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(71) Applicant: THE REGENTS OF THE UNIVERSITY
OF CALIFORNIA [US/US]; 1111 Franklin Street, 12th
Floor, Oakland, CA 94607-5200 (US).

(72) Inventor: VOSKUHL, Rhonda, R.; 859 Warner Avenue,
Los Angeles, CA 90024 (US).

(74) Agents: HALSTEAD, David, P. et al.; Foley Hoag LLP,
Seaport West, 155 Seaport Blvd., Boston, MA 02210-2600
(US).

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(54) Title: METHODS OF PROVIDING NEUROPROTECTIVE THERAPY RELATED TO ADMINISTERING AN ESTROGEN
RECEPTOR BETA LIGAND

(57) Abstract: Provided are methods for treating a neurodegenerative disease, such as multiple sclerosis, in a subject who does not
present with active lesions (e.g., gadolinium-enhancing lesions), comprising administering an estrogen receptor β ligand to the sub-
ject without conjointly administering a second immunotherapeutic agent. For example, any immunotherapeutic agent that the subject
is receiving may be discontinued, e.g., upon determining that the brain of the subject does not present with active lesions. The meth-
od may include determining whether the brain of the subject presents with active lesions, e.g., by gadolinium-enhanced MRI.



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Methods of Providing Neuroprotective Therapy Related to Administering an Estrogen Receptor β Ligand

RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application No. 62/207,163, filed August 19, 2015, which is hereby incorporated by reference in its entirety.

BACKGROUND

Multiple sclerosis (MS) is a chronic, often debilitating disease affecting the central nervous system (brain and spinal cord). MS affects more than 1 million people worldwide and is the most common neurological disease among young adults, particularly women. The exact cause of MS is still unknown. MS is an autoimmune disease in which myelin sheaths surrounding neuronal axons are destroyed. This condition can cause weakness, impaired vision, loss of balance, and poor muscle coordination.

MS takes several forms, with new symptoms either occurring in isolated attacks (relapsing forms) or building up over time (progressive forms). Between attacks, symptoms may disappear completely; however, permanent neurological problems often occur, especially as the disease advances.

In 1996, the United States National Multiple Sclerosis Society described four clinical subtypes of MS: (i) relapsing-remitting; (ii) secondary-progressive; (iii) primary-progressive; and (iv) progressive-relapsing.

Relapsing-remitting MS is characterized by unpredictable relapses followed by periods of months to years of relative quiet (remission) with no new signs of disease activity. Deficits that occur during attacks may either resolve or leave sequelae, the latter in about 40% of attacks and being more common the longer a person has had the disease. This describes the initial course of 80% of individuals with MS. When deficits always resolve between attacks, this is sometimes referred to as benign MS, although people will still build up some degree of disability in the long term. On the other hand, the term malignant multiple sclerosis is used to describe people with MS having reached a significant level of disability in a short period of time. The relapsing-remitting subtype usually begins with a clinically isolated syndrome (CIS). In CIS, a person has an attack suggestive of demyelination but does not fulfill the criteria for multiple sclerosis; 30 to 70% of persons experiencing CIS go on to develop MS.

Secondary-progressive MS occurs in around 65% of those with initial relapsing-remitting MS, who eventually have progressive neurologic decline between acute attacks without any definite periods of remission. Occasional relapses and minor remissions may appear. The median length of time between disease onset and conversion from relapsing-remitting to secondary progressive MS is 19 years.

Primary-progressive MS occurs in approximately 10–20% of individuals, with no remission after the initial symptoms. It is characterized by progression of disability from onset, with no, or only occasional and minor, remissions and improvements. The usual age of onset for the primary progressive subtype is later than of the relapsing-remitting subtype, but similar to the age that secondary-progressive MS usually begins in relapsing-remitting MS, around 40 years of age.

Progressive-relapsing MS describes those individuals who, from onset, have a steady neurologic decline but also have clear superimposed attacks. This is the least common of all subtypes.

The following agents are approved by the U.S. Food and Drug Administration (FDA) to reduce disease activity and disease progression for many people with relapsing forms of MS, including relapsing-remitting MS, as well as secondary-progressive and progressive-relapsing MS in those people who continue to have relapses: dimethyl fumarate (Tecfidera®), fingolimod (Gilenya®), glatiramer acetate (Copaxone®), interferon beta-1a (Avonex® and Rebif®), interferon beta-1b (Betaseron® and Extavia®), peginterferon beta-1a (Plegridy®), mitoxantrone (Novantrone®), natalizumab (Tysabri®), alemtuzumab (Lemtrada®), and teriflunomide (Aubagio®). However, many of these therapies fail to successfully treat all patients or all symptoms in treated patients, and many of these therapies are associated with undesirable side effects. Accordingly, alternative therapies are needed.

SUMMARY

In some aspects, the invention relates to a method for treating multiple sclerosis in a subject who does not present with active lesions (*e.g.*, gadolinium-enhancing lesions), comprising administering an estrogen receptor β ligand (“ER β ligand”) to the subject without conjointly administering a second immunotherapeutic agent for treating multiple sclerosis.

In some aspects, the invention relates to a method for treating multiple sclerosis in a subject receiving treatment with an immunotherapeutic agent who does not present with active

lesions (*e.g.*, gadolinium-enhancing lesions), comprising administering an ER β ligand to the subject and discontinuing treatment with the immunotherapeutic agent.

In some aspects, the invention relates to a method for treating multiple sclerosis in a subject, comprising determining whether the brain of the subject presents with active lesions (*e.g.*, gadolinium-enhancing lesions), administering an ER β ligand to the subject, and discontinuing any second immunotherapeutic agent that the subject is receiving if the subject lacks active lesions.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Study overview. Figure 1 includes two panels, identified as panels (A) and (B). Panel A shows the disposition of subjects enrolled in a clinical trial of estriol for treating multiple sclerosis. Panel B shows the study design. “Taper” indicates a period of reduction of either estriol or placebo over the course of 4 weeks at end of study, after month 24 clinic visit. Specifically, the dose of estriol was reduced by half (from 8 mg to 4 mg) for 2 weeks, then reduced by half again (from 4 mg to 2 mg) for 2 weeks, then discontinued. “x” indicates the administration of a progestin (0.7 mg norethindrone) orally each day for 2 weeks every three months, beginning at study month 6. “o” indicates the administration of a placebo for the progestin orally each day for 2 weeks every three months, beginning at study month 6.

Figure 2. Estriol levels and relapsing disease activity in Estriol + glatiramer acetate (GA) as compared to Placebo + GA treatment groups. Figure 2 includes three panels, identified as panels (A), (B), and (C). Panel (A) shows that serum estriol concentrations are significantly increased at each time point after baseline (month 0) in the Estriol + GA group (x), while remaining below the assay detection limit in the Placebo + GA group (—). However, in the Estriol + GA group, estriol levels decreased by one-third at month 24 compared to month 3 (month 3 vs month 24, $p = 0.003$; month 3 vs month 18, $p = 0.065$). Estriol levels are expressed as mean $\pm$ SE in ng/mL. Panel (B) shows the annualized confirmed relapse rates at months 0-12 and at months 0-24. Relapse rates decreased by 47% ($p = 0.021$) in the Estriol + GA group compared to the Placebo + GA group at month 12 and decreased by 32% ($p = 0.098$) at month 24. Panel (C) shows the proportion of subjects with confirmed relapses over 24 months, the between groups trend favored Estriol + GA ($p = 0.096$).

Figure 3. Disabilities and Brain Volumes. Figure 3 includes nine panels, identified as panels (A), (B), (C), (D), (E), (F), (G), (H), and (I). Panel (A) shows that EDSS improvement

was observed at 24 months in the Estriol + GA within group comparison (median = -0.5, $P = 0.03$), with no change in the Placebo + GA group (median = 0, $P = \text{NS}$), and between groups comparison not reaching significance. (B) MFIS score (Fatigue) improvement was observed at 24 months in the Estriol + GA within group comparison (median = -10.0, $P = 0.014$), with no change in the Placebo + GA group (median = 0, $p = \text{NS}$), and between groups comparison significant ($P = 0.03$). (C) PASAT score (Cognition) improvement was observed at 12 months in the Estriol + GA within group comparison ($P = 0.005$), with no change in the Placebo + GA group, and between group comparison significant ($P = 0.04$), however scores assessed at the 24 month time point were no different between groups. All data are expressed as change in mean absolute scores over time as compared to baseline. (D-G) Change in volume from baseline for cortical gray matter in D; for whole gray matter in E; for whole white matter in F; and for whole brain in G. (H-I) Change in cortical gray matter (CGM), whole gray matter (GM) and whole white matter (WM) in subjects that were enhancing lesion positive in H, or enhancing lesion negative in I. Lower right: Significant voxel-wise gray matter loss from baseline to month 12 was more in Placebo + GA (top left subpanel) than in Estriol + GA (top right panel), with regions showing significant between group differences demonstrated by intensity heat map (bottom panel). Disabilities are expressed as means $\pm$ SE. Negative values indicate improvement for EDSS and MFIS scores. Positive values indicate improvement for PASAT scores. EDSS = Expanded Disability Status Scale; MFIS = Modified Fatigue Impact Scale; PASAT = Paced Auditory Serial Addition Test (at 3 seconds). Volumes are expressed as mean percent change $\pm$ SE from baseline. * = $P < 0.10$, ** = $P < 0.05$. VBM results are visualized on the mean template and thresholded at $P \leq 0.05$, FDR corrected. Black indicates Placebo + GA, while Gray indicates Estriol + GA.

Figure 4. Trends for MS Quality of Life and Depression. Figure 4 includes three panels, identified as panels (A), (B), and (C). Panel (A) shows MSQOL composite scores for Physical outcomes were improved in the Estriol + GA group ($p = 0.02$), with no change in the Placebo + GA group, between group comparisons not reaching significance. Panel (B) shows MSQOL composite scores for Mental outcomes had trends similar to MSQOL Physical. Panel (C) shows Beck Depression Inventory (BDI) score improvement was observed at 24 months in the Estriol + GA group (median = -4.0, $p = 0.03$), with no significant change in the Placebo + GA group (median = -3.5, $p = \text{NS}$), between groups not significant. All data are expressed as

change in mean absolute scores over time as compared to baseline. Positive values indicate improvement for MSQOL Physical and Mental scores (panels (A) and (B)), while negative values indicate improvement for Depression scores (panel (C)).

Figure 5. Change in PASAT: Subgroups by Baseline Performance. Figure 5 shows the percent change in PASAT scores at 12 months from baseline for all subjects (All, left bars), those with disability scores of less than 55 at baseline (<55, middle bars), and those with scores from 55 to the maximum of 60 at baseline (>55, right bars). A perfect PASAT score is 60, and scores lower than 55 depict disability. The estriol group displayed a significant benefit as assessed by PASAT scores, and the subgroup of estriol subjects with PASAT scores less than 55 at baseline displayed a significant benefit. The data is expressed as mean % change $\pm$ SE.

** indicates $P = 0.054$, * indicates $P = 0.011$.

Figure 6. Voxel-wise Gray Matter Atrophy. Figure 6 shows maximum intensity projections of voxel-wise gray matter atrophy superimposed onto 3 orthogonal planes through the brain. At 12 months, significant localized gray matter loss was observed in the Placebo + GA group as compared to baseline (top) and in the Estriol + GA group as compared to baseline (middle), each shown in gray against a black background in the 3 planes. Regions of significantly more gray matter loss in the Placebo + GA group as compared to the Estriol + GA group on between group comparisons are shown in white in the 3 planes (bottom). Gray matter loss is also visualized as projected onto a surface rendering of the mean template (lower right corner of each panel). All results are corrected for multiple comparisons by controlling the FDR at $P \leq 0.05$.

Figure 7. EAE scores versus time for all treatment groups in female C57BL/6 mice.

Figure 8. EAE scores separated by different doses of AC-186 dose. Animals that received high dose AC-186 treatments (30 mg/kg) had significantly less severe EAE scores as compared to the sesame oil vehicle alone ($p = 0.0299$), while lower doses (10 mg/kg and 3 mg/kg) were not efficacious.

Figure 9. Rotarod times for female C57BL/6 mice that received high dose AC-186 treatments (30 mg/kg) in sesame oil versus control animals (Veh). No differences in AC-186 treatment using the sesame oil vehicle.

Figure 10. EAE scores (Fig. 10A) and rotarod times (Fig. 10B) for a positive control DPN in the vehicle of 10% ethanol / miglyol solution.

Figure 11. EAE scores for male C57BL/6 mice that received high dose AC-186 treatments (30 mg/kg) in either sesame oil or miglyol or the vehicle alone. Miglyol carrier with AC-186 shows benefit while sesame oil carrier with AC-186 does not.

Figure 12. Rotarod times for male C57BL/6 mice that received high dose AC-186 treatments (30 mg/kg) in either sesame oil or miglyol or the vehicle alone.

Figure 13. EAE scores for animals that received either the sesame oil or miglyol vehicle and no ER β ligand, showing that efficacy differences are due to the ER β ligand and not inherent to the type of vehicle in and of itself.

Figure 14. EAE scores for NOD females under various treatment conditions, to extend efficacy findings to another EAE progressive model on another genetic background.

Figure 15. Rotarod times for NOD females under various treatment conditions, showing this is an insensitive outcome measure in this genetic background.

Figure 16. EAE scores for NOD males under various treatment conditions.

Figure 17. Rotarod times for NOD males under various treatment conditions.

Figure 18. EAE scores for C57BL/6 females under various treatment conditions.

Figure 19. Rotarod times for C57BL/6 females under various treatment conditions.

Figure 20. AC-186 post-treatment during EAE: effects on axonal densities, beta-APP, and myelin in spinal cord. (A, top row) Representative 10 $\times$ captures of spinal cord sections at the dorsal column of matched healthy control (left), vehicle-treated EAE (2nd from left), AC-186 10 mg/kg post-treated EAE (3rd from left), and AC-186 30 mg/kg post-treated EAE (right). EAE mice were sacrificed at EAE day 60. Axons and myelin were stained with NF200 and MBP, respectively (A, top three rows). Representative 10 $\times$ confocal images of spinal cord sections were stained for axonal damage using beta-APP (A, bottom row). (B) Quantification of axonal densities (left), beta-APP expression (middle), and myelin staining intensity (right). Vehicle treated EAE (Veh) as compared to matched healthy controls (Cont) showed significantly reduced axon numbers (left), increased beta-APP (middle) and reduced myelin (right). AC-186 10mg/kg, and 30mg/kg treated EAE groups each showed significantly more axon numbers compared with the Vehicle treated EAE group ($p < 0.03$, Veh vs AC-186 10mg/kg and Veh vs AC-186 30mg/kg). The beta-APP staining showed that the AC-186 30 mg/kg treatment group had significantly less expression of beta-APP compared with the Vehicle treated EAE group ($p = 0.0296$, Veh vs AC-186 30mg/kg). The AC-186 10mg/kg treatment group

showed a trend of less beta-APP staining compared with the Vehicle treated EAE group, but the difference with this dose did not reach significance. MBP staining showed that both AC-186 treatment groups had a trend for somewhat higher MBP staining intensity as compared to vehicle but this did not reach statistical significance. Four mice in each treatment group were examined for each treatment group. p-values were determined by one-way ANOVA.

