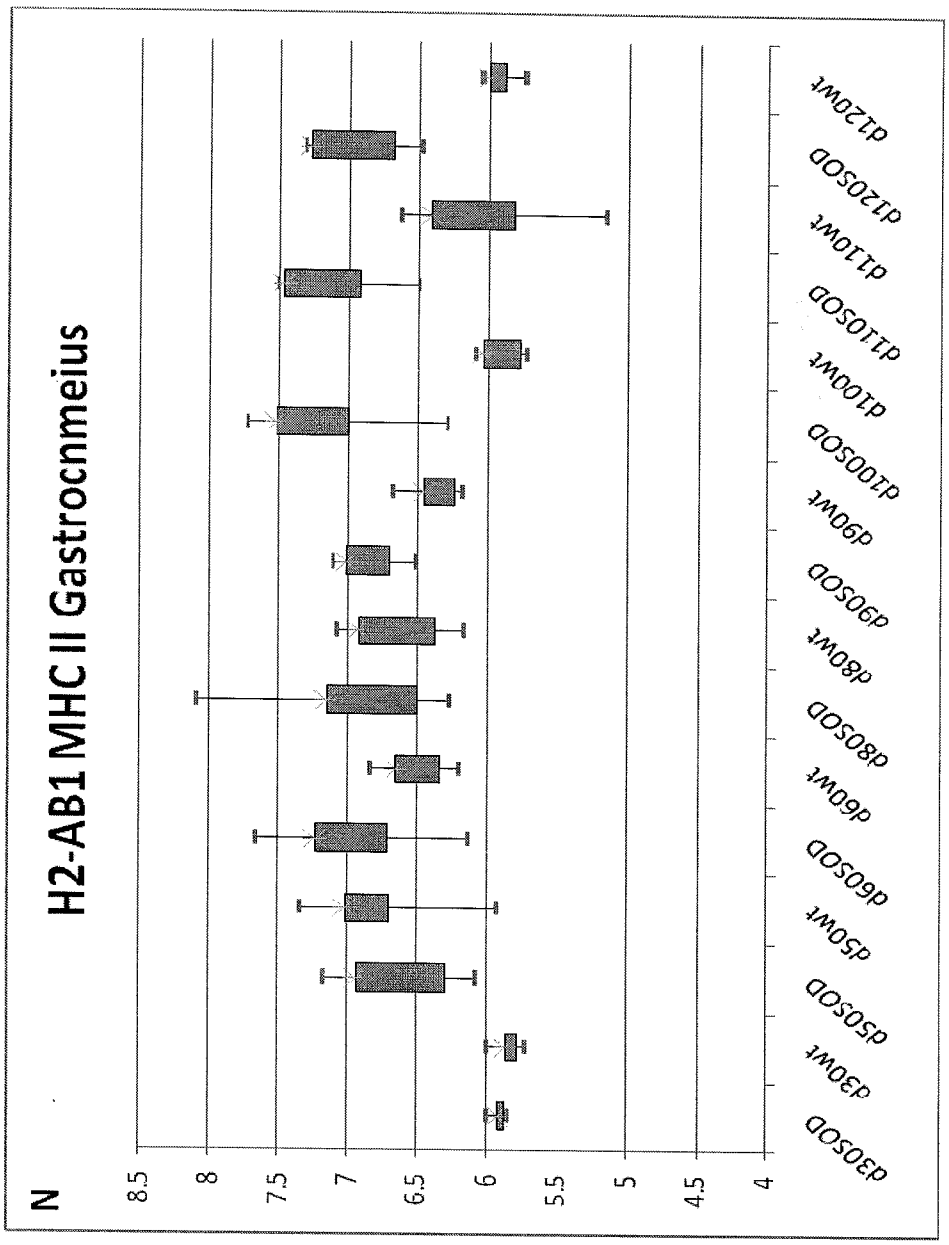

N
H2-AB1 MHC II Gastrocnmeius
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
d30SOD
d30wt
d50SOD
d50wt
d60SOD
d60wt
d80SOD
d80wt
d90SOD
d90wt
d100SOD
d100wt
d110SOD
d110wt
d120SOD
d120wt