Figure 21. AC-186 post-treatment during EAE: effects on macrophages and T cells in spinal cord. (A, top row) Representative 10× captures of spinal cord sections at the dorsal column of matched healthy controls (left), vehicle-treated EAE (2nd from left), AC-186 10

mg/kg post-treated EAE (3rd from left), and AC-186 30 mg/kg post-treated EAE (right). EAE mice were sacrificed at EAE day 60. Immune cells in the CNS were stained with pan-leucocyte marker CD45. The tissues were counterstained by DAPI. (A, middle row) Representative

10×confocal images of spinal cord sections stained with Iba-1. (A, bottom row) Representative 10×confocal images of spinal cord sections stained with CD3. (B) Quantification of CD45 immunoreactivity (left), Iba-1 globoid to quantify macrophage like cells (middle), and CD3 to

quantify T lymphocytes (right) as shown in (A). Vehicle treated EAE (Veh) as compared to matched healthy controls (Cont) showed significantly increased CD45 staining (left), increased Iba-1 globoid cells (middle), and increased CD3 cells (right). AC-186 10mg/kg and 30mg/kg post-treatment EAE groups each showed a reduction of CD45 expression compared with the Vehicle treated EAE group ($p=0.001$, Veh vs AC-186 10mg/kg, $p=0.005$, Veh vs AC-186

30mg/kg). Each of the AC-186 treatment groups also showed a significant reduction in the number of Iba-1 stained cells with globoid morphology as compared with the Vehicle treated EAE group ($p<0.0001$, Veh vs AC-186 10mg/kg, $p=0.0001$, Veh vs AC-186 30mg/kg). There were no differences in the number of CD3 stained cells between either of the AC-186 treatment groups as compared to the Vehicle EAE treatment group. Four mice in each treatment group (or three mice for Veh and AC-186 10mg/kg groups in CD45 staining) were examined for each treatment group. p-values were determined by one-way ANOVA.

Figure 22. EAE scores and rotarod times for female C57BL/6 mice who received 30 mg/kg AC-186 in miglyol for mice that were subject to MRI and neuropathologic analyses of cerebrum and cerebellum. (Top) Standard EAE disease scores were ameliorated in female C57BL/6 mice treated with AC-186 at 30 mg/kg as compared to vehicle (miglyol), $p<0.0001$.

(Bottom) Rotarod testing showed significant improvement with the AC-186 30mg/kg as compared to vehicle (miglyol), $p < 0.0001$.

Figure 23. Whole Brain, Cortical and Cerebellar Atrophy in EAE is Ameliorated by AC-186 Treatment. (A) A graph of the mean whole brain volume in healthy controls (data points with least decay in each graph), AC-186-treated mice with EAE (data points with steepest decay in each graph) and vehicle-treated mice with EAE (data points with intermediate decay in each graph) at d0, d30 and d60. AC-186-treated EAE mice exhibit less brain atrophy than vehicle-treated EAE mice as early as d30. (B) A graph of the mean cerebral cortex volume in healthy controls, AC-186-treated mice with EAE and vehicle-treated mice with EAE at d0, d30 and d60. AC-186-treated EAE mice exhibit less atrophy in the cerebral cortex than vehicle-treated EAE mice by d60. (C) A graph of the mean cerebellar volume in healthy controls, AC-186-treated mice with EAE and vehicle-treated mice with EAE at d0, d30 and d60. AC-186-treated EAE mice exhibit less cerebellar atrophy than vehicle-treated EAE mice by d60.

Figure 24. AC-186 treatment protects against loss of cerebral and cerebellar neurons and synapses in gray matter. (Top row) Quantification of NeuN⁺ neuronal cells in the cerebral cortex gray matter (left), and percent area of Post-Synaptic Density-95 (PSD-95, right) positivity in healthy controls (Cont), Vehicle (Veh), and AC-186-treated EAE mice. Vehicle treated EAE mice, as compared to age-matched healthy controls, had fewer numbers of NeuN⁺ cortical neurons and less PSD-95 staining ($p = 0.02$, NeuN; $p = 0.0328$, PSD-95, one-way ANOVA). AC-186 treated EAE mice, as compared to Vehicle, had higher numbers of NeuN⁺ cortical neurons and greater PSD-95 staining, with values comparable to age-matched healthy controls ($p = 0.02$, NeuN, $p = 0.0049$, PSD-95, one-way ANOVA). (Bottom row) Quantification of Calbindin⁺ Purkinje cells (left) in the cerebellar gray matter, and PSD-95 (right) in cerebellar gray matter of age-matched healthy controls, Vehicle, and AC-186-treated EAE mice. Vehicle treated EAE mice, as compared to age-matched healthy controls, had fewer numbers of Calbindin⁺ Purkinje cells and less PSD-95 staining ($p = 0.02$, Calbindin; $p = 0.0328$, PSD-95; one-way ANOVA). AC-186 treated EAE mice, as compared to Vehicle, had higher numbers of Calbindin⁺ Purkinje cells and greater PSD-95 staining, with values comparable to age-matched healthy controls ($p = 0.04$, Calbindin; $p = 0.0005$, PSD-95; one-way ANOVA).

Figure 25. AC-186 treatment during EAE: effects on axonal loss, axonal damage, and myelin in spinal cord. EAE mice were sacrificed at the end of the experiments depicted in

Figures 22 (clinical data) and 23 (MRI data). Quantification is shown of axonal densities by NF200 staining (left), beta-APP expression for axonal damage (middle), and myelin staining intensity by MBP (right). The vehicle treated (Veh) group as compared to matched healthy controls (Cont) showed significantly reduced axon numbers (left), increased beta-APP (middle) and reduced myelin (right). The AC-186 30mg/kg (AC186) treated EAE group showed significantly more axon numbers compared with the Vehicle (Veh) treated EAE group ($p < 0.05$, Veh vs AC-186 30mg/kg). The beta-APP staining showed that the AC-186 30 mg/kg (AC186) treated group had significantly less expression of beta-APP compared with the Vehicle (Veh) treated EAE group ($p < 0.04$, Veh vs AC-186 30mg/kg). MBP staining showed that the AC-186 treatment group had higher MBP staining intensity as compared to vehicle ($p < 0.004$, Veh vs AC-186 30mg/kg). Three to five mice were examined for each treatment group. p-values were determined by one-way ANOVA.

Figure 26. AC-186 treatment during EAE: effects on macrophages and T cells in spinal cord. EAE mice were sacrificed at end of the experiments depicted in Figure 22 (clinical data) and 23 (MRI data). Quantification of CD45 immunoreactivity to quantify all immune cells (left), Iba-1 globoid to quantify macrophage like cells (middle), and CD3 to quantify T lymphocytes (right) was done. Vehicle treated EAE (Veh) as compared to matched healthy controls (Cont) showed significantly increased CD45 staining (left), increased Iba-1 globoid cells (middle), and increased CD3 cells (right). AC-186 30mg/kg treated EAE groups showed a reduction of CD45 expression compared with the Vehicle treated EAE group ($p = 0.029$, Veh vs AC-186 30mg/kg). The AC-186 treatment group also showed a significant reduction in the number of Iba-1 stained cells with globoid morphology as compared with the Vehicle treated EAE group ($p = 0.037$, Veh vs AC-186 30mg/kg). There were no differences in the number of CD3 stained cells between the AC-186 treated group as compared to the Vehicle EAE treatment group. Three to five mice were examined for each treatment group. p-values were determined by one-way ANOVA.

Figure 27. AC-186 treatment during EAE: effects on cerebellar white matter. EAE mice were sacrificed at end of the experiments depicted in Figures 22 (clinical data) and 23 (MRI data). Quantification is shown of myelin staining intensity by MBP (left) and axonal densities by NF200 staining (right). The vehicle treated (Veh) group as compared to matched healthy controls (Cont) showed significantly reduced myelin (left) and reduced axon numbers (right).

MBP staining showed that the AC-186 treated group had higher MBP staining intensity as compared to vehicle ($p < 0.0176$, Veh vs AC-186 30mg/kg) (left). The AC-186 30mg/kg treated EAE group showed significantly more axon numbers compared with the Vehicle treated EAE group ($p < 0.01$, Veh vs AC-186 30mg/kg) (right). Three to five mice were examined for each treatment group. p-values were determined by one-way ANOVA.

Figure 28. AC-186 treatment during EAE: effects on cerebral white matter. EAE mice were sacrificed at end of the experiments depicted in Figure 22 (clinical data) and 23 (MRI data). Quantification is shown of myelin staining intensity by MBP (left) and axonal densities by NF200 staining (right) in the splenium of the corpus callosum of the cerebrum. The vehicle treated (Veh) group as compared to matched healthy controls (Cont) showed a trend for reduced myelin, but this did not reach significance (left), and a significant reduction in axon numbers ($p = 0.0025$, Cont vs Veh) (right). Regarding the effect of AC-186 treatment, MBP staining showed that the AC-186 treated group had a trend for higher MBP staining intensity as compared to vehicle (left), but this did not reach significance. The AC-186 30mg/kg treated EAE group showed significantly more axon numbers compared with the Vehicle treated EAE group ($p = 0.0286$, Veh vs AC-186 30mg/kg) (right). Three to five mice were examined for each treatment group. p-values were determined by one-way ANOVA.

Figure 29 consists of four panels, labeled panels A, B, C, and D. Panel A shows that 30 mg/kg AC-186 administered every other day significantly improved EAE clinical severity scores and rotarod performance in female mice ($p < 0.0001$). Panel B shows that 30 mg/kg AC-186 administered every other day significantly improved EAE clinical severity scores and rotarod performance in male mice ($p < 0.0001$). Panel C shows that 30 mg/kg AC-186 administered every other day significantly reduced spinal cord white matter axonal loss, as evidenced by NF200 staining ($p < 0.05$), axonal damage, as evidenced by beta-APP staining ($p < 0.05$), and demyelination, as evidenced by MBP staining ($p < 0.005$) relative to vehicle-treated controls. Panel D shows that 30 mg/kg AC-186 administered every other day significantly reduced inflammation, as evidenced by CD45 staining ($p < 0.05$), and macrophage/activated microglia, as evidenced by Iba-1 globoid staining ($p < 0.05$), but not T lymphocyte counts relative to vehicle-treated controls.

DETAILED DESCRIPTION**I. OVERVIEW**

Some aspects of the invention are based on the finding that the estrogen receptor β ligand estriol protects gray matter (*e.g.*, cortical gray matter) in subjects with relapsing-remitting multiple sclerosis who do not present with active lesions (*see, e.g.*, Figure 3, Panel I). This finding suggests that, in contrast with other therapeutics for treating relapsing multiple sclerosis, ER β ligand therapies are efficacious for treating multiple disease states, not only relapsing-remitting multiple sclerosis, but also secondary progressive multiple sclerosis and primary progressive multiple sclerosis. Furthermore, unlike other therapeutics for treating multiple sclerosis that target immune responses and inflammation, ER β ligand therapies are likely generally efficacious for treating various other forms of neurodegenerative disease that are not primarily autoimmune response driven.

In some aspects, the invention relates to a method for treating a neurodegenerative disease, such as multiple sclerosis, in a subject who does not present with active lesions (*e.g.*, gadolinium-enhancing lesions), comprising administering an ER β ligand to the subject without conjointly administering a second immunotherapeutic agent.

In some aspects, the invention relates to a method for treating a neurodegenerative disease, such as multiple sclerosis, in a subject receiving treatment with an immunotherapeutic agent who does not present with active lesions (*e.g.*, gadolinium-enhancing lesions), comprising administering an ER β ligand to the subject and discontinuing treatment with the immunotherapeutic agent.

In some aspects, the invention relates to a method for treating a neurodegenerative disease, such as multiple sclerosis, in a subject, comprising determining whether the brain of the subject presents with active lesions (*e.g.*, gadolinium-enhancing lesions), administering an ER β ligand to the subject, and discontinuing any second immunotherapeutic agent that the subject is receiving if the subject lacks active lesions.

In some aspects, the invention relates to a method for treating a neurodegenerative disease, such as multiple sclerosis, in a subject, comprising administering an ER β ligand to the subject, determining whether the brain of the subject presents with active lesions, and conjointly

administering to the subject a second immunotherapeutic agent if the brain of the subject presents with active lesions.

II. SUBJECTS PRESENTING WITH ACTIVE LESIONS

The term “active lesion” as used herein, refers to inflammation in the central nervous system associated with immune cells, such as T lymphocytes. For example, during a relapse, T lymphocytes may cross the blood-brain barrier in a relapsing-remitting multiple sclerosis patient. In multiple sclerosis, T cells may mount an autoimmune response against myelin. Additionally, other blood cells may cross the blood-brain barrier, such as white blood cells, *e.g.*, other lymphocytes and monocytes. An active lesion may be detected, for example, by gadolinium-enhanced magnetic resonance imaging (MRI). Gadolinium-based contrast agents generally do not cross the blood-brain barrier, and thus a gadolinium-enhanced MRI scan may be used to detect an active lesion, termed a “gadolinium-enhancing lesion” or simply an “enhancing lesion”. Additionally, active lesions may be diagnosed by other methods. For example, an active lesion may be detected by MRI without a gadolinium-based contrast agent, *e.g.*, by identifying a new or enlarging T2 hyperintensity in the brain, relative to a previous MRI, indicating that the subject had a recent active lesion. Active lesions may also be identified by diagnosing a relapse in a multiple sclerosis patient (*e.g.*, by significant worsening on a walking, balance, or visual acuity test that has an acute onset, such as over 1-7 days).

The term “central nervous system” is used herein as defined in the art and includes the brain and spinal cord. As used herein, the term “brain” is used synonymously with “central nervous system” and thus, unless otherwise apparent from context, the term “brain” includes both the brain as defined in the art and the spinal cord.

A subject presents with active lesions if the subject has been recently diagnosed (*e.g.*, within the last two months, preferably within the last month) with at least one active lesion, *e.g.*, by gadolinium-enhanced MRI.

A subject does not present with active lesions if the subject has not been recently diagnosed with any active lesions. For example, a subject does not present with active lesions if a gadolinium-enhanced MRI scan of the brain of the subject suggests that no gadolinium crossed the blood-brain barrier of the subject, *e.g.*, relative to a control scan or control region of the brain. Additionally, a patient diagnosed with secondary progressive multiple sclerosis or primary progressive multiple sclerosis presents with few or no active lesions. Similarly, a

subject with relapsing-remitting multiple sclerosis who is in clinical remission may or may not present with active lesions on an MRI scan.

Whether the brain of a subject presents with active lesions may be determined directly, *e.g.*, by diagnosing the subject, or indirectly, by obtaining a prior diagnosis. For example, a physician may determine whether the brain of a subject presents with active lesions by identifying a breach in the blood-brain barrier of the subject, *e.g.*, by gadolinium-enhanced MRI or by assaying the cerebrospinal fluid of the subject for a biomarker. Similarly, a physician may determine whether the brain of a subject presents with active lesions by diagnosing the subject with a relapse of multiple sclerosis, *e.g.*, by identifying a new onset walking or other difficulty.

Alternatively, whether the brain of a subject presents with active lesions may be determined indirectly, *e.g.*, by obtaining a diagnosis from a physician, technician, nurse, medical chart, MRI scan, or from the subject. For example, a doctor or other prescriber may administer an ER β ligand to a subject after determining whether the brain of a subject presents with active lesions by reviewing the medical records of the subject and/or discussing the medical history of the subject with the subject or another caretaker of the subject.

An active lesion may be, for example, an infratentorial lesion, juxtacortical lesion, periventricular lesion, or spinal cord lesion.

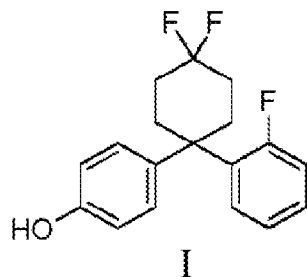
In some embodiments, determining whether the brain of a subject presents with an active lesion comprises evaluating the cerebrospinal fluid of the subject, *e.g.* for immunoglobulin abnormalities (assessing the IgG Index in cerebrospinal fluid).

The subject may display evidence of cognitive decline. The evidence of cognitive decline may be worsening performance on the Paced Auditory Serial Addition Test (“PASAT”). The subject may display evidence of brain atrophy. The evidence of brain atrophy may be determined by MRI. The brain atrophy may be cortical gray matter atrophy. The brain atrophy may be a decrease in whole brain volume. In some embodiments, the subject may present with a cognitive disability. The evidence of cognitive disability may be determined by performance on the Paced Auditory Serial Addition Test (“PASAT”) or on a Symbol Digit Modalities Test (SDMT).

III. ADMINISTERING AN ER β ligand

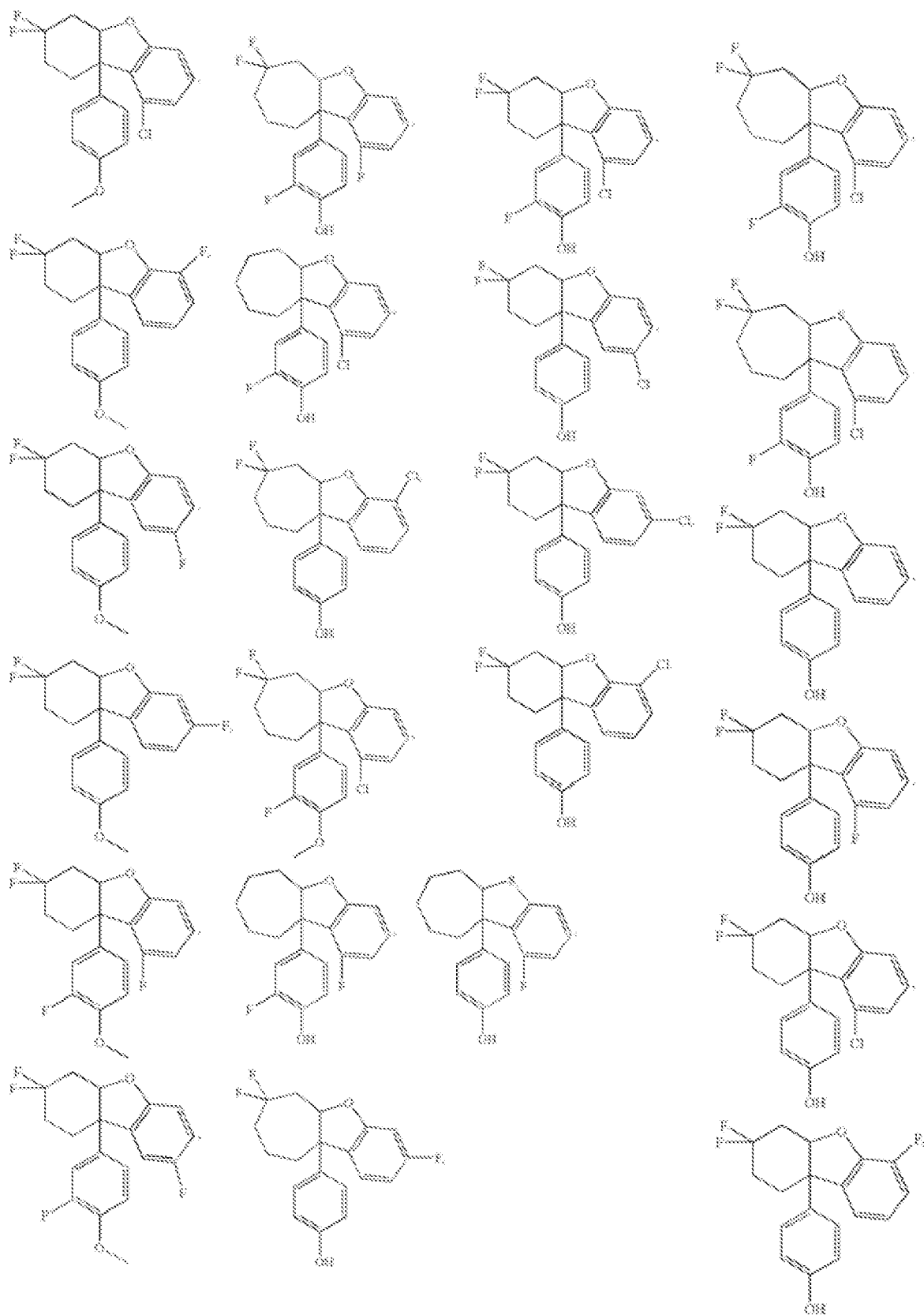
In some aspects, the method includes the steps of administering an ER β ligand to a subject. The ER β ligand may be administered on a continuous basis, *e.g.*, daily.

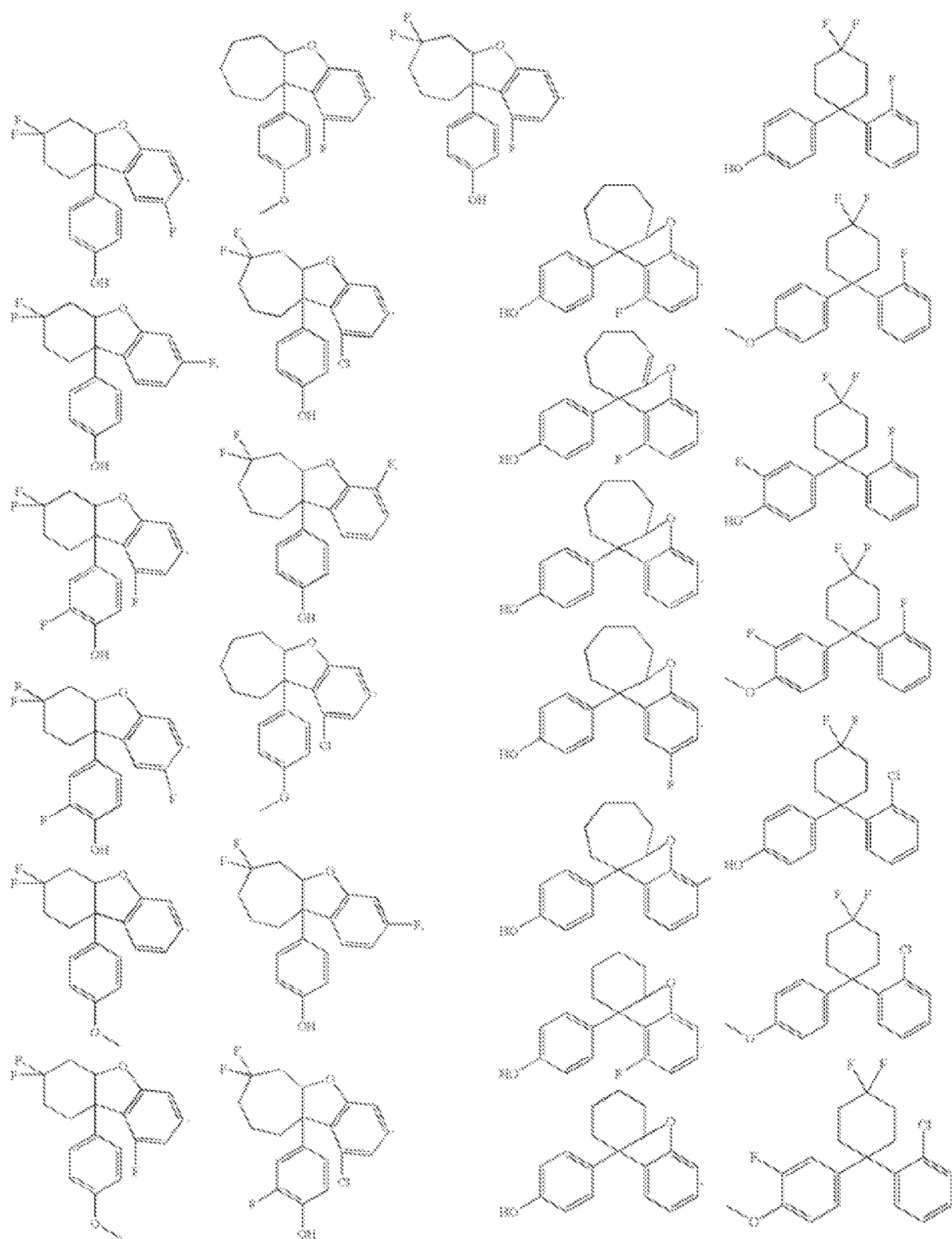
The term “ER β ligand” as used herein refers to an estrogen receptor β agonist, including steroidal and non-steroidal agents that bind to and/or cause a change in activity or binding of the estrogen receptor β . One agent useful in this invention is the ER β ligand known as AC-186 (Compound I), or compounds substantially similar in structure and function thereto (such as those compounds disclosed in U.S. Patent Application Publication No’s 2009/0131510 and 2014/0275284, and PCT Patent Application Publication No. WO 2014/125121, hereby incorporated by reference, especially for the molecules disclosed therein).



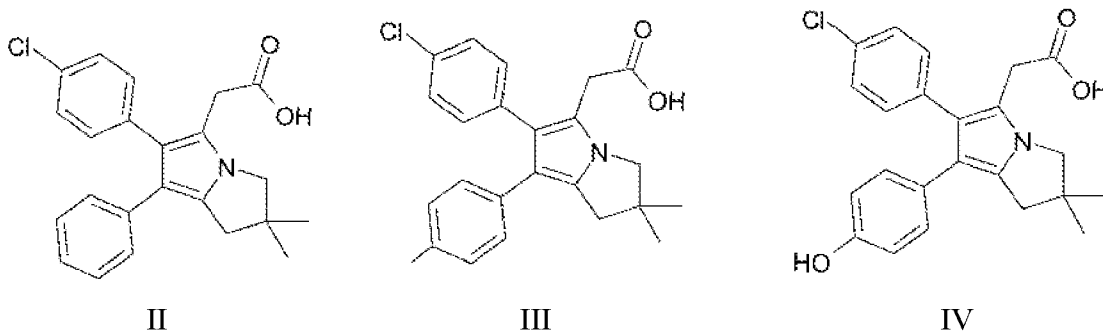
In some embodiments, the ER β ligand is a compound selected from the compounds disclosed in U.S. Patent Application Publication No’s 2012/0128435, 2012/0202853, 2012/0202861, 2013/0131061, or 2014/0323518 and PCT Patent Application Publication No’s WO 2012/022776 and 2012/136772 (hereby incorporated by reference, especially for the molecules disclosed therein). In some embodiments, the ER β ligand is KBRV1 or KBRV2 (Karo Bio, Huddinge, Sweden).

The ER β ligand may be any one of the following compounds:





In some embodiments, the ER β ligand is Compound **II**, **III**, or **IV**.



The ER β ligand may be 5-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-1-(4-hydroxyphenyl)-3-methyl-1H-pyrazole-4-carboximidamide; 5-(3,5-dimethylisoxazol-4-yl)-1-(4-hydroxyphenyl)-3-methyl-1H-pyrazole-4-carboxamide; 5-(3,5-dimethylisoxazol-4-yl)-1-(4-hydroxyphenyl)-3-methyl-1H-pyrazole-4-carbaldehyde oxime; 5-(3,5-dimethylisoxazol-4-yl)-1-(4-hydroxyphenyl)-3-propyl-1H-pyrazole-4-carbaldehyde oxime; 5-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-1-(4-hydroxyphenyl)-3-propyl-1H-pyrazole-4-carboximidamide; 5-(2,5-dimethyl-1H-pyrrol-1-yl)-1-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-3-methyl-1H-pyrazole-4-carboximidamide; 5-(diethylamino)-3-ethyl-N'-hydroxy-1-(4-hydroxyphenyl)-1H-pyrazole-4-carboximidamide; 5-(1,3-dimethyl-1H-pyrrol-2-yl)-1-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-3-methyl-1H-pyrazole-4-carboximidamide; 5-(5-bromo-1,3-dimethyl-1H-pyrrol-2-yl)-1-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-3-methyl-1H-pyrazole-4-carboximidamide; 3-ethyl-N'-hydroxy-1-(4-hydroxyphenyl)-5-(3-methylfuran-2-yl)-1H-pyrazole-4-carboximidamide; 4-(4-(3,5-dimethylisoxazol-4-yl)-5-(hydroxymethyl)-1-methyl-1H-pyrazol-3-yl)phenol; 4-(3,5-dimethylisoxazol-4-yl)-3-(4-hydroxyphenyl)-1-methyl-1H-pyrazole-5-carbaldehyde oxime; 4-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-3-(4-hydroxyphenyl)-1-methyl-1H-pyrazole-5-carboximidamide; 4-(3,5-dimethylisoxazol-4-yl)-3-(4-hydroxyphenyl)-1-methyl-1H-pyrazole-5-carboxamide; 4-(4-(3,5-dimethylisoxazol-4-yl)-5-(2-hydroxyethyl)-1-methyl-1H-pyrazol-3-yl)phenol; 4-(2,6-difluorophenyl)-N'-hydroxy-3-(4-hydroxyphenyl)-1-methyl-1H-pyrazole-5-carboximidamide; 5-(3,5-dimethylisoxazol-4-yl)-1-(4-hydroxyphenyl)-3-methyl-1H-pyrazole-4-carbonitrile; 5-(3,5-dimethylisoxazol-4-yl)-1-(4-hydroxyphenyl)-3-isopropyl-1H-pyrazole-4-carbaldehyde oxime; 5-(3,5-dimethylisoxazol-4-yl)-3-ethyl-1-(4-hydroxyphenyl)-1H-pyrazole-4-carbaldehyde oxime; 5-(3,5-dimethylisoxazol-4-yl)-3-ethyl-N'-hydroxy-1-(4-hydroxyphenyl)-1H-pyrazole-4-carboximidamide; 1-(3,5-difluoro-4-hydroxyphenyl)-5-(3,5-dimethylisoxazol-4-yl)-3-ethyl-N'-