FIG. 1O

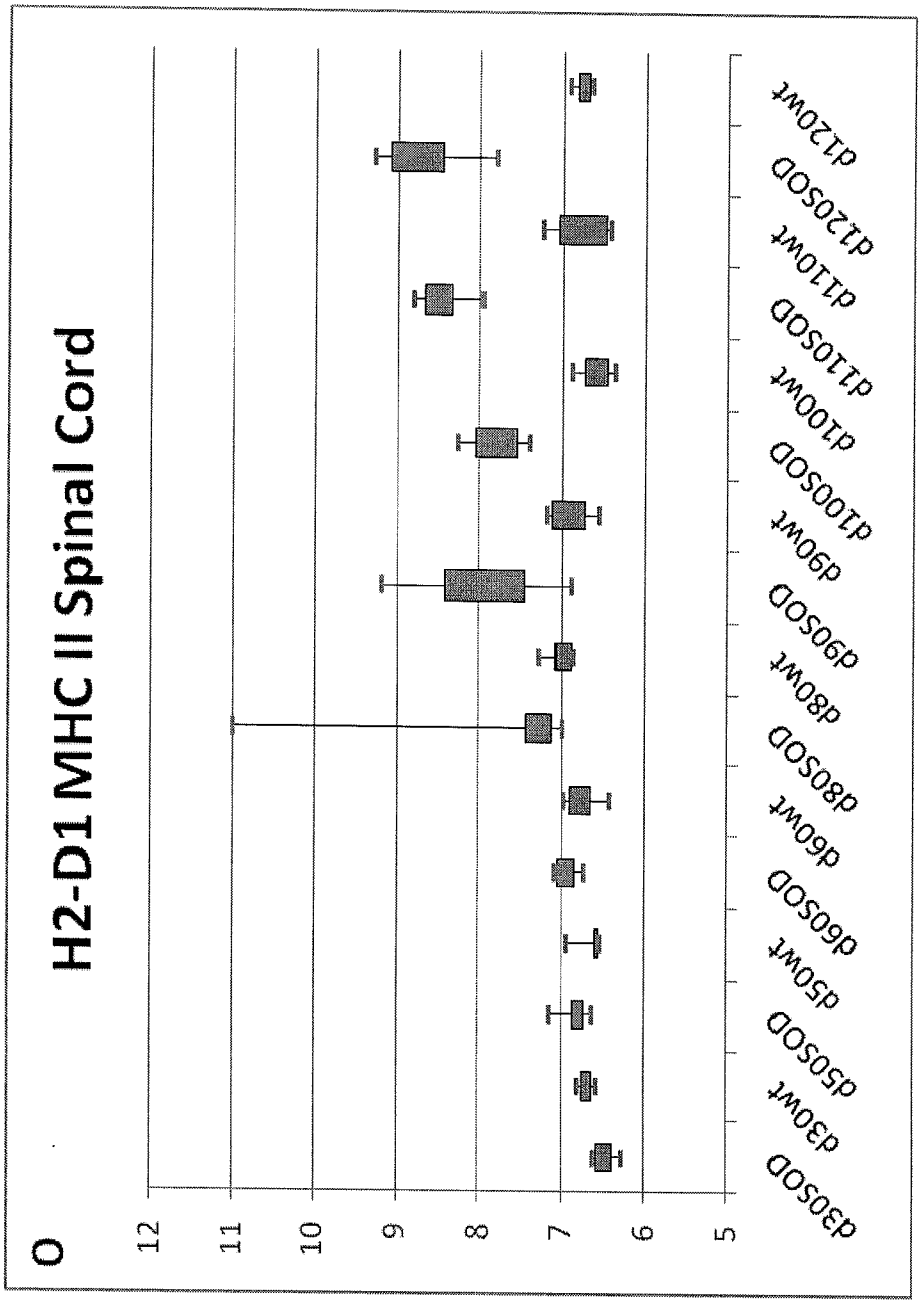

O
H2-D1 MHC II Spinal Cord
12
11
10
9
8
7
6
5
d30SOD
d30wt
d50SOD
d50wt
d60SOD
d60wt
d80SOD
d80wt
d90SOD
d90wt
d100SOD
d100wt
d110SOD
d110wt
d120SOD
d120wt

FIG. 1P

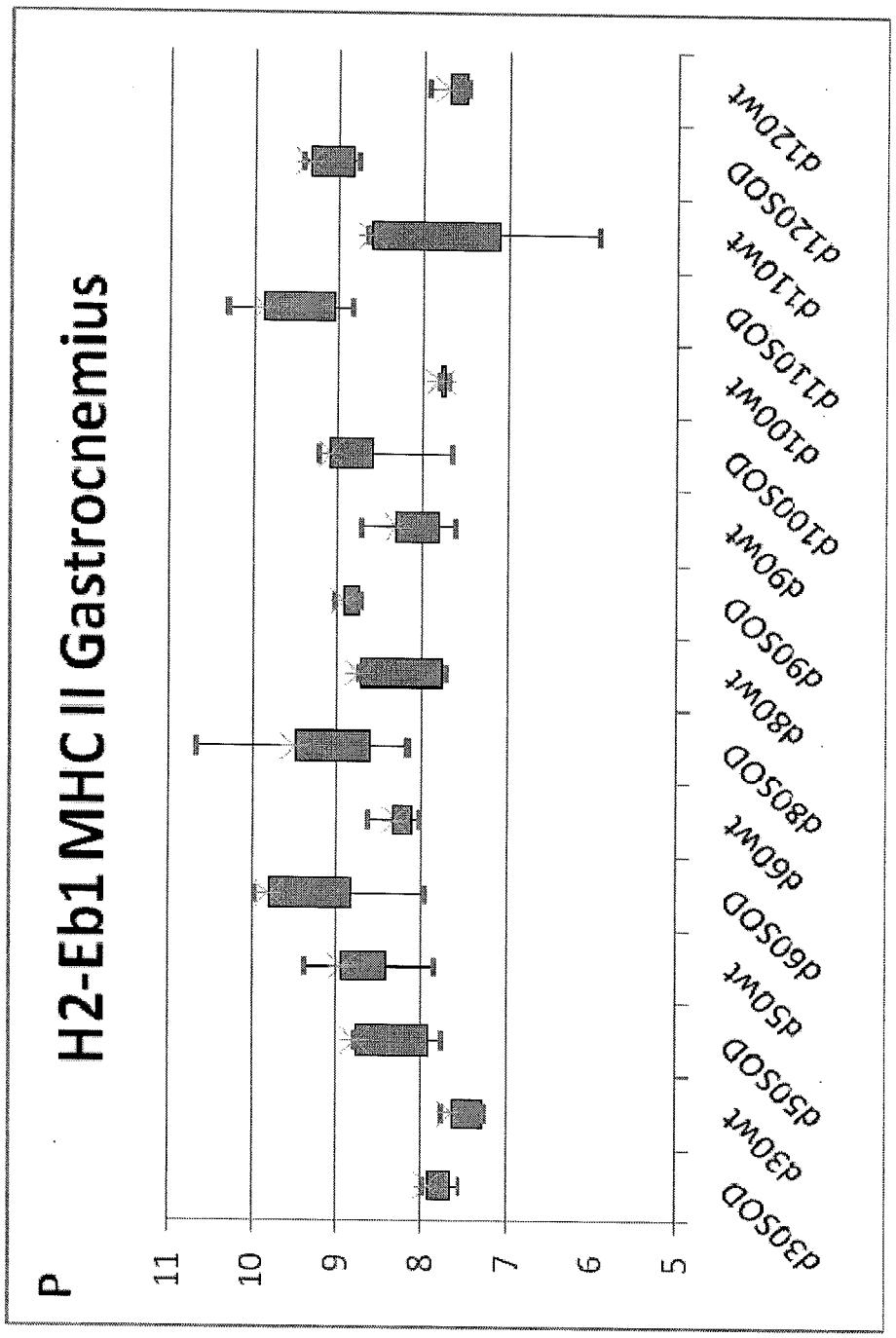

P
H2-Eb1 MHC II Gastrocnemius
11
10
9
8
7
6
5
d30SOD
d30wt
d50SOD
d50wt
d60SOD
d60wt
d80SOD
d80wt
d90SOD
d90wt
d100SOD
d100wt
d110SOD
d110wt
d120SOD
d120wt

FIG. 2

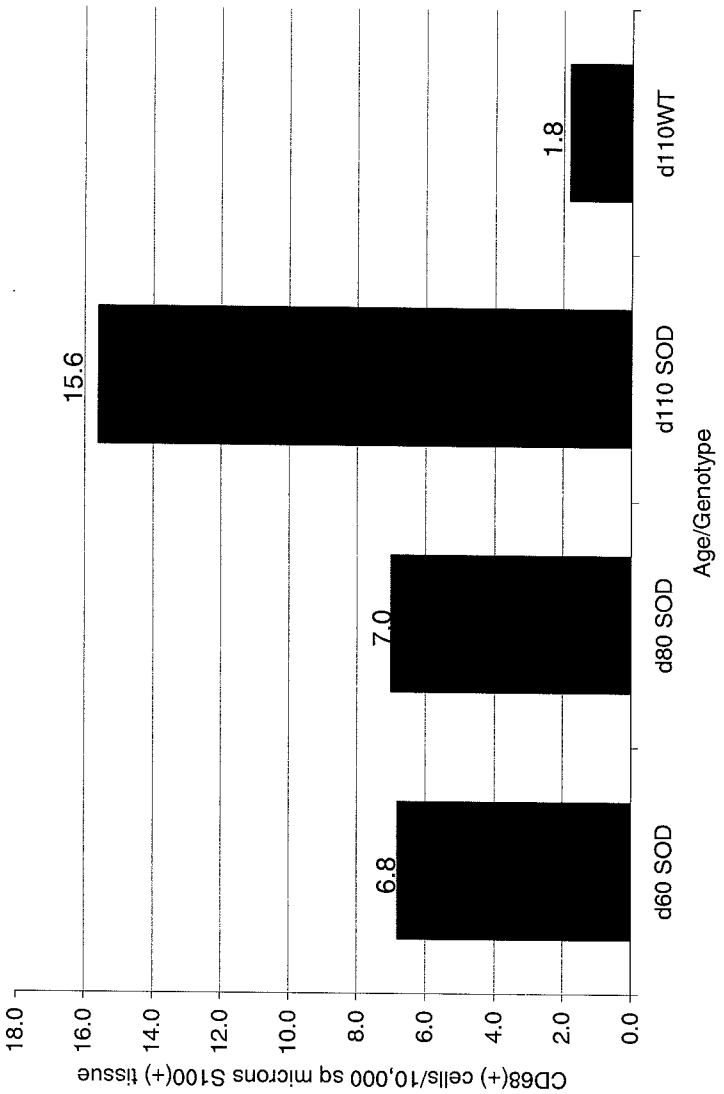
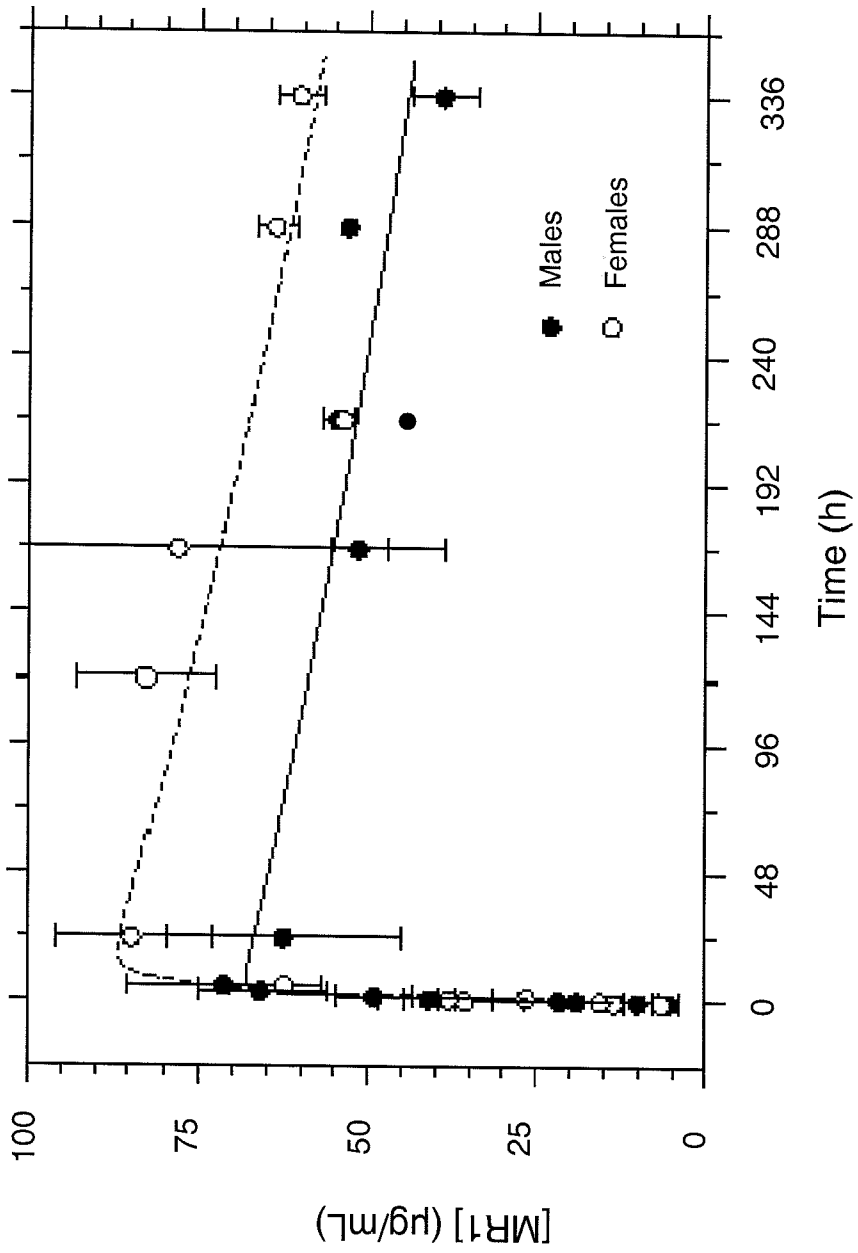

CD68(+) cells/10,000 sq microns S100(+) tissue
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
6.8
7.0
15.6
1.8
d60 SOD
d80 SOD
d110 SOD
d110WT
Age/Genotype