hydroxy-1H-pyrazole-4-carboximidamide; 5-(3,5-dimethylisoxazol-4-yl)-3-ethyl-1-(3-fluoro-4-hydroxyphenyl)-N'-hydroxy-1H-pyrazole-4-carboximidamide; 1-(2,3-difluoro-4-hydroxyphenyl)-5-(3,5-dimethylisoxazol-4-yl)-3-ethyl-N'-hydroxy-1H-pyrazole-4-carboximidamide; 5-(3,5-dimethylisoxazol-4-yl)-3-ethyl-1-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-1H-pyrazole-4-carboximidamide; 1-(2,3-difluoro-4-hydroxyphenyl)-5-(3,5-dimethylisoxazol-4-yl)-3-ethyl-1H-pyrazole-4-carbaldehyde oxime (isomer A); 1-(2,3-difluoro-4-hydroxyphenyl)-5-(3,5-dimethylisoxazol-4-yl)-3-ethyl-1H-pyrazole-4-carbaldehyde oxime (isomer B); 5-(3,5-dimethylisoxazol-4-yl)-3-ethyl-1-(2-fluoro-4-hydroxyphenyl)-1H-pyrazole-4-carbaldehyde oxime; 1-(3,5-difluoro-4-hydroxyphenyl)-5-(3,5-dimethylisoxazol-4-yl)-3-ethyl-1H-pyrazole-4-carbaldehyde oxime; 1-(2,5-difluoro-4-hydroxyphenyl)-5-(3,5-dimethylisoxazol-4-yl)-3-ethyl-N'-hydroxy-1H-pyrazole-4-carboximidamide; 1-(2,5-difluoro-4-hydroxyphenyl)-5-(3,5-dimethylisoxazol-4-yl)-3-ethyl-1H-pyrazole-4-carbaldehyde oxime; 5-(3,5-dimethylisoxazol-4-yl)-1-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-3-methyl-1H-pyrazole-4-carboximidamide; 5-(3,5-dimethylisoxazol-4-yl)-1-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-3-propyl-1H-pyrazole-4-carboximidamide; 5-(3,5-dimethylisoxazol-4-yl)-1-(2-fluoro-4-hydroxyphenyl)-3-methyl-1H-pyrazole-4-carbaldehyde oxime; 1-(2,3-difluoro-4-hydroxyphenyl)-3-ethyl-5-(1-methyl-1H-pyrrol-2-yl)-1H-pyrazole-4-carbaldehyde oxime; 2-(2-fluoro-4-hydroxyphenyl)-2',4',5-trimethyl-2H,2'H-3,3'-bipyrazole-4-carbaldehyde oxime; 2-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-2',4',5-trimethyl-2H,2'H-3,3'-bipyrazole-4-carboximidamide; 5-(2,6-dimethylphenyl)-1-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-3-methyl-1H-pyrazole-4-carboximidamide; 5-(2,5-dimethyl-1H-pyrrol-1-yl)-3-ethyl-N'-hydroxy-1-(4-hydroxyphenyl)-1H-pyrazole-4-carboximidamide; 5-(2,6-dimethylphenyl)-1-(2-fluoro-4-hydroxyphenyl)-3-methyl-1H-pyrazole-4-carbaldehyde oxime; 5-(2,5-dimethyl-1H-pyrrol-1-yl)-1-(3-fluoro-4-hydroxyphenyl)-N'-hydroxy-3-methyl-1H-pyrazole-4-carboximidamide; 1-(2,3-difluoro-4-hydroxyphenyl)-5-(2,5-dimethyl-1H-pyrrol-1-yl)-N'-hydroxy-3-methyl-1H-pyrazole-4-carboximidamide; 1-(3,5-difluoro-4-hydroxyphenyl)-5-(2,5-dimethyl-1H-pyrrol-1-yl)-N'-hydroxy-3-methyl-1H-pyrazole-4-carboximidamide; 3-(3-chloro-4-hydroxyphenyl)-4-(3,5-dimethylisoxazol-4-yl)-1-methyl-1H-pyrazole-5-carboxamide; 4-(2,6-difluorophenyl)-3-(4-hydroxyphenyl)-1-methyl-1H-pyrazole-5-carbaldehyde oxime; 4-(2,6-dichlorophenyl)-3-(4-hydroxyphenyl)-1-methyl-1H-pyrazole-5-carbaldehyde oxime; 4-(2,6-dichlorophenyl)-3-(4-hydroxyphenyl)-1-methyl-1H-pyrazole-5-carboxamide; 4-(2,6-dichlorophenyl)-N'-hydroxy-3-(4-

hydroxyphenyl)-1-methyl-1H-pyrazole-5-carboximidamide; 4-(3,5-dimethylisoxazol-4-yl)-3-(2-fluoro-4-hydroxyphenyl)-1-methyl-1H-pyrazole-5-carbaldehyde oxime; 4-(3,5-dimethylisoxazol-4-yl)-3-(2-fluoro-4-hydroxyphenyl)-1-methyl-1H-pyrazole-5-carboxamide; 4-(3,5-dimethylisoxazol-4-yl)-3-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-1-methyl-1H-pyrazole-5-carboximidamide; 5-((Z)-but-2-en-2-yl)-1-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-3-methyl-1H-pyrazole-4-carboximidamide; 5-(2,4-dimethylthiophen-3-yl)-1-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-3-methyl-1H-pyrazole-4-carboximidamide; 5-(3,5-dimethylpyridin-4-yl)-1-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-3-methyl-1H-pyrazole-4-carboximidamide; 5-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-1-(4-hydroxy-2-methylphenyl)-3-methyl-1H-pyrazole-4-carboximidamide; 5-(3,5-dimethylisoxazol-4-yl)-3-ethyl-N'-hydroxy-1-(4-hydroxy-2-methylphenyl)-1H-pyrazole-4-carboximidamide; 5-(2,5-dimethyl-1H-pyrrol-1-yl)-N'-hydroxy-1-(4-hydroxy-2-methylphenyl)-3-methyl-1H-pyrazole-4-carboximidamide; N'-hydroxy-1-(4-hydroxy-2-methylphenyl)-3-methyl-5-(2-methyl-5-propyl-1H-pyrrol-1-yl)-1H-pyrazole-4-carboximidamide; 4-(2,4-dimethylthiophen-3-yl)-3-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-1-methyl-1H-pyrazole-5-carboximidamide; 4-(3,5-dimethylisoxazol-4-yl)-3-(3-fluoro-4-hydroxyphenyl)-1-methyl-1H-pyrazole-5-carbaldehyde oxime; 4-(3,5-dimethylisoxazol-4-yl)-3-(3-fluoro-4-hydroxyphenyl)-1-methyl-1H-pyrazole-5-carboxamide; 4-(3,5-dimethylisoxazol-4-yl)-3-(3-fluoro-4-hydroxyphenyl)-N'-hydroxy-1-methyl-1H-pyrazole-5-carboximidamide; (Z)-4-(3,5-dimethylisoxazol-4-yl)-3-(4-hydroxy-2-methylphenyl)-1-methyl-1H-pyrazole-5-carbaldehyde oxime; (E)-4-(3,5-dimethylisoxazol-4-yl)-3-(4-hydroxy-2-methylphenyl)-1-methyl-1H-pyrazole-5-carbaldehyde oxime; 4-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-3-(4-hydroxy-2-methylphenyl)-1-methyl-1H-pyrazole-5-carboximidamide; 4-(3,5-dimethylisoxazol-4-yl)-3-(4-hydroxy-2-methylphenyl)-1-methyl-1H-pyrazole-5-carboxamide; 4-(2,4-dimethylthiophen-3-yl)-N'-hydroxy-3-(4-hydroxy-2-methylphenyl)-1-methyl-1H-pyrazole-5-carboximidamide; 4-(3,5-dimethylisothiazol-4-yl)-3-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-1-methyl-1H-pyrazole-5-carboximidamide; 4-(2,6-dimethylphenyl)-3-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-1-methyl-1H-pyrazole-5-carboximidamide; 4-(2,4-dimethylfuran-3-yl)-3-(4-hydroxyphenyl)-1-methyl-1H-pyrazole-5-carbaldehyde oxime; 4-(3,5-dimethylisothiazol-4-yl)-3-(4-hydroxyphenyl)-1-methyl-1H-pyrazole-5-carbaldehyde oxime; or 4-(3,5-dimethylisothiazol-4-yl)-N'-hydroxy-3-(4-hydroxyphenyl)-1-methyl-1H-pyrazole-5-carboximidamide.

The ER β ligand may be 2-(3,5-dimethylisoxazol-4-yl)-3-(4-hydroxyphenyl)-1H-indole-1-carbonitrile; 2-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-3-(4-hydroxyphenyl)-1H-indole-1-carboximidamide; 2-(3,5-dimethylisoxazol-4-yl)-3-(4-hydroxyphenyl)-1H-indole-1-carboxamide; 2-(3,5-dimethylisoxazol-4-yl)-3-(4-hydroxyphenyl)-N,N-dimethyl-1H-indole-1-sulfonamide; 2-(3,5-dimethylisoxazol-4-yl)-3-(4-hydroxyphenyl)-1H-indole-1-carbaldehyde oxime; 4-(2-(3,5-dimethylisoxazol-4-yl)-1-(methylsulfonyl)-1H-indol-3-yl)phenol; 2-((Z)-but-2-en-2-yl)-N'-hydroxy-3-(4-hydroxyphenyl)-1H-indole-1-carboximidamide; 2-(3,5-dimethylisoxazol-4-yl)-N-ethyl-3-(4-hydroxyphenyl)-1H-indole-1-carboxamide; 2-(3,5-dimethylisoxazol-4-yl)-3-(4-hydroxyphenyl)-N-methyl-1H-indole-1-carboxamide; 2-(2,4-dimethylthiophen-3-yl)-N'-hydroxy-3-(4-hydroxyphenyl)-1H-indole-1-carboximidamide; 2-(2,4-dimethylthiophen-3-yl)-3-(4-hydroxyphenyl)-1H-indole-1-carboxamide; 2-(2,6-dimethylphenyl)-N'-hydroxy-3-(4-hydroxyphenyl)-1H-indole-1-carboximidamide; 2-(3,5-dimethylisoxazol-4-yl)-3-(4-hydroxyphenyl)-N-isopropyl-1H-indole-1-carboxamide; 2-(3,5-dimethylisoxazol-4-yl)-3-(4-hydroxyphenyl)-N-pentyl-1H-indole-1-carboxamide; 2-(2,4-dimethylthiophen-3-yl)-N-ethyl-3-(4-hydroxyphenyl)-1H-indole-1-carboxamide; 3-(3,5-difluoro-4-hydroxyphenyl)-2-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-1H-indole-1-carboximidamide; 3-(2,3-difluoro-4-hydroxyphenyl)-2-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-1H-indole-1-carboximidamide; 2-(3,5-dimethylisoxazol-4-yl)-3-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-1H-indole-1-carboximidamide; 3-(2,5-difluoro-4-hydroxyphenyl)-2-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-1H-indole-1-carboximidamide; 2-(3,5-dimethylisoxazol-4-yl)-3-(3-fluoro-4-hydroxyphenyl)-N'-hydroxy-1H-indole-1-carboximidamide; 5-chloro-2-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-3-(4-hydroxyphenyl)-1H-indole-1-carboximidamide; 2-(2,4-dimethylfuran-3-yl)-N'-hydroxy-3-(4-hydroxyphenyl)-1H-indole-1-carboximidamide; 2-(3,5-dimethylisothiazol-4-yl)-N'-hydroxy-3-(4-hydroxyphenyl)-1H-indole-1-carboximidamide; 2-(3,5-dimethylisothiazol-4-yl)-3-(4-hydroxyphenyl)-1H-indole-1-carboxamide; 5-chloro-2-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-3-(4-hydroxyphenyl)-1H-indole-1-carboximidamide; 2-(2,4-dimethylfuran-3-yl)-N'-hydroxy-3-(4-hydroxyphenyl)-1H-indole-1-carboximidamide; 2-(3,5-dimethylisothiazol-4-yl)-N'-hydroxy-3-(4-hydroxyphenyl)-1H-indole-1-carboximidamide; or 2-(3,5-dimethylisothiazol-4-yl)-3-(4-hydroxyphenyl)-1H-indole-1-carboxamide

The ER β ligand may be 2-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-3-(4-hydroxyphenyl)-2,4,5,6-tetrahydrocyclopenta[c]pyrrole-1-carboximidamide; 2-(3,5-dimethylisoxazol-4-yl)-3-(2-

fluoro-4-hydroxyphenyl)-N'-hydroxy-5,6-dihydrocyclopenta[b]pyrrole-1 (4H)-carboximidamide; 3-(2,6-difluoro-4-hydroxyphenyl)-2-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-5,6-dihydrocyclopenta[b]pyrrole-1 (4H)-carboximidamide; 3-(2,5-difluoro-4-hydroxyphenyl)-2-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-5,6-dihydrocyclopenta[b]pyrrole-1 (4H)-carboximidamide; 3-(2-chloro-4-hydroxyphenyl)-2-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-5,6-dihydrocyclopenta[b]pyrrole-1 (4H)-carboximidamide; 3-(2-chloro-6-fluoro-4-hydroxyphenyl)-2-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-5,6-dihydrocyclopenta[b]pyrrole-1 (4H)-carboximidamide; 3-(2,3-difluoro-4-hydroxyphenyl)-2-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-5,6-dihydrocyclopenta[b]pyrrole-1 (4H)-carboximidamide; 3-(3,5-difluoro-4-hydroxyphenyl)-2-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-5,6-dihydrocyclopenta[b]pyrrole-1 (4H)-carboximidamide; 2-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-3-(4-hydroxyphenyl)-4,5,6,7-tetrahydro-1H-indole-1-carboximidamide; 2-(3,5-dimethylisoxazol-4-yl)-3-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-4,5,6,7-tetrahydro-1H-indole-1-carboximidamide; 3-(2,6-difluoro-4-hydroxyphenyl)-2-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-4,5,6,7-tetrahydro-1H-indole-1-carboximidamide; 3-(2,5-difluoro-4-hydroxyphenyl)-2-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-4,5,6,7-tetrahydro-1H-indole-1-carboximidamide; 3-(2-chloro-4-hydroxyphenyl)-2-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-4,5,6,7-tetrahydro-1H-indole-1-carboximidamide; 3-(2,3-difluoro-4-hydroxyphenyl)-2-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-4,5,6,7-tetrahydro-1H-indole-1-carboximidamide; or 3-(3,5-difluoro-4-hydroxyphenyl)-2-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-4,5,6,7-tetrahydro-1H-indole-1-carboximidamide.