FIG. 3A


[MR1] (µg/mL)
100
75
50
25
0
0
48
96
144
192
240
288
336
Time (h)
Males
Females

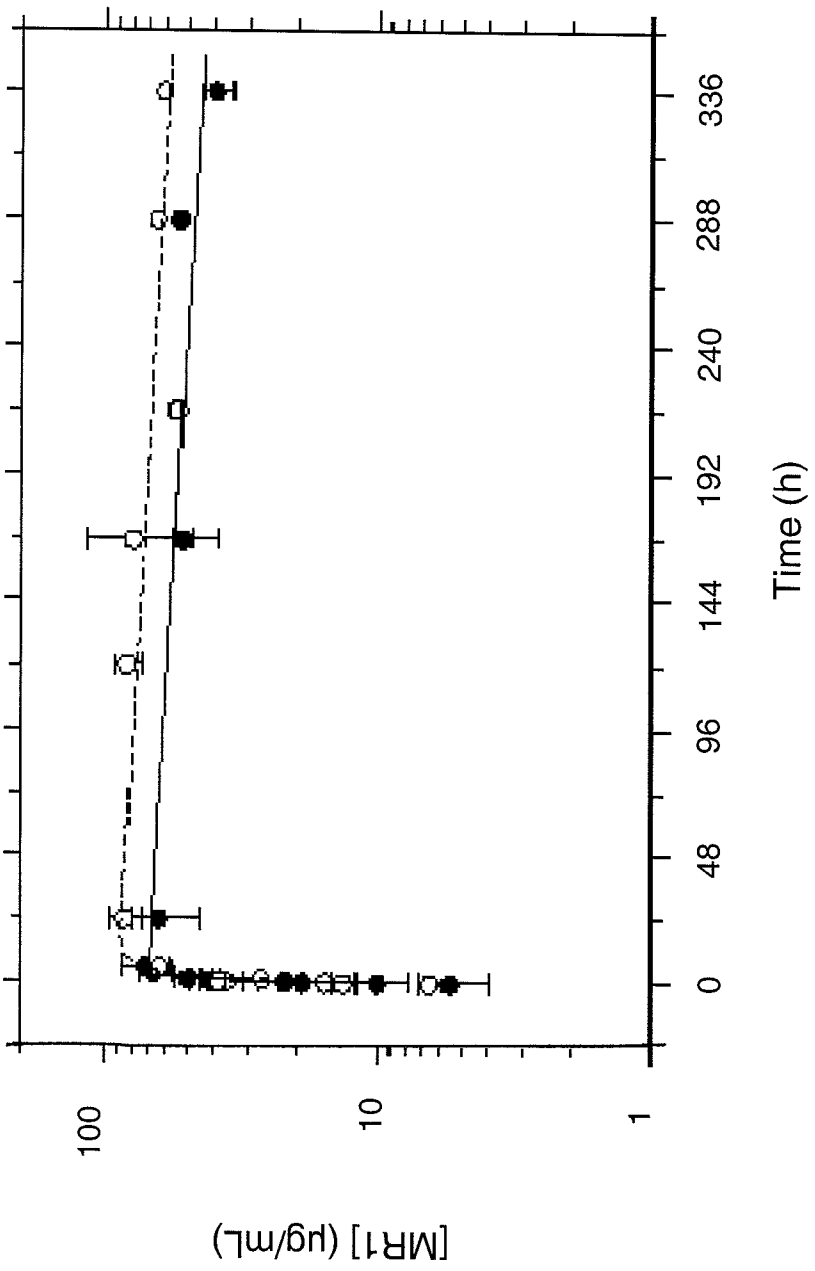


FIG. 3B

FIG. 4

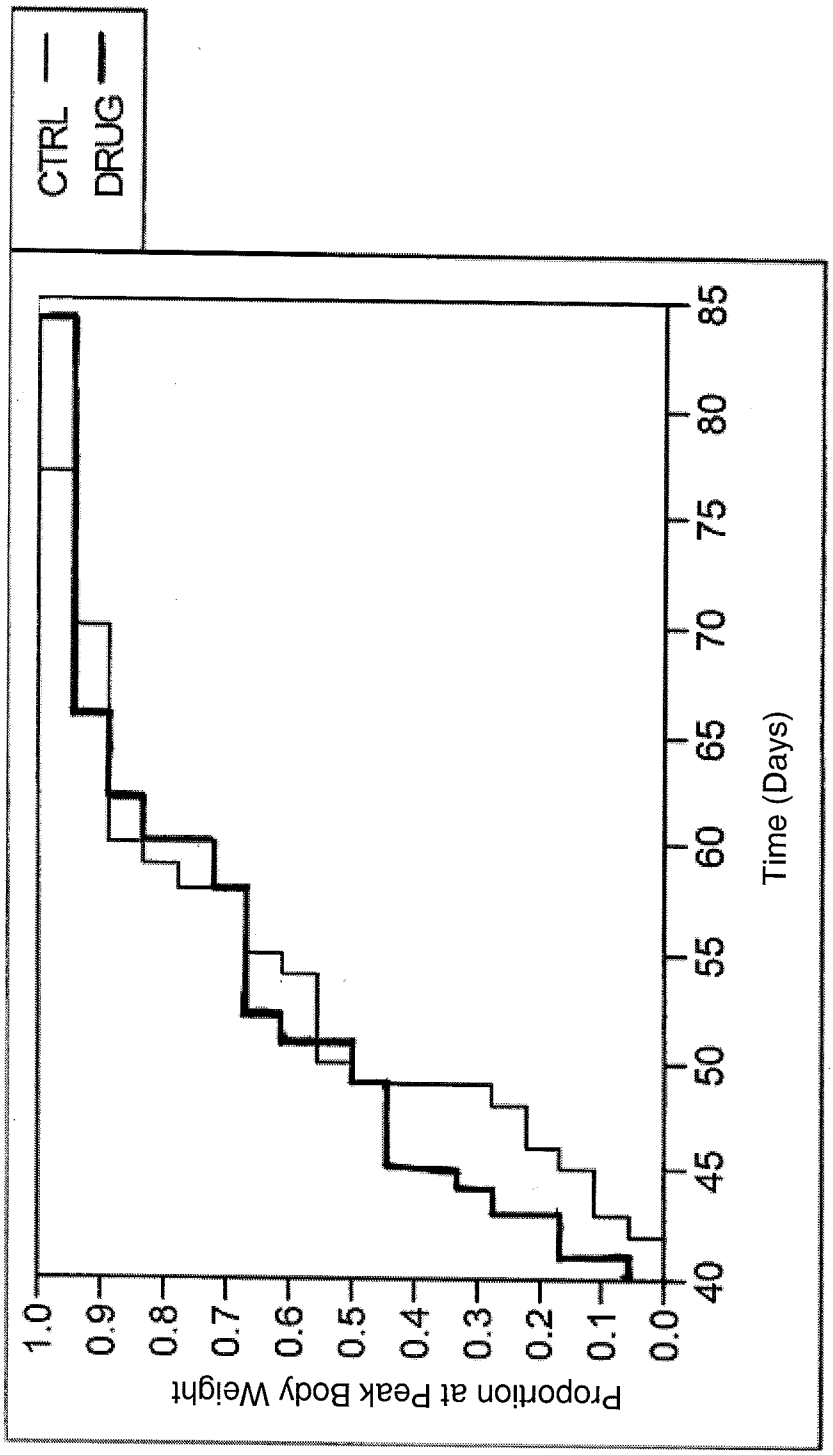

CTRL
DRUG
Proportion at Peak Body Weight
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
40
45
50
55
60
65
70
75
80
85
Time (Days)