The ERβ ligand may be N',4'-dihydroxy-2-(3-methylthiophen-2-yl)-5-propyl-[1,1'-biphenyl]-3-carboximidamide; 2-(3,5-dimethylisoxazol-4-yl)-N',4'-dihydroxy-5-methyl-[1,1'-biphenyl]-3-carboximidamide; 2-(3,5-dimethylisoxazol-4-yl)-N',4'-dihydroxy-5-propyl-[1,1'-biphenyl]-3-carboximidamide; 2-(3,5-dimethylisoxazol-4-yl)-4'-hydroxy-5-propyl-[1,1'-biphenyl]-3-carboxamide; 2-(3,5-dimethylisoxazol-4-yl)-4'-hydroxy-5-propyl-[1,1'-biphenyl]-3-carbonitrile; N',4'-dihydroxy-5-methyl-2-(3-methylthiophen-2-yl)-[1,1'-biphenyl]-3-carboximidamide; 3',5'-difluoro-N',4'-dihydroxy-2-(3-methylthiophen-2-yl)-5-propyl-[1,1'-biphenyl]-3-carboximidamide; 2-bromo-3',5'-difluoro-N',4'-dihydroxy-5-propyl-[1,1'-biphenyl]-3-carboximidamide; 2-(3,5-dimethylisoxazol-4-yl)-3',5'-difluoro-N',4'-dihydroxy-5-propyl-[1,1'-biphenyl]-3-carboximidamide; 5-bromo-N',4'-dihydroxy-2-(3-methylthiophen-2-yl)-[1,1'-biphenyl]-3-carboximidamide; 5-bromo-N',4'-dihydroxy-2-iodo-[1,1'-biphenyl]-3-

carboximidamide; 5'-bromo-N',4-dihydroxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5''-fluoro-N',4-dihydroxy-2'',5'-dimethyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5''-fluoro-4-hydroxy-2'',5'-dimethyl-[1,1':2',1''-terphenyl]-3'-carboxamide; 5-chloro-2-(3,5-dimethylisoxazol-4-yl)-N',4'-dihydroxy-[1,1'-biphenyl]-3-carboximidamide; 5-chloro-2-(3,5-dimethylisoxazol-4-yl)-4'-hydroxy-[1,1'-biphenyl]-3-carboxamide; 2-(3,5-dimethylisoxazol-4-yl)-3'-fluoro-N',4'-dihydroxy-5-propyl-[1,1'-biphenyl]-3-carboximidamide; 2-(2,4-dimethylfuran-3-yl)-4'-hydroxy-5-propyl-[1,1'-biphenyl]-3-carbonitrile; 2-(2,4-dimethylfuran-3-yl)-N',4'-dihydroxy-5-propyl-[1,1'-biphenyl]-3-carboximidamide; 2-(2,4-dimethylfuran-3-yl)-4'-hydroxy-5-propyl-[1,1'-biphenyl]-3-carboxamide; N',4'-dihydroxy-2-iodo-5-propyl-[1,1'-biphenyl]-3-carboximidamide; N',4'-dihydroxy-2-(4-methylthiophen-3-yl)-5-propyl-[1,1'-biphenyl]-3-carboximidamide; N',4'-dihydroxy-5'-propyl-2''-(trifluoromethoxy)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2-(2,4-dimethylthiophen-3-yl)-N',4'-dihydroxy-5-propyl-[1,1'-biphenyl]-3-carboximidamide; N',4'-dihydroxy-5'-propyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2-((E)-2-cyclopropylvinyl)-N',4'-dihydroxy-5-propyl-[1,1'-biphenyl]-3-carboximidamide; N',4'-dihydroxy-2-(3-methylbut-2-en-2-yl)-5-propyl-[1,1'-biphenyl]-3-carboximidamide; N',4'-dihydroxy-3''-methyl-5'-propyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5''-fluoro-N',4-dihydroxy-2''-methyl-5'-propyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2''-ethyl-N',4-dihydroxy-5'-propyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; N',4'-dihydroxy-5-propyl-2-(thiophen-2-yl)-[1,1'-biphenyl]-3-carboximidamide; N',4'-dihydroxy-5-propyl-2-(quinolin-5-yl)-[1,1'-biphenyl]-3-carboximidamide; 3''-chloro-N',4-dihydroxy-5'-propyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; N',4'-dihydroxy-5-propyl-2-(pyridin-3-yl)-[1,1'-biphenyl]-3-carboximidamide; 2-(benzofuran-5-yl)-N',4'-dihydroxy-5-propyl-[1,1'-biphenyl]-3-carboximidamide; 4''-chloro-N',4-dihydroxy-5'-propyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; N',4'-dihydroxy-5-propyl-2-(pyridin-4-yl)-[1,1'-biphenyl]-3-carboximidamide; N',4'-dihydroxy-2-(1-phenylvinyl)-5-propyl-[1,1'-biphenyl]-3-carboximidamide; 2-(5-chlorothiophen-2-yl)-N',4'-dihydroxy-5-propyl-[1,1'-biphenyl]-3-carboximidamide; 5''-fluoro-N',4-dihydroxy-2''-methoxy-5'-propyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; N',4'-dihydroxy-2-(isoquinolin-6-yl)-5-propyl-[1,1'-biphenyl]-3-carboximidamide; 2-(benzofuran-3-yl)-N',4'-dihydroxy-5-propyl-[1,1'-biphenyl]-3-carboximidamide; 5''-fluoro-N',4-dihydroxy-2''-methoxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5''-fluoro-N',4-dihydroxy-2''-methyl-5'-(trifluoromethyl)-

[1,1':2',1''-terphenyl]-3'-carboximidamide; N',4'-dihydroxy-2''-methyl-5'-(trifluoromethyl)-
[1,1':2',1''-terphenyl]-3'-carboximidamide; N',4'-dihydroxy-5'-(trifluoromethyl)-[1,1':2',1''-
terphenyl]-3'-carboximidamide; N',4'-dihydroxy-2-(4-methylthiophen-3-yl)-5-(trifluoromethyl)-
[1,1'-biphenyl]-3-carboximidamide; 2-(2,4-dimethylthiophen-3-yl)-N',4'-dihydroxy-5-
5 (trifluoromethyl)-[1,1'-biphenyl]-3-carboximidamide; N',4'-dihydroxy-2-iodo-5-
(trifluoromethyl)-[1,1'-biphenyl]-3-carboximidamide; 2'',5''-difluoro-N',4'-dihydroxy-5'-propyl-
[1,1':2',1''-terphenyl]-3'-carboximidamide; 2-(1,3-dimethyl-1H-pyrrol-2-yl)-N',4'-dihydroxy-5-
propyl-[1,1'-biphenyl]-3-carboximidamide; 3'',5''-difluoro-N',4'-dihydroxy-5'-propyl-[1,1':2',1''-
terphenyl]-3'-carboximidamide; 2-(2,4-dimethylfuran-3-yl)-N',4'-dihydroxy-5-(trifluoromethyl)-
10 [1,1'-biphenyl]-3-carboximidamide; 3'-chloro-5'-fluoro-N',4'-dihydroxy-2-(3-methylthiophen-2-
yl)-5-propyl-[1,1'-biphenyl]-3-carboximidamide; N'-hydroxy-3-(1H-indazol-5-yl)-2-(3-
methylthiophen-2-yl)-5-propylbenzimidamide; 3'-fluoro-N',4'-dihydroxy-2-(3-methylthiophen-2-
yl)-5-propyl-[1,1'-biphenyl]-3-carboximidamide; 3'-chloro-N',4'-dihydroxy-2-(3-
methylthiophen-2-yl)-5-propyl-[1,1'-biphenyl]-3-carboximidamide; 3',5'-dichloro-N',4'-
15 dihydroxy-2-(3-methylthiophen-2-yl)-5-propyl-[1,1'-biphenyl]-3-carboximidamide; N',4'-
dihydroxy-3'-methyl-2-(3-methylthiophen-2-yl)-5-propyl-[1,1'-biphenyl]-3-carboximidamide; 2'-
fluoro-N',4'-dihydroxy-2-(3-methylthiophen-2-yl)-5-propyl-[1,1'-biphenyl]-3-carboximidamide;
2',3'-difluoro-N',4'-dihydroxy-2-(3-methylthiophen-2-yl)-5-propyl-[1,1'-biphenyl]-3-
carboximidamide; 2',5'-difluoro-N',4'-dihydroxy-2-(3-methylthiophen-2-yl)-5-propyl-[1,1'-
20 biphenyl]-3-carboximidamide; N',4'-dihydroxy-2-(2-methylallyl)-5-propyl-[1,1'-biphenyl]-3-
carboximidamide; 2-allyl-N',4'-dihydroxy-5-propyl-[1,1'-biphenyl]-3-carboximidamide; N',4'-
dihydroxy-5-propyl-2-vinyl-[1,1'-biphenyl]-3-carboximidamide; 5-bromo-N',4'-dihydroxy-2-(1-
methyl-1H-imidazol-5-yl)-[1,1'-biphenyl]-3-carboximidamide; N',4'-dihydroxy-5-propyl-2-
(pyridin-2-yl)-[1,1'-biphenyl]-3-carboximidamide; N',4'-dihydroxy-2-(2-methoxythiazol-4-yl)-5-
25 propyl-[1,1'-biphenyl]-3-carboximidamide; N',4'-dihydroxy-5-propyl-2-(thiazol-5-yl)-[1,1'-
biphenyl]-3-carboximidamide; N',4'-dihydroxy-5-propyl-2-(thiazol-2-yl)-[1,1'-biphenyl]-3-
carboximidamide; 5'-ethyl-N',4'-dihydroxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; N',4'-
dihydroxy-5'-isobutyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; N',4'-dihydroxy-5'-((E)-prop-1-
en-1-yl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-allyl-N',4'-dihydroxy-[1,1':2',1''-
30 terphenyl]-3'-carboximidamide; 5'-butyl-N',4'-dihydroxy-[1,1':2',1''-terphenyl]-3'-
carboximidamide; 2-(2,5-dimethyl-1H-pyrrol-1-yl)-N',4'-dihydroxy-5-(trifluoromethyl)-[1,1'-

biphenyl]-3-carboximidamide; 4-hydroxy-5'-propyl-[1,1':2',1''-terphenyl]-3'-carbaldehyde oxime
5'-propyl-3'-(1H-pyrazol-4-yl)-[1,1':2',1''-terphenyl]-4-ol; 5-chloro-2-(3,5-dimethylisoxazol-4-
yl)-N',4'-dihydroxy-3'-nitro-[1,1'-biphenyl]-3-carboximidamide; 5'-chloro-5''-fluoro-N',4'-
dihydroxy-2''-methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-chloro-5''-fluoro-N',4'-
5 dihydroxy-2''-methoxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5-chloro-N',4'-dihydroxy-2-
(4-methylthiophen-3-yl)-[1,1'-biphenyl]-3-carboximidamide; 5'-chloro-N',4'-dihydroxy-2''-
methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2-(2,4-dimethylthiophen-3-yl)-N',4'-
dihydroxy-[1,1'-biphenyl]-3-carboximidamide; 5-chloro-2-(2,4-dimethylthiophen-3-yl)-N',4'-
dihydroxy-[1,1'-biphenyl]-3-carboximidamide; 2''-chloro-5''-fluoro-N',4'-dihydroxy-5'-propyl-
10 [1,1':2',1''-terphenyl]-3'-carboximidamide; 6'-chloro-N',4'-dihydroxy-5'-propyl-[1,1':2',1''-
terphenyl]-3'-carboximidamide; N',4'-dihydroxy-5',6'-dipropyl-[1,1':2',1''-terphenyl]-3'-
carboximidamide; N',4'-dihydroxy-2-(3-methylthiophen-2-yl)-[1,1'-biphenyl]-3-
carboximidamide; 5'-bromo-6'-chloro-N',4'-dihydroxy-[1,1':2',1''-terphenyl]-3'-carboximidamide;
6'-chloro-N',4'-dihydroxy-5'-phenyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 6'-chloro-N',4'-
15 dihydroxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; N',4'-dihydroxy-6'-methyl-5'-propyl-
[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'',6'-difluoro-N',4'-dihydroxy-2''-methyl-5'-propyl-
[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'',6'-difluoro-4-hydroxy-2''-methyl-5'-propyl-
[1,1':2',1''-terphenyl]-3'-carboxamide; 4-hydroxy-6'-methyl-5'-propyl-[1,1':2',1''-terphenyl]-3'-
carbaldehyde oxime 5'',6'-difluoro-N',4'-dihydroxy-2''-methoxy-5'-propyl-[1,1':2',1''-terphenyl]-
20 3'-carboximidamide; 5'',6'-difluoro-4-hydroxy-2''-methoxy-5'-propyl-[1,1':2',1''-terphenyl]-3'-
carboxamide; 6'-fluoro-4-hydroxy-5'-propyl-[1,1':2',1''-terphenyl]-3'-carboxamide; 6'-fluoro-
N',4'-dihydroxy-2''-methyl-5'-propyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 6-fluoro-N',4'-
dihydroxy-2-(4-methylthiophen-3-yl)-5-propyl-[1,1'-biphenyl]-3-carboximidamide; 6'-fluoro-4-
hydroxy-2''-methyl-5'-propyl-[1,1':2',1''-terphenyl]-3'-carboxamide; 6-fluoro-4'-hydroxy-2-(4-
25 methylthiophen-3-yl)-5-propyl-[1,1'-biphenyl]-3-carboxamide; 2-(2,4-dimethylthiophen-3-yl)-6-
fluoro-N',4'-dihydroxy-5-propyl-[1,1'-biphenyl]-3-carboximidamide; 5'-chloro-5''-fluoro-4-
hydroxy-2''-methoxy-[1,1':2',1''-terphenyl]-3'-carboxamide; 6'-fluoro-N',4'-dihydroxy-5'-propyl-
[1,1':2',1''-terphenyl]-3'-carboximidamide; 5''-fluoro-N',4'-dihydroxy-2''-methoxy-[1,1':2',1''-
terphenyl]-3'-carboximidamide; N',4'-dihydroxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5''-
30 fluoro-N',4'-dihydroxy-2''-methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; N',4'-dihydroxy-5'-
(trifluoromethyl)-2''-vinyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2''-ethyl-N',4'-dihydroxy-