FIG. 5

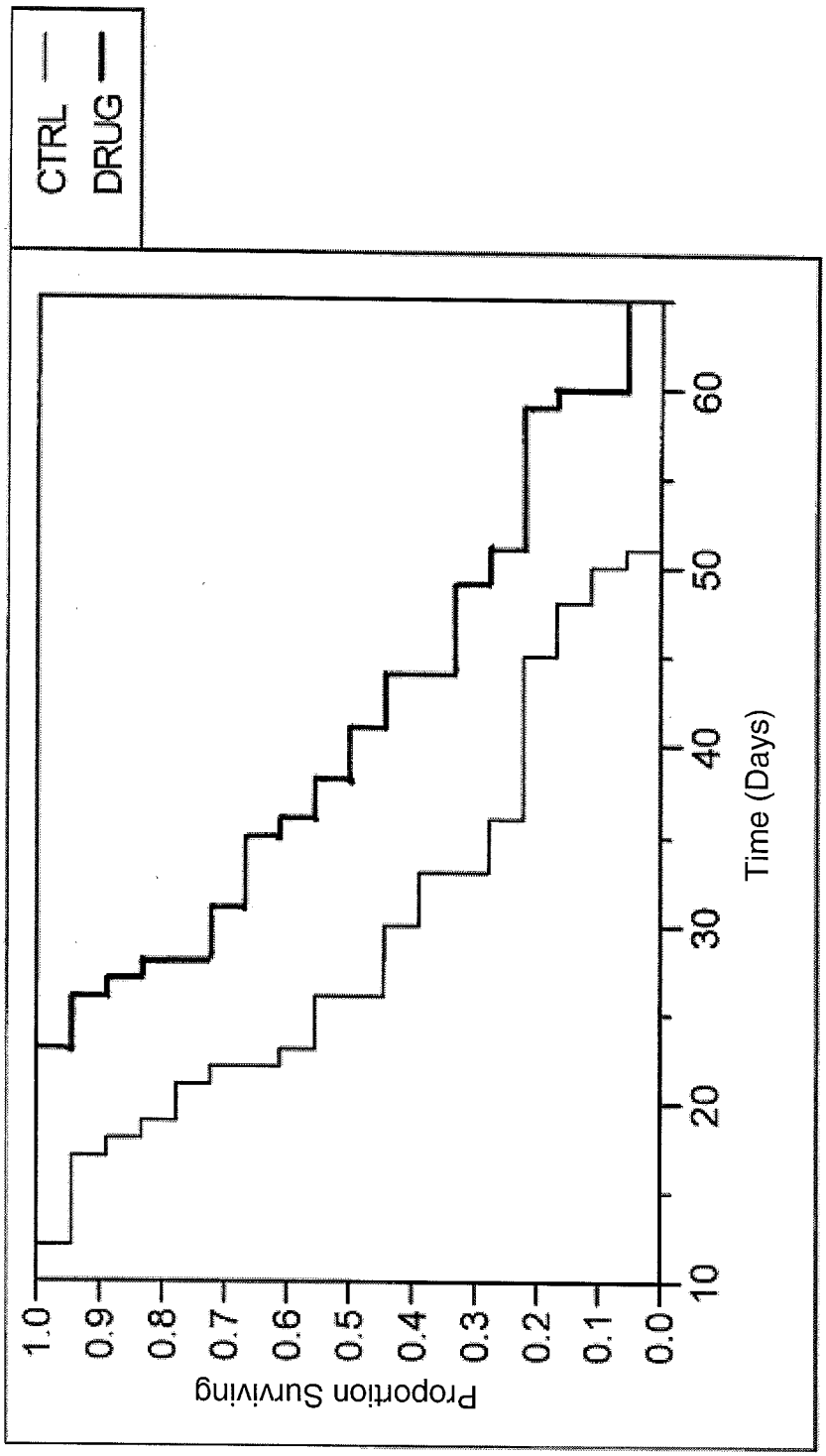

CTRL
DRUG
Proportion Surviving
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
10
20
30
40
50
60
Time (Days)