5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 3''-fluoro-N',4-dihydroxy-2''-methyl-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 3''-fluoro-N',4-dihydroxy-2''-methoxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5',6'-dichloro-5''-fluoro-N',4-dihydroxy-2''-methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 3'',5''-difluoro-N',4-dihydroxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5''-chloro-N',4-dihydroxy-2''-methoxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2''-ethynyl-N',4-dihydroxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 3''-chloro-5''-fluoro-N',4-dihydroxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5''-fluoro-N',4-dihydroxy-2'',5'-bis(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 3'',5''-difluoro-N',4-dihydroxy-2''-methoxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-methyl-3'-(1H-pyrazol-4-yl)-[1,1':2',1''-terphenyl]-4-ol; 3'',5''-difluoro-5'-propyl-3'-(1H-pyrazol-4-yl)-[1,1':2',1''-terphenyl]-4-ol; N',4-dihydroxy-5'-phenyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-benzyl-N',4-dihydroxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; N',4-dihydroxy-5'-phenethyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2,5''-difluoro-N',4-dihydroxy-2''-methyl-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2,5''-difluoro-N',4-dihydroxy-2''-methoxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2-fluoro-N',4-dihydroxy-2''-methyl-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2-fluoro-N',4-dihydroxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5''-chloro-2-fluoro-N',4-dihydroxy-2''-methoxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2,3''-difluoro-N',4-dihydroxy-2''-methoxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2,3'',5''-trifluoro-N',4-dihydroxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2-fluoro-N',4-dihydroxy-5'-(trifluoromethyl)-2''-vinyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5''-chloro-2-fluoro-4-hydroxy-2''-methoxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboxamide; 2-fluoro-4-hydroxy-5'-(trifluoromethyl)-2''-vinyl-[1,1':2',1''-terphenyl]-3'-carboxamide; 3''-chloro-2,5''-difluoro-N',4-dihydroxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 3''-chloro-2,5''-difluoro-4-hydroxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboxamide; 2''-ethynyl-2-fluoro-N',4-dihydroxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-chloro-3'',5''-difluoro-N',4-dihydroxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-chloro-3''-fluoro-N',4-dihydroxy-2''-methoxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5',5''-dichloro-N',4-dihydroxy-2''-methoxy-[1,1':2',1''-

terphenyl]-3'-carboximidamide; 3''-chloro-2-fluoro-N',4-dihydroxy-2''-methyl-5'-
 (trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 3''-chloro-2-fluoro-4-hydroxy-2''-
 methyl-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboxamide; N',4-dihydroxy-5'-methyl-2''-
 vinyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 3'',5''-difluoro-N',4-dihydroxy-2''-methoxy-5'-
 5 methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 3''-fluoro-N',4-dihydroxy-2''-methoxy-5'-
 methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5''-fluoro-N',4-dihydroxy-2''-methoxy-5'-
 methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 3''-chloro-5''-fluoro-N',4-dihydroxy-5'-
 methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5''-chloro-N',4-dihydroxy-2'',5'-dimethyl-
 [1,1':2',1''-terphenyl]-3'-carboximidamide; 5''-chloro-N',4-dihydroxy-2''-methyl-5'-
 10 (trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 3''-chloro-N',4-dihydroxy-2''-
 methyl-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5''-chloro-N',4-
 dihydroxy-2''-methoxy-5'-methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2''-chloro-5''-
 fluoro-N',4-dihydroxy-5'-methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; N',4-dihydroxy-5'-
 methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; N',4-dihydroxy-3'',5'-dimethyl-[1,1':2',1''-
 15 terphenyl]-3'-carboximidamide; 3''-chloro-N',4-dihydroxy-5'-methyl-[1,1':2',1''-terphenyl]-3'-
 carboximidamide; N',4-dihydroxy-3''-methoxy-5'-methyl-[1,1':2',1''-terphenyl]-3'-
 carboximidamide; 4''-fluoro-N',4-dihydroxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-
 carboximidamide; 4''-chloro-N',4-dihydroxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-
 carboximidamide; N',4-dihydroxy-2''-methoxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-
 20 carboximidamide; 4''-fluoro-N',4-dihydroxy-2''-methoxy-5'-(trifluoromethyl)-[1,1':2',1''-
 terphenyl]-3'-carboximidamide; N',4-dihydroxy-2''-isopropyl-5'-(trifluoromethyl)-[1,1':2',1''-
 terphenyl]-3'-carboximidamide; 2-(3,5-dimethylisoxazol-4-yl)-N',4'-dihydroxy-5-
 (trifluoromethyl)-[1,1'-biphenyl]-3-carboximidamide; 2-(3,5-dimethylisoxazol-4-yl)-4'-hydroxy-
 5-(trifluoromethyl)-[1,1'-biphenyl]-3-carboxamide; 3''-chloro-4-hydroxy-2''-methyl-5'-
 25 (trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboxamide; N',4-dihydroxy-2'',5''-dimethyl-5'-
 (trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; N',4-dihydroxy-2''-methoxy-5''-
 methyl-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2''-chloro-N',4-
 dihydroxy-5''-methyl-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-fluoro-
 N',4-dihydroxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5''-chloro-5'-fluoro-N',4-dihydroxy-
 30 2''-methoxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 3'',5'',5''-trifluoro-N',4-dihydroxy-[1,1':2',1''-
 methoxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 3'',5'',5''-trifluoro-N',4-dihydroxy-[1,1':2',1''-

terphenyl]-3'-carboximidamide; 3''-chloro-5',5''-difluoro-N',4-dihydroxy-[1,1':2',1''-terphenyl]-
3'-carboximidamide; 5'-fluoro-N',4-dihydroxy-2''-vinyl-[1,1':2',1''-terphenyl]-3'-
carboximidamide; 5'-chloro-N',4-dihydroxy-2'',5''-dimethyl-[1,1':2',1''-terphenyl]-3'-
carboximidamide; N',4-dihydroxy-2'',5''-dimethyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2''-
5 chloro-4-hydroxy-5''-methyl-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboxamide; 3''-
chloro-N',4-dihydroxy-5''-methyl-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide;
2'',5''-dichloro-N',4-dihydroxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide;
5''-chloro-N',4-dihydroxy-2''-methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5''-chloro-N',4-
dihydroxy-2''-methoxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 3'',5''-difluoro-N',4-
10 dihydroxy-5'-methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 3'',5''-difluoro-4-hydroxy-5'-
methyl-[1,1':2',1''-terphenyl]-3'-carboxamide; 3''-chloro-4-hydroxy-5''-methyl-5'-
(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboxamide; 2'',5''-dichloro-4-hydroxy-5'-
(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboxamide; 3'',5''-dichloro-N',4-dihydroxy-5'-
(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 3'',5''-dichloro-N',4-dihydroxy-5'-
15 methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2-(3,5-dimethylisoxazol-4-yl)-2'-fluoro-N',4'-
dihydroxy-5-(trifluoromethyl)-[1,1'-biphenyl]-3-carboximidamide; 2-(3,5-dimethylisoxazol-4-
yl)-2'-fluoro-4'-hydroxy-5-(trifluoromethyl)-[1,1'-biphenyl]-3-carboxamide; 3'',5''-difluoro-N',4-
dihydroxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 3'',5''-difluoro-4-hydroxy-[1,1':2',1''-
terphenyl]-3'-carboxamide; 3''-fluoro-N',4-dihydroxy-2''-methoxy-[1,1':2',1''-terphenyl]-3'-
20 carboximidamide; 3''-fluoro-4-hydroxy-2''-methoxy-[1,1':2',1''-terphenyl]-3'-carboxamide; 2''-
ethyl-N',4-dihydroxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 4''-chloro-2''-fluoro-N',4-
dihydroxy-5'-methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 4''-chloro-3''-fluoro-N',4-
dihydroxy-5'-methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; N',4-dihydroxy-2''-methoxy-
[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-chloro-N',4-dihydroxy-2''-methoxy-[1,1':2',1''-
25 terphenyl]-3'-carboximidamide; 4''-fluoro-N',4-dihydroxy-2''-methoxy-[1,1':2',1''-terphenyl]-3'-
carboximidamide; 5'-chloro-4''-fluoro-N',4-dihydroxy-2''-methoxy-[1,1':2',1''-terphenyl]-3'-
carboximidamide; 3''-chloro-N',4-dihydroxy-5',5''-dimethyl-[1,1':2',1''-terphenyl]-3'-
carboximidamide; 2'',5''-dichloro-N',4-dihydroxy-5'-methyl-[1,1':2',1''-terphenyl]-3'-
carboximidamide; 3'',5''-difluoro-4-hydroxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-
30 carboxamide; N',4-dihydroxy-4'',5'-dimethyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; N',4-
dihydroxy-2'',4'',5'-trimethyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 4''-fluoro-N',4-

dihydroxy-5'-methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2'',4''-difluoro-N',4-dihydroxy-5'-methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5''-chloro-5'-fluoro-N',4-dihydroxy-2''-methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5',5''-difluoro-N',4-dihydroxy-2''-methoxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2''-chloro-5'-fluoro-N',4-dihydroxy-5''-methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2'',5''-dichloro-5'-fluoro-N',4-dihydroxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-fluoro-N',4-dihydroxy-2''-methoxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 4'',5'-difluoro-N',4-dihydroxy-2''-methoxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-fluoro-N',4-dihydroxy-2'',5''-dimethyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-fluoro-N',4-dihydroxy-2''-methoxy-5''-methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; N',4-dihydroxy-2''-methoxy-5''-methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; N',4-dihydroxy-2''-methoxy-5',5''-dimethyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 4''-fluoro-N',4-dihydroxy-2''-methoxy-5'-methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2''-chloro-N',4-dihydroxy-5',5''-dimethyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; N',4-dihydroxy-5'-methyl-2''-(trifluoromethoxy)-[1,1':2',1''-terphenyl]-3'-carboximidamide; N',4-dihydroxy-2''-methoxy-5'-methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 3'',5''-difluoro-N',4-dihydroxy-5'-methoxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5''-fluoro-N',4-dihydroxy-2'',5'-dimethoxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2-(5-fluoro-2-methoxypyridin-3-yl)-N',4'-dihydroxy-5-methyl-[1,1'-biphenyl]-3-carboximidamide; N',4'-dihydroxy-5-methyl-2-(2-methylpyridin-3-yl)-[1,1'-biphenyl]-3-carboximidamide; N',4'-dihydroxy-2-(2-methoxypyridin-3-yl)-5-methyl-[1,1'-biphenyl]-3-carboximidamide; 2-(3,5-dimethylisothiazol-4-yl)-N',4'-dihydroxy-5-methyl-[1,1'-biphenyl]-3-carboximidamide; N',4-dihydroxy-5'-methoxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2-(3,5-dimethylisothiazol-4-yl)-4'-hydroxy-5-methyl-[1,1'-biphenyl]-3-carboxamide; 5-chloro-2-(5-fluoro-2-methoxypyridin-3-yl)-N',4'-dihydroxy-[1,1'-biphenyl]-3-carboximidamide; 2-(5-fluoro-2-methoxypyridin-3-yl)-4'-hydroxy-5-methyl-[1,1'-biphenyl]-3-carboxamide; 2-(3,5-dimethylisothiazol-4-yl)-N',4'-dihydroxy-5-(trifluoromethyl)-[1,1'-biphenyl]-3-carboximidamide; 5-chloro-2-(3,5-dimethylisothiazol-4-yl)-N',4'-dihydroxy-[1,1'-biphenyl]-3-carboximidamide; 4''-fluoro-N',4-dihydroxy-2''-methyl-5''-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2'',4''-difluoro-N',4-dihydroxy-5''-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; N',4'-dihydroxy-2-(4-methylpyridin-3-yl)-5-(trifluoromethyl)-[1,1'-biphenyl]-3-carboximidamide; 2-(2,5-dimethylpyridin-3-yl)-N',4'-

5 dihydroxy-5-(trifluoromethyl)-[1,1'-biphenyl]-3-carboximidamide; 2''-chloro-4''-fluoro-N',4'-
dihydroxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 4'',5''-difluoro-N',4'-
dihydroxy-2''-methyl-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2'',5''-
difluoro-N',4'-dihydroxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; N',4'-
10 dihydroxy-2-(pyridin-3-yl)-5-(trifluoromethyl)-[1,1'-biphenyl]-3-carboximidamide; 2-(2,3-
dihydrobenzofuran-7-yl)-N',4'-dihydroxy-5-(trifluoromethyl)-[1,1'-biphenyl]-3-
carboximidamide; 2-(benzofuran-7-yl)-N',4'-dihydroxy-5-(trifluoromethyl)-[1,1'-biphenyl]-3-
carboximidamide; N',4'-dihydroxy-2-(1-methyl-1H-indol-7-yl)-5-(trifluoromethyl)-[1,1'-
biphenyl]-3-carboximidamide; 2''-chloro-5''-fluoro-N',4'-dihydroxy-5'-(trifluoromethyl)-
15 [1,1':2',1''-terphenyl]-3'-carboximidamide; 5''-chloro-2''-fluoro-N',4'-dihydroxy-5'-
(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2-(4-fluorobenzofuran-7-yl)-N',4'-
dihydroxy-5-(trifluoromethyl)-[1,1'-biphenyl]-3-carboximidamide; 3'',4''-difluoro-N',4'-
dihydroxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-chloro-2'',4''-
difluoro-N',4'-dihydroxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-chloro-2'',5''-difluoro-
20 N',4'-dihydroxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2-(benzo[d][1,3]dioxol-4-yl)-N',4'-
dihydroxy-5-(trifluoromethyl)-[1,1'-biphenyl]-3-carboximidamide; 3'',4'',5''-trifluoro-N',4'-
dihydroxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-chloro-3'',4''-
difluoro-N',4'-dihydroxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-chloro-3'',4'',5''-trifluoro-
N',4'-dihydroxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5-chloro-N',4'-dihydroxy-2-(4-
25 methylpyridin-3-yl)-[1,1'-biphenyl]-3-carboximidamide; 5',5''-dichloro-2''-fluoro-N',4'-
dihydroxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2'',5'-dichloro-5''-fluoro-N',4'-dihydroxy-
[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-chloro-2''-fluoro-N',4'-dihydroxy-5''-methyl-
[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-chloro-2''-fluoro-N',4'-dihydroxy-4''-methyl-
[1,1':2',1''-terphenyl]-3'-carboximidamide; 2-(5-fluoro-2-methoxypyridin-3-yl)-N',4'-dihydroxy-
30 5-(trifluoromethyl)-[1,1'-biphenyl]-3-carboximidamide; 5'-chloro-N',4'-dihydroxy-2'',4''-
dimethoxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-chloro-4-hydroxy-2'',4''-dimethoxy-
[1,1':2',1''-terphenyl]-3'-carbonitrile; 5-chloro-2-(5-chloro-2-methoxypyridin-3-yl)-N',4'-
dihydroxy-[1,1'-biphenyl]-3-carboximidamide; 5-chloro-2-(5-chloro-2-methoxypyridin-3-yl)-4'-
hydroxy-[1,1'-biphenyl]-3-carboxamide; 5-chloro-2-(2,5-dimethylpyridin-3-yl)-N',4'-dihydroxy-
30 [1,1'-biphenyl]-3-carboximidamide; 5-chloro-2-(2,5-dimethylpyridin-3-yl)-4'-hydroxy-[1,1'-
biphenyl]-3-carboxamide; 2-(benzo[d][1,3]dioxol-4-yl)-5-chloro-N',4'-dihydroxy-[1,1'-