FIG. 6

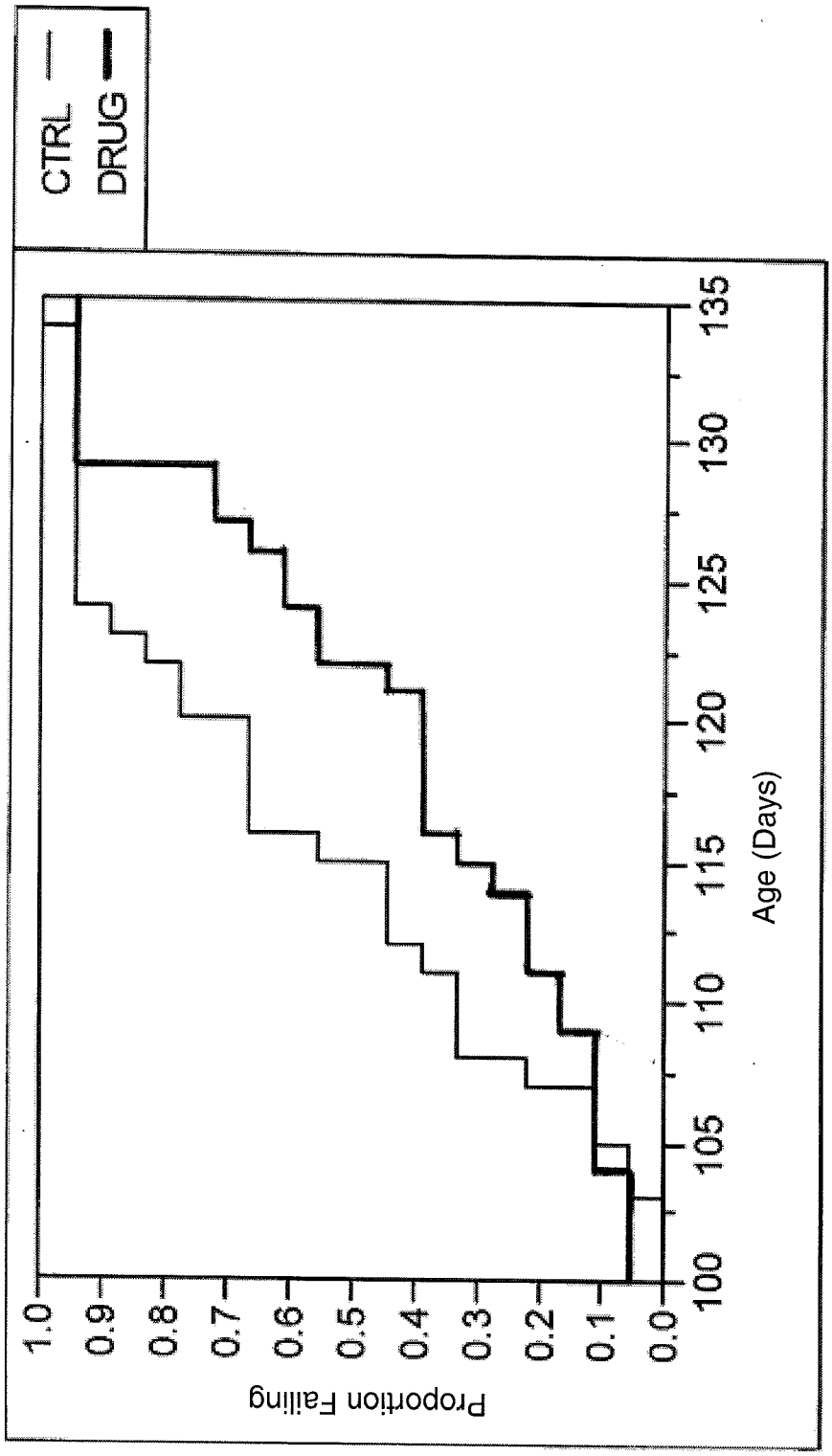

CTRL
DRUG
Proportion Failing
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
100
105
110
115
120
125
130
135
Age (Days)

FIG. 7

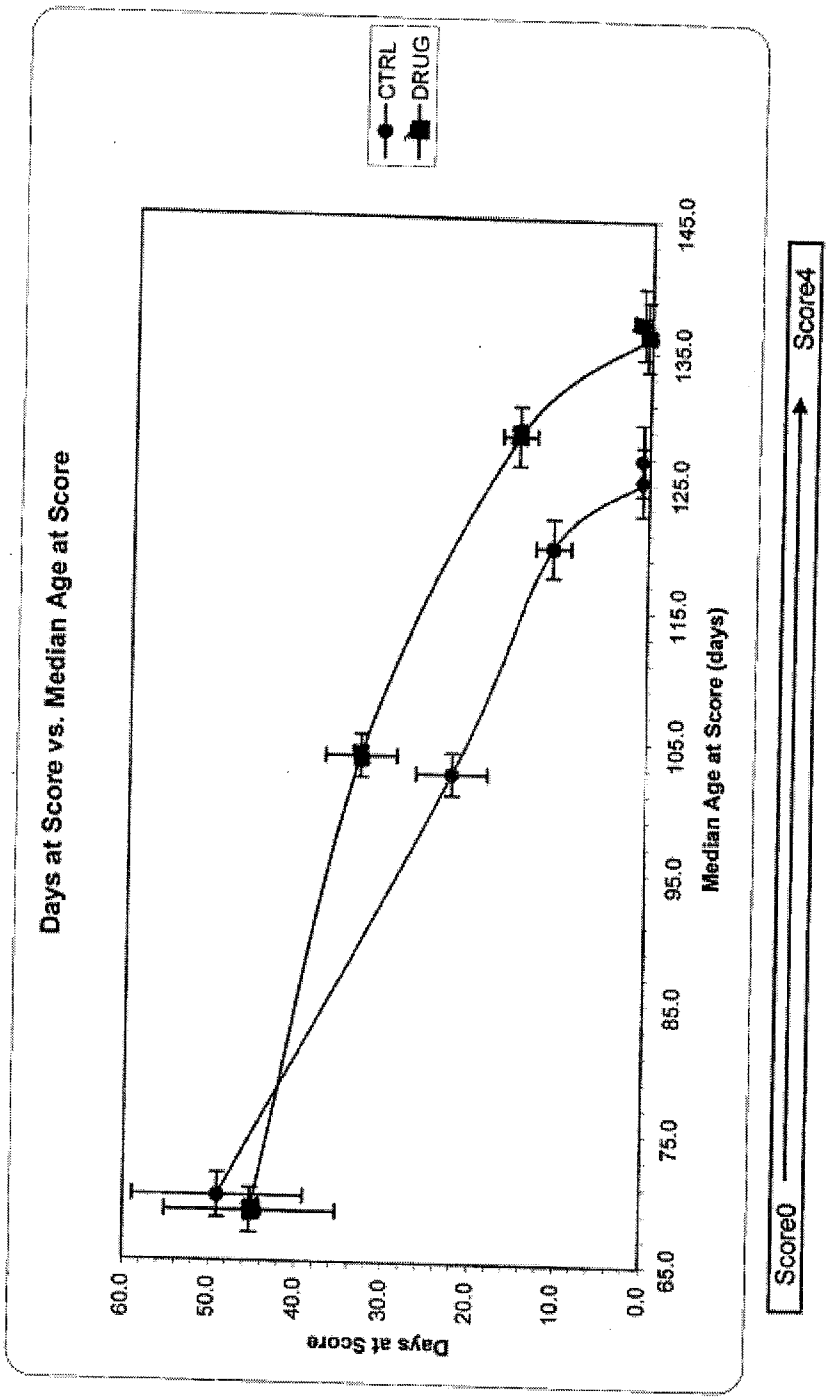

Days at Score vs. Median Age at Score
CTRL
DRUG
Days at Score
60.0
50.0
40.0
30.0
20.0
10.0
0.0
65.0
75.0
85.0
95.0
105.0
115.0
125.0
135.0
145.0
Median Age at Score (days)
Score0
Score4

FIG. 8

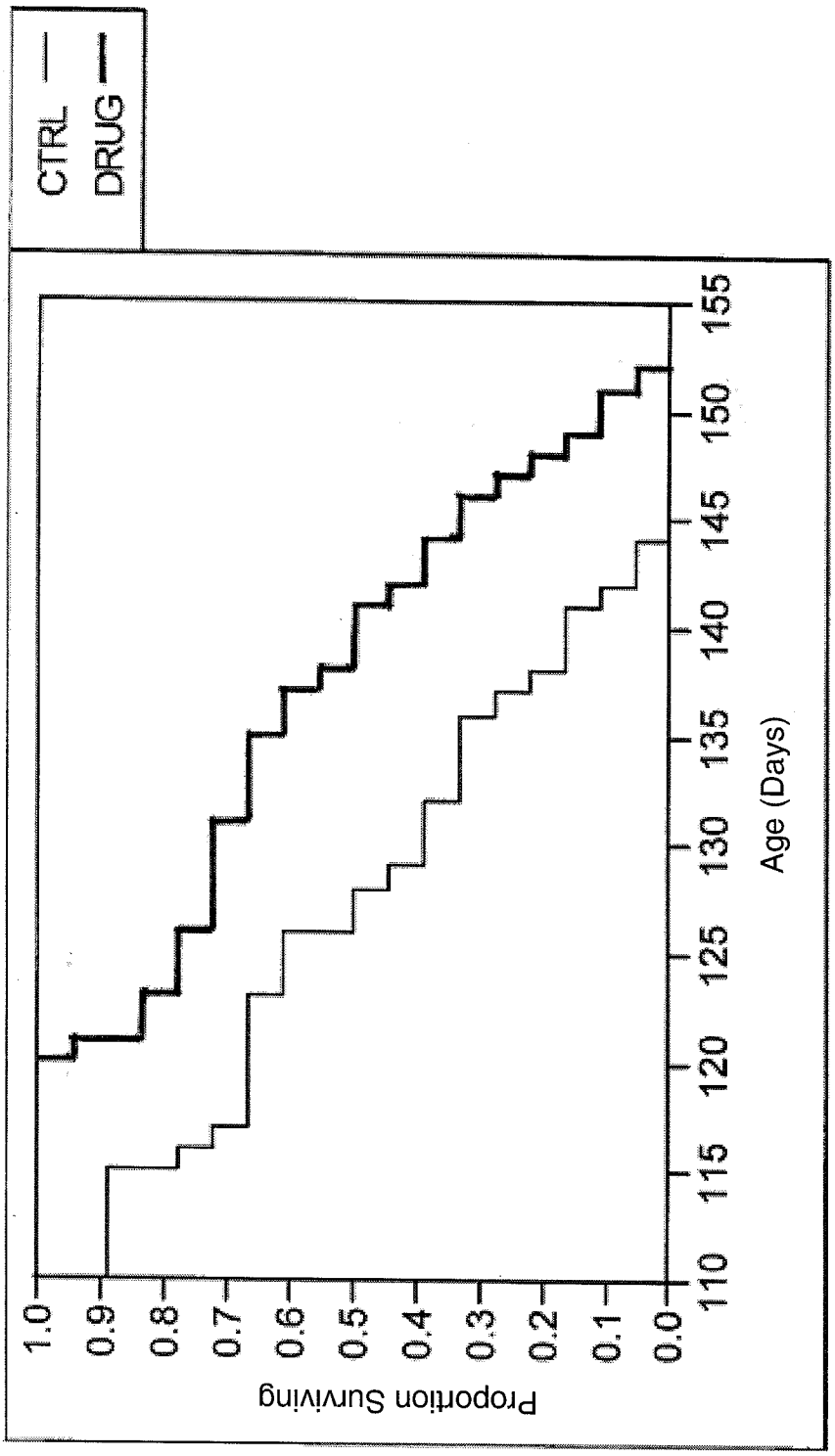

CTRL
DRUG
Proportion Surviving
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
110
115
120
125
130
135
140
145
150
155
Age (Days)