biphenyl]-3-carboximidamide; 5-chloro-N',4'-dihydroxy-2-(naphthalen-2-yl)-[1,1'-biphenyl]-3-carboximidamide; 5-chloro-N',4'-dihydroxy-2-(isoquinolin-6-yl)-[1,1'-biphenyl]-3-carboximidamide; 5-chloro-N',4'-dihydroxy-2-(quinolin-6-yl)-[1,1'-biphenyl]-3-carboximidamide; 5-chloro-N',4'-dihydroxy-2-(1-methyl-H-benzo[d]imidazol-5-yl)-[1,1'-biphenyl]-3-carboximidamide; 5-chloro-4'-hydroxy-2-(1-methyl-1H-benzo[d]imidazol-5-yl)-[1,1'-biphenyl]-3-carboxamide; 2-(5-chloro-2-methoxypyridin-3-yl)-N',4'-dihydroxy-5-(trifluoromethyl)-[1,1'-biphenyl]-3-carboximidamide; 2''-fluoro-N',4'-dihydroxy-5''-methyl-5''-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5-chloro-2-(6-chloro-2-methoxypyridin-3-yl)-N',4'-dihydroxy-[1,1'-biphenyl]-3-carboximidamide; 5-chloro-N',4'-dihydroxy-2-(2-methoxy-5-methylpyridin-3-yl)-[1,1'-biphenyl]-3-carboximidamide; 5-chloro-2-(cyclopent-1-en-1-yl)-N',4'-dihydroxy-[1,1'-biphenyl]-3-carboximidamide; 5-chloro-2-(cyclopent-1-en-1-yl)-4'-hydroxy-[1,1'-biphenyl]-3-carboxamide; 2-(cyclopent-1-en-1-yl)-N',4'-dihydroxy-5-(trifluoromethyl)-[1,1'-biphenyl]-3-carboximidamide; 2-(cyclopent-1-en-1-yl)-4'-hydroxy-5-(trifluoromethyl)-[1,1'-biphenyl]-3-carboxamide; 5'-bromo-5''-fluoro-N',4'-dihydroxy-2''-methoxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-bromo-5''-fluoro-N',4'-dihydroxy-2''-methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-bromo-3''-fluoro-N',4'-dihydroxy-2''-methoxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-bromo-N',4'-dihydroxy-2'',5''-dimethyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-bromo-N',4'-dihydroxy-5''-methoxy-2''-methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-bromo-N',4'-dihydroxy-2''-methoxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-bromo-4''-fluoro-N',4'-dihydroxy-2''-methoxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5''-fluoro-N',4'-dihydroxy-5'-(trifluoromethyl)-2''-vinyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2,5''-difluoro-N',4'-dihydroxy-5'-(trifluoromethyl)-2''-vinyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5''-chloro-N',4'-dihydroxy-5'-(trifluoromethyl)-2''-vinyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2''-ethynyl-5''-fluoro-N',4'-dihydroxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 4-hydroxy-2''-methoxy-5''-methyl-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboxamide; 5'-bromo-5''-chloro-N',4'-dihydroxy-2''-methyl-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-bromo-3'',5''-difluoro-N',4'-dihydroxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-bromo-3''-chloro-5''-fluoro-N',4'-dihydroxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-bromo-2''-chloro-5''-fluoro-N',4'-dihydroxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-bromo-4''-chloro-N',4'-dihydroxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-bromo-2'',5''-dichloro-N',4'-dihydroxy-[1,1':2',1''-terphenyl]-

3'-carboximidamide; 5'-bromo-2''-chloro-N',4-dihydroxy-5''-methyl-[1,1':2',1''-terphenyl]-3'-
carboximidamide; 5-bromo-2-(3,5-dimethylisoxazol-4-yl)-N',4'-dihydroxy-[1,1'-biphenyl]-3-
carboximidamide; 3-chloro-5-fluoro-N',4-dihydroxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-
3'-carboximidamide; 3-chloro-5-fluoro-4-hydroxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-
5 carboxamide; 3,5'-dichloro-3'',5''-trifluoro-N',4-dihydroxy-[1,1':2',1''-terphenyl]-3'-
carboximidamide; 3'-chloro-2-(3,5-dimethylisoxazol-4-yl)-5'-fluoro-N',4'-dihydroxy-5-
(trifluoromethyl)-[1,1'-biphenyl]-3-carboximidamide; 3-chloro-3'',5''-trifluoro-N',4-dihydroxy-
5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 3-chloro-5,5''-difluoro-N',4-
dihydroxy-2''-methoxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 3-chloro-
10 5-fluoro-N',4-dihydroxy-2''-methoxy-5'-(trifluoromethyl)-[1':2',1''-terphenyl]-3'-
carboximidamide; 5'-chloro-3'',5''-difluoro-N'-hydroxy-4-methoxy-3-methyl-[1,1':2',1''-
terphenyl]-3'-carboximidamide; 5'-chloro-3'',5''-difluoro-N',4-dihydroxy-3-methyl-[1,1':2',1''-
terphenyl]-3'-carboximidamide; 5-chloro-2-(3,5-dimethylisoxazol-4-yl)-N',4'-dihydroxy-3'-
methyl-[1,1'-biphenyl]-3-carboximidamide; 5'-chloro-N',4-dihydroxy-2'',3-dimethyl-[1,1':2',1''-
15 terphenyl]-3'-carboximidamide; 5'-chloro-5''-fluoro-N',4-dihydroxy-2''-methoxy-3-methyl-
[1,1':2',1''-terphenyl]-3'-carboximidamide; 5'-chloro-5''-fluoro-4-hydroxy-2''-methoxy-3-methyl-
[1,1':2',1''-terphenyl]-3'-carboxamide; 5'-chloro-3'',5''-difluoro-N',4-dihydroxy-3-isopropyl-
[1,1':2',1''-terphenyl]-3'-carboximidamide; 2'-(3,5-dimethylisoxazol-4-yl)-3'-(1H-1,2,3-triazol-4-
yl)-5'-(trifluoromethyl)-[1,1'-biphenyl]-4-ol; N',4-dihydroxy-5'-(trifluoromethoxy)-[1,1':2',1''-
20 terphenyl]-3'-carboximidamide; 3'',5''-difluoro-N',4-dihydroxy-5'-(trifluoromethoxy)-[1,1':2',1''-
terphenyl]-3'-carboximidamide; 5''-chloro-N',4-dihydroxy-2''-methoxy-5'-(trifluoromethoxy)-
[1,1':2',1''-terphenyl]-3'-carboximidamide; 4-hydroxy-5'-(trifluoromethoxy)-[1,1':2',1''-
terphenyl]-3'-carboxamide; 3'',5''-difluoro-4-hydroxy-5'-(trifluoromethoxy)-[1,1':2',1''-
terphenyl]-3'-carboxamide; N',4-dihydroxy-5'-isopropyl-[1,1':2',1''-terphenyl]-3'-
25 carboximidamide; 3'',5''-difluoro-N',4-dihydroxy-5'-isopropyl-[1,1':2',1''-terphenyl]-3'-
carboximidamide; 5''-chloro-N',4-dihydroxy-5'-isopropyl-2''-methoxy-[1,1':2',1''-terphenyl]-3'-
carboximidamide; 4-amino-5'-isopropyl-[1,1':2',1''-terphenyl]-3'-carboxamide; 3'',5''-difluoro-4-
hydroxy-5'-isopropyl-[1,1':2',1''-terphenyl]-3'-carboxamide; 2-(3-cyanofuran-2-yl)-N',4'-
dihydroxy-5-(trifluoromethyl)-[1,1'-biphenyl]-3-carboximidamide; 2-(4-cyano-1-methyl-1H-
30 pyrazol-5-yl)-N',4'-dihydroxy-5-(trifluoromethyl)-[1,1'-biphenyl]-3-carboximidamide; 2''-cyano-
N',4-dihydroxy-5'-(trifluoromethyl)-[1,1':2',1''-terphenyl]-3'-carboximidamide; 2-(3-cyano-1-

methyl-1H-pyrrol-2-yl)-N',4'-dihydroxy-5-(trifluoromethyl)-[1,1'-biphenyl]-3-carboximidamide;
2'-(3,5-dimethylisoxazol-4-yl)-3'-(hydroxymethyl)-5'-(trifluoromethyl)-[1,1'-biphenyl]-4-ol; 5'-
cyano-3'',5''-difluoro-N',4'-dihydroxy-[1,1':2',1''-terphenyl]-3'-carboximidamide; N',4'-
dihydroxy-2-(pyrrolidin-1-yl)-5-(trifluoromethyl)-[1,1'-biphenyl]-3-carboximidamide; 4-chloro-
5 3',5'-difluoro-N'-hydroxy-6-(1H-indazol-5-yl)-[1,1'-biphenyl]-2-carboximidamide; 6-(1H-
benzo[d]imidazol-5-yl)-4-chloro-N'-hydroxy-[1,1'-biphenyl]-2-carboximidamide; 4-chloro-3',5'-
difluoro-N'-hydroxy-6-(1H-indol-5-yl)-[1,1'-biphenyl]-2-carboximidamide; 4-chloro-3',5'-
difluoro-6-(1H-indol-5-yl)-[1,1'-biphenyl]-2-carboxamide; 4-chloro-3',5'-difluoro-6-(1H-
indazol-5-yl)-[1,1'-biphenyl]-2-carboxamide; 6-(1H-benzo[d]imidazol-5-yl)-4-chloro-3',5'-
10 difluoro-[1,1'-biphenyl]-2-carboxamide; 3',5'-difluoro-N'-hydroxy-6-(1H-indol-5-yl)-4-methyl-
[1,1'-biphenyl]-2-carboximidamide; 3',5'-difluoro-N'-hydroxy-6-(1H-indol-6-yl)-4-methyl-[1,1'-
biphenyl]-2-carboximidamide; 3',5'-difluoro-N'-hydroxy-6-(1H-indazol-5-yl)-4-methyl-[1,1'-
biphenyl]-2-carboximidamide; 3',5'-difluoro-N'-hydroxy-6-(1H-indazol-5-yl)-4-propyl-[1,1'-
biphenyl]-2-carboximidamide; 3',5'-difluoro-N'-hydroxy-6-(1H-indol-5-yl)-4-propyl-[1,1'-
15 biphenyl]-2-carboximidamide; 3',5'-difluoro-N'-hydroxy-6-(1H-indazol-5-yl)-4-
(trifluoromethyl)-[1,1'-biphenyl]-2-carboximidamide; 3',5'-difluoro-N'-hydroxy-6-(1H-indol-6-
yl)-4-(trifluoromethyl)-[1,1'-biphenyl]-2-carboximidamide; 3',5'-difluoro-N'-hydroxy-6-(1H-
indazol-6-yl)-4-(trifluoromethyl)-[1,16'-biphenyl]-2-carboximidamide; 3',5'-difluoro-N'-
hydroxy-6-(1H-indol-5-yl)-4-(trifluoromethyl)-[1,1'-biphenyl]-2-carboximidamide; 3',5'-
20 difluoro-6-(1H-indol-5-yl)-4-(trifluoromethyl)-[1,1'-biphenyl]-2-carboxamide; 3',5'-difluoro-N'-
hydroxy-6-(1H-indazol-6-yl)-4-propyl-[1,1'-biphenyl]-2-carboximidamide; N'-hydroxy-6-(1H-
indazol-5-yl)-2'-methoxy-5'-methyl-4-(trifluoromethyl)-[1,1'-biphenyl]-2-carboximidamide; 5'-
chloro-N'-hydroxy-6-(1H-indol-5-yl)-2'-methoxy-4-(trifluoromethyl)-[1,1'-biphenyl]-2-
carboximidamide; 5'-chloro-N'-hydroxy-6-(1H-indazol-5-yl)-2'-methoxy-4-(trifluoromethyl)-
25 [1,1'-biphenyl]-2-carboximidamide; 2',5'-difluoro-N'-hydroxy-6-(1H-indazol-5-yl)-4-
(trifluoromethyl)-[1,1'-biphenyl]-2-carboximidamide; 3',5'-difluoro-6-(6-fluoro-1H-indol-5-yl)-
N'-hydroxy-4-(trifluoromethyl)-[1,1'-biphenyl]-2-carboximidamide; N'-hydroxy-3-(1H-indazol-
5-yl)-2-(naphthalen-1-yl)-5-(trifluoromethyl)benzimidamide; 2-(benzo[d][1,3]dioxol-4-yl)-N'-
hydroxy-3-(1H-indazol-5-yl)-5-(trifluoromethyl)benzimidamide; 4'-fluoro-N'-hydroxy-6-(1H-
30 indazol-5-yl)-2'-methoxy-4-(trifluoromethyl)-[1,1'-biphenyl]-2-carboximidamide; N'-hydroxy-6-
(1H-indazol-5-yl)-3'-methyl-4-(trifluoromethyl)-[1,1'-biphenyl]-2-carboximidamide; 3',4',5'-

trifluoro-N'-hydroxy-6-(1H-indazol-5-yl)-4-(trifluoromethyl)-[1,1'-biphenyl]-2-carboximidamide; 3',4'-difluoro-N'-hydroxy-6-(1H-indazol-5-yl)-4-(trifluoromethyl)-[1,1'-biphenyl]-2-carboximidamide; N'-hydroxy-3-(1H-indazol-5-yl)-2-(2-methoxypyridin-3-yl)-5-(trifluoromethyl)benzimidamide; 2-(cyclopent-1-en-1-yl)-N'-hydroxy-3-(1H-indazol-5-yl)-5-(trifluoromethyl)benzimidamide; 2-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-3-(1H-indazol-5-yl)-5-(trifluoromethyl)benzimidamide; or 2',4'-difluoro-N'-hydroxy-6-(1H-indazol-5-yl)-4-(trifluoromethyl)-[1,1'-biphenyl]-2-carboximidamide.