FIG. 9A

FIG. 9B

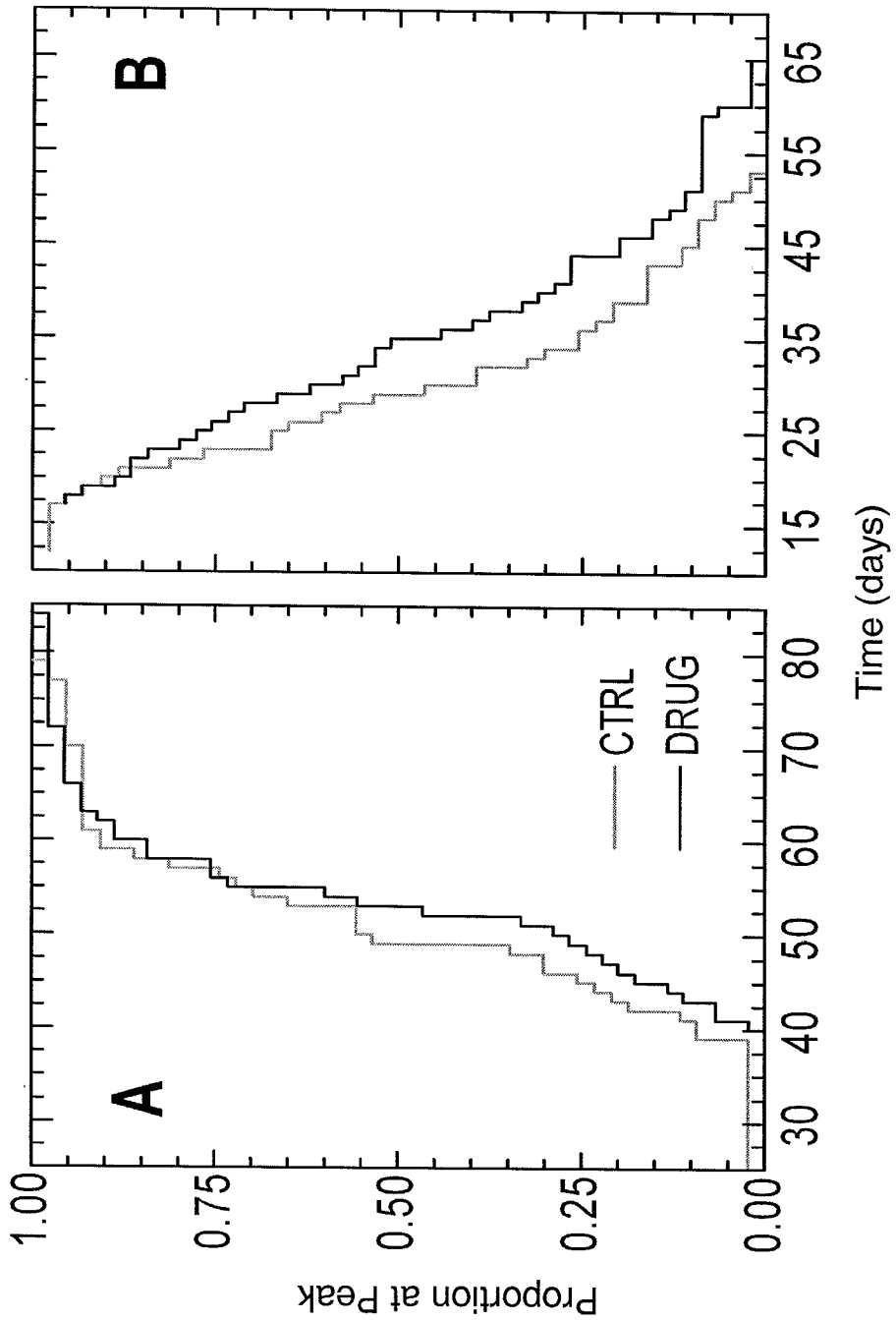
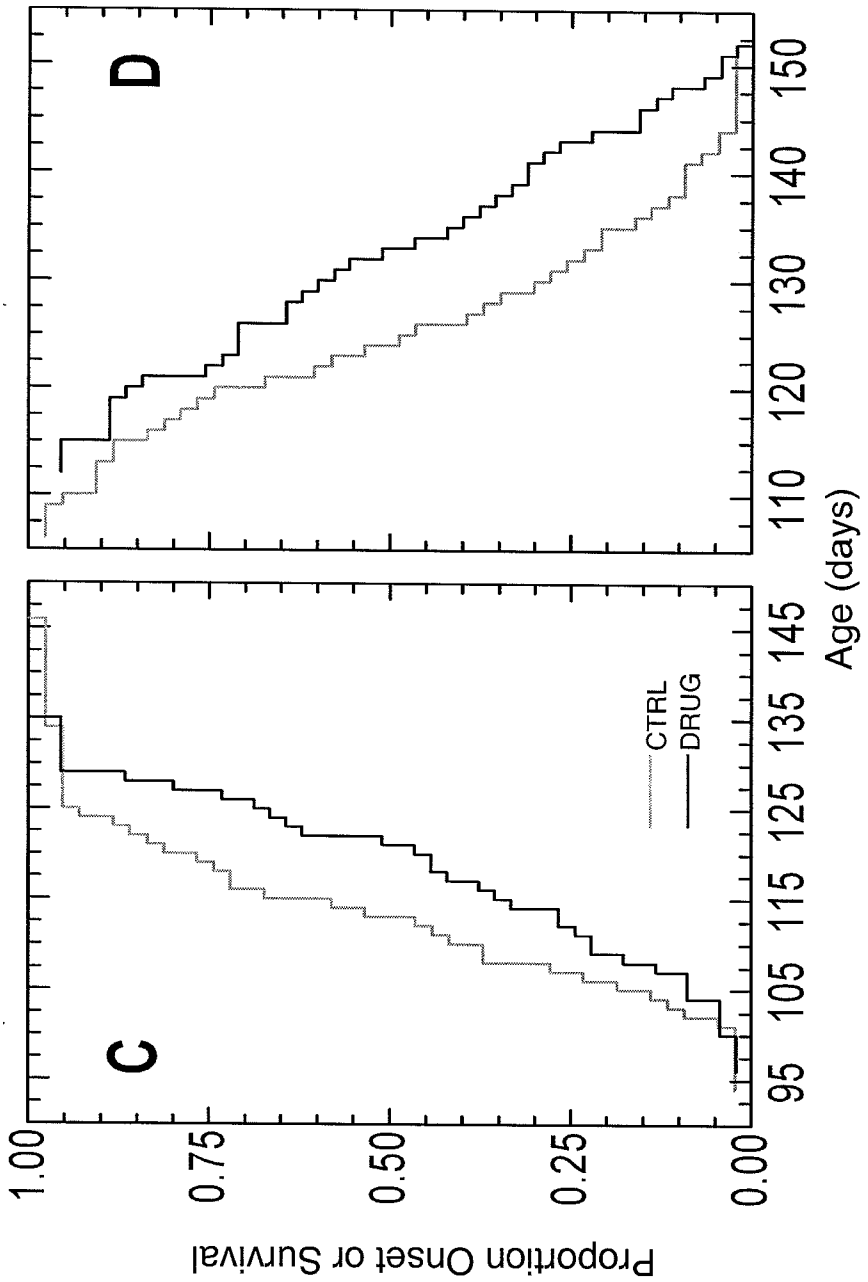

A
B
CTRL
DRUG
Proportion at Peak
1.00
0.75
0.50
0.25
0.00
30
40
50
60
70
80
15
25
35
45
55
65
Time (days)

FIG. 9C

FIG. 9D


C
D
CTRL
DRUG
Proportion Onset or Survival
1.00
0.75
0.50
0.25
0.00
95
105
115
125
135
145
110
120
130
140
150
Age (days)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2009/066715

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, EMBASE, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/138316 A2 (BIOGEN IDEC INC [US]; LINCECUM JOHN M [US]; PERRIN STEVEN N [US]) 28 December 2006 (2006-12-28)	1-14
Y	page 1, line 19 - page 2, line 8	15-24
X	WO 2004/037204 A2 (UNIV SOUTH FLORIDA [US]; TAN JUN [US]; TOWN TERRENCE C [US]; MULLAN MI) 6 May 2004 (2004-05-06)	1-14
Y	claims 54, 55, 63	15-24

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 March 2010	09/04/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Weikl, Martina

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KE Z J ET AL: "CD40-CD40L interactions promote neuronal death in a model of neurodegeneration due to mild impairment of oxidative metabolism" NEUROCHEMISTRY INTERNATIONAL, PERGAMON PRESS, OXFORD, GB, vol. 47, no. 3, 1 August 2005 (2005-08-01) , pages 204-215, XP004953076 ISSN: 0197-0186	1-14
Y	the whole document	15-24
X	KIAEI MAHMOUD ET AL: "Celastrol blocks neuronal cell death and extends life in transgenic mouse model of amyotrophic lateral sclerosis" NEURODEGENERATIVE DISEASES, KARGER AG, BASEL, CH, vol. 2, no. 5, 1 January 2005 (2005-01-01) , pages 246-254, XP009131255 ISSN: 1660-2854	1-4,6
Y	the whole document	15-17
Y	VIGLIETTA ET AL: "CTLA4Ig treatment in patients with multiple sclerosis" NEUROLOGY, vol. 71, no. 12, September 2008 (2008-09), pages 917-924, XP002574319 ISSN: 0028-3878 the whole document	15-24
A	WO 01/83755 A2 (GEMINI SCIENCE INC [US]; MIKAYAMA TOSHIFUMI [US]; TAKAHASHI NOBUAKI [J) 8 November 2001 (2001-11-08) claims 1,10	1-24

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2009/066715

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2006138316 A2	28-12-2006	NONE	
WO 2004037204 A2	06-05-2004	AU 2003284968 A1	13-05-2004
		US 2004146949 A1	29-07-2004
WO 0183755 A2	08-11-2001	AU 5921501 A	12-11-2001
		TW 264467 B	21-10-2006