The ER β ligand may be 2-Bromo-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-thiophen-3-yl-1H-indole-3-carbonitrile; 2-(3-Cyano-furan-2-yl)-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-pyrrol-1-yl-1H-indole-3-carbonitrile; 2-Dimethylamino-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-isopropyl-1H-indole-3-carbonitrile; 2-Acetyl-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 2-(3,5-Dimethyl-isoxazol-4-yl)-1-(4-hydroxy-phenyl)-1H-indole-3-carboxylic acid; 1-[1-(4-Hydroxy-phenyl)-2-phenyl-1H-indol-3-yl]-ethanone; 1-(4-Hydroxy-phenyl)-2-phenyl-1H-indole-3-carboxylic acid, amide; (Z)-2-(3,5-dimethyl isoxazol-4-yl)-N'-hydroxy-1-(4-hydroxyphenyl)-1H-indole-3-carboximidamide; [2-(3,5-Dimethyl-isoxazol-4-yl)-1-(4-hydroxyphenyl)-1H-indol-3-yl]-carbamic acid tert-butyl ester; 4-[3-Amino-2-(3,5-dimethyl-isoxazol-4-yl)-indol-1-yl]-phenol; (Z)-2-(3,5-dimethylisoxazol-4-yl)-7-fluoro-N'-hydroxy-1-(4-hydroxyphenyl)-1H-indole-3-carboximidamide; (Z)-2-(5-chlorothiophen-2-yl)-N'-hydroxy-1-(4-hydroxyphenyl)-1H-indole-3-carboximidamide; 1-(2,3-Difluoro-4-hydroxy-phenyl)-2-(3,5-dimethyl-isoxazol-4-yl)-1H-indole-3-carbonitrile; 2-(3,5-dimethylisoxazol-4-yl)-1-(4-hydroxyphenyl)-1H-indole-3-carbohydrazonamide; 4-(2-(3,5-dimethylisoxazol-4-yl)-3-(1,2,4-oxadiazol-3-yl)-1H-indol-1-yl)phenol; 2-(3,5-Dimethyl-isoxazol-4-yl)-1-(4-hydroxy-phenyl)-1H-indole-3-carboxylic acid, methyl ester; 2-(3,5-Dimethyl-isoxazol-4-yl)-1-(4-hydroxyphenyl)-1H-indole-3-carboxylic acid, hydroxyamide; 4-[2-(3,5-Dimethyl-isoxazol-4-yl)-3-methanesulfonyl-indol-1-yl]-phenol; 1-[2-(3,5-Dimethyl-isoxazol-4-yl)-1-(4-hydroxy-phenyl)-1H-indol-3-yl]-2,2,2-trifluoro-ethanone; 4-(3-bromo-2-(3,5-dimethylisoxazol-4-yl)-1H-indol-1-yl)phenol; 2-Bromo-5-fluoro-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; (Z)-2-(4-fluorophenoxy)-N'-hydroxy-1-(4-hydroxyphenyl)-1H-indole-3-carboximidamide; 4-(2-(3,5-dimethylisoxazol-4-yl)-3-nitro-1H-indol-1-yl)phenol; 4-(3-(dihydroxyamino)-2-(3,5-dimethylisoxazol-4-yl)-1H-indol-1-yl)phenol; N-(2-(3,5-dimethylisoxazol-4-yl)-1-(4-

hydroxyphenyl)-1H-indol-3-yl)acetamide N-(2-(3,5-dimethylisoxazol-4-yl)-1-(4-hydroxyphenyl)-1H-indol-3-yl)methanesulfonamide; 1-(2-(3,5-dimethylisoxazol-4-yl)-1-(4-hydroxyphenyl)-1H-indol-3-yl)urea; 4-(2-(3,5-dimethylisoxazol-4-yl)-3-thiocyanato-1H-indol-1-yl)phenol; (E)-2-(3,5-dimethylisoxazol-4-yl)-1-(4-hydroxyphenyl)-1H-indol-3-yl N'-hydroxycarbamimidothioate; 4-(3-benzyl-2-phenyl-1H-indol-1-yl)phenol; 2-(2-(3,5-dimethylisoxazol-4-yl)-1-(4-hydroxyphenyl)-1H-indol-3-yl)-2-oxoacetamide; (Z)-2-(2-(3,5-dimethylisoxazol-4-yl)-1-(4-hydroxyphenyl)-1H-indol-3-yl)-2-(hydroxyimino)acetamide; 2-(2-(3,5-dimethylisoxazol-4-yl)-1-(4-hydroxyphenyl)-1H-indol-3-yl)-2-hydroxyacetamide; 2-(2-(3,5-dimethylisoxazol-4-yl)-1-(4-hydroxyphenyl)-1H-indol-3-yl)acetamide; 2-((Z)-But-1-enyl)-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-(2-methyl-prop-1-enyl)-1H-indole-3-carbonitrile; 1-(2,3-Difluoro-4-hydroxy-phenyl)-2-(2-methyl-allyl)-1H-indole-3-carbonitrile; (Z)-2-(5-ethyl-3-methylisoxazol-4-yl)-N'-hydroxy-1-(4-hydroxyphenyl)-1H-indole-3-carboximidamide; 4-(2-(3,5-dimethylisoxazol-4-yl)-3-phenyl-1H-indol-1-yl)phenol; 4-(3-chloro-2-(3,5-dimethylisoxazol-4-yl)-1H-indol-1-yl)phenol; 2-(3,5-dimethylisoxazol-4-yl)-1-(4-hydroxyphenyl)-1H-indole-3-sulfonamide; 2-(3,5-dimethylisoxazol-4-yl)-1-(2-fluoro-4-hydroxyphenyl)-1H-indole-3-carboximidamide; 1-(4-Hydroxy-phenyl)-2-phenyl-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-methyl-1H-indole-3-carbonitrile; 2-(3-Cyano-thiophen-2-yl)-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-((E)-propenyl)-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-thiophen-2-yl-1H-indole-3-carbonitrile; 2-(3,5-Dimethyl-isoxazol-4-yl)-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-pyridin-4-yl-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-(1-methyl-1H-pyrrol-2-yl)-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-(3-methyl-thiophen-2-yl)-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-isopropylamino-1H-indole-3-carbonitrile; 2-Ethylamino-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 2-Butylamino-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-piperidin-1-yl-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-pyrrolidin-1-yl-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-morpholin-4-yl-1H-indole-3-carbonitrile; 2-Diethylamino-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 2-Ethynyl-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-vinyl-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-1H-indole-2,3-dicarbonitrile; 1-(4-Hydroxy-phenyl)-2-prop-1-ynyl-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-pyridin-2-yl-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-(2-methyl-allyl)-1H-indole-3-carbonitrile; 1-(4-

Hydroxy-phenyl)-2-((Z)-propenyl)-1H-indole-3-carbonitrile; 2-(Butyl-methyl-amino)-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-((Z)-1-methyl-propenyl)-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-imidazol-1-yl-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-[1,2,4]triazol-1-yl-1H-indole-3-carbonitrile; 2-(3,5-Dimethyl-pyrazol-1-yl)-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-pyrazol-1-yl-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-(5-methyl-imidazol-1-yl)-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-(5-methyl-pyrazol-1-yl)-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-(3-methyl-pyrazol-1-yl)-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-thiazol-2-yl-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-(2-methoxy-thiazol-4-yl)-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-thiazol-4-yl-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-(3-methyl-but-2-enyl)-1H-indole-3-carbonitrile; 2-((E)-But-1-enyl)-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-(5-methyl-thiophen-2-yl)-1H-indole-3-carbonitrile; 2-(5-Acetyl-thiophen-2-yl)-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-(1-methyl-1H-pyrazol-4-yl)-1H-indole-3-carbonitrile; 2-(5-Chloro-thiophen-2-yl)-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-(4-methyl-thiophen-3-yl)-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-(4-methyl-thiophen-2-yl)-1H-indole-3-carbonitrile; 2-(4-Cyano-thiophen-3-yl)-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-(2-methyl-2H-pyrazol-3-yl)-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-(1,3,5-trimethyl-1H-pyrazol-4-yl)-1H-indole-3-carbonitrile; 2-(2-Acetyl-pyrrol-1-yl)-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 2-(2-Ethyl-pyrrol-1-yl)-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 2-(2-Cyano-pyrrol-1-yl)-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 1-(4-Hydroxy-phenyl)-2-(2-methyl-pyrrol-1-yl)-1H-indole-3-carbonitrile; 1-(3-Chloro-5-fluoro-4-hydroxy-phenyl)-2-phenyl-1H-indole-3-carbonitrile; 1-(3-Chloro-5-fluoro-4-hydroxy-phenyl)-2-(3-cyano-thiophen-2-yl)-1H-indole-3-carbonitrile; 1-(3-Chloro-5-fluoro-4-hydroxy-phenyl)-2-(3-cyano-furan-2-yl)-1H-indole-3-carbonitrile; 2-Bromo-1-(3-chloro-5-fluoro-4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 2-Bromo-1-(2-fluoro-4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 1-(2-Fluoro-4-hydroxy-phenyl)-2-phenyl-1H-indole-3-carbonitrile; 2-Bromo-1-(3-fluoro-4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 2-Bromo-1-(2,3-difluoro-4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 2-Bromo-1-(2,5-difluoro-4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 2-Bromo-1-(3-chloro-4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 2-Bromo-1-(3,5-difluoro-4-hydroxy-phenyl)-1H-indole-3-

carbonitrile; 1-(3-Fluoro-4-hydroxy-phenyl)-2-phenyl-1H-indole-3-carbonitrile; 1-(3,5-Difluoro-4-hydroxy-phenyl)-2-phenyl-1H-indole-3-carbonitrile; 1-(3-Chloro-4-hydroxy-phenyl)-2-phenyl-1H-indole-3-carbonitrile; 1-(2,3-Difluoro-4-hydroxy-phenyl)-2-phenyl-1H-indole-3-carbonitrile; 1-(2,5-Difluoro-4-hydroxy-phenyl)-2-phenyl-1H-indole-3-carbonitrile; 1-(3,5-Difluoro-4-hydroxy-phenyl)-2-thiophen-3-yl-1H-indole-3-carbonitrile; 1-(3,5-Difluoro-4-hydroxy-phenyl)-2-thiophen-2-yl-1H-indole-3-carbonitrile; 1-(3,5-Difluoro-4-hydroxy-phenyl)-2-(3,5-dimethyl-isoxazol-4-yl)-1H-indole-3-carbonitrile; 1-(3,5-Difluoro-4-hydroxy-phenyl)-2-(1-methyl-1H-pyrrol-2-yl)-1H-indole-3-carbonitrile; 1-(3,5-Difluoro-4-hydroxy-phenyl)-2-(3-methyl-thiophen-2-yl)-1H-indole-3-carbonitrile; 1-(3,5-Difluoro-4-hydroxy-phenyl)-2-(1-methyl-1H-pyrazol-4-yl)-1H-indole-3-carbonitrile; 1-(3,5-Difluoro-4-hydroxy-phenyl)-2-pyridin-4-yl-1H-indole-3-carbonitrile; 1-(3-Chloro-4-hydroxy-phenyl)-2-thiophen-3-yl-1H-indole-3-carbonitrile; 1-(3-Chloro-4-hydroxy-phenyl)-2-thiophen-2-yl-1H-indole-3-carbonitrile; 1-(3-Chloro-4-hydroxy-phenyl)-2-(3,5-dimethyl-isoxazol-4-yl)-1H-indole-3-carbonitrile; 1-(3-Chloro-4-hydroxy-phenyl)-2-(1-methyl-1H-pyrrol-2-yl)-1H-indole-3-carbonitrile; 1-(3-Chloro-4-hydroxy-phenyl)-2-(3-methyl-thiophen-2-yl)-1H-indole-3-carbonitrile; 1-(3-Chloro-4-hydroxy-phenyl)-2-pyridin-4-yl-1H-indole-3-carbonitrile; 1-(3-Fluoro-4-hydroxy-phenyl)-2-thiophen-3-yl-1H-indole-3-carbonitrile; 1-(3-Fluoro-4-hydroxy-phenyl)-2-thiophen-2-yl-1H-indole-3-carbonitrile; 2-(3,5-Dimethyl-isoxazol-4-yl)-1-(3-fluoro-4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 1-(3-Fluoro-4-hydroxy-phenyl)-2-(1-methyl-1H-pyrrol-2-yl)-1H-indole-3-carbonitrile; 1-(3-Fluoro-4-hydroxy-phenyl)-2-(3-methyl-thiophen-2-yl)-1H-indole-3-carbonitrile; 1-(3-Fluoro-4-hydroxy-phenyl)-2-(1-methyl-1H-pyrazol-4-yl)-1H-indole-3-carbonitrile; 1-(3-Fluoro-4-hydroxy-phenyl)-2-pyridin-4-yl-1H-indole-3-carbonitrile; 2-Dimethylamino-1-(2-fluoro-4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 2-(3,5-Dimethyl-isoxazol-4-yl)-1-(2-fluoro-4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 1-(3-Fluoro-4-hydroxy-phenyl)-2-((E)-propenyl)-1H-indole-3-carbonitrile; 1-(3-Fluoro-4-hydroxy-phenyl)-2-((Z)-propenyl)-1H-indole-3-carbonitrile; 1-(2,3-Difluoro-4-hydroxy-phenyl)-2-((Z)-propenyl)-1H-indole-3-carbonitrile; 1-(2,3-Difluoro-4-hydroxy-phenyl)-2-vinyl-1H-indole-3-carbonitrile; 1-(2,3-Difluoro-4-hydroxy-phenyl)-2-thiophen-3-yl-1H-indole-3-carbonitrile; 1-(2,3-Difluoro-4-hydroxy-phenyl)-2-thiophen-2-yl-1H-indole-3-carbonitrile; 1-(2,3-Difluoro-4-hydroxy-phenyl)-2-(3-methyl-thiophen-2-yl)-1H-indole-3-carbonitrile; 1-(2,3-Difluoro-4-hydroxy-phenyl)-2-(1-methyl-1H-pyrrol-2-yl)-1H-indole-3-carbonitrile; 2-(2-Acetyl-pyrrol-1-yl)-1-(3-fluoro-4-

hydroxy-phenyl)-1H-indole-3-carbonitrile; 1-(3-Fluoro-4-hydroxy-phenyl)-2-pyrrol-1-yl-1H-indole-3-carbonitrile; 1-(2,3-Difluoro-4-hydroxy-phenyl)-2-pyrrol-1-yl-1H-indole-3-carbonitrile; 1-(2,3-Difluoro-4-hydroxy-phenyl)-2-prop-1-ynyl-1H-indole-3-carbonitrile; 1-(3-Fluoro-4-hydroxy-phenyl)-2-(2-methyl-prop-1-enyl)-1H-indole-3-carbonitrile; 1-(2,3-Difluoro-4-hydroxy-phenyl)-2-(2-methyl-prop-1-enyl)-1H-indole-3-carbonitrile; 2-(2-Acetyl-pyrrol-1-yl)-1-(2,3-difluoro-4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 1-(3-Fluoro-4-hydroxy-phenyl)-2-pyrazol-1-yl-1H-indole-3-carbonitrile; 1-(2,3-Difluoro-4-hydroxy-phenyl)-2-pyrazol-1-yl-1H-indole-3-carbonitrile; 2-(2,5-Dimethyl-pyrrol-1-yl)-1-(3-fluoro-4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 2-(2-Ethyl-pyrrol-1-yl)-1-(3-fluoro-4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 2-(2-Cyano-pyrrol-1-yl)-1-(3-fluoro-4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 1-(3-Fluoro-4-hydroxy-phenyl)-2-(2-methyl-pyrrol-1-yl)-1H-indole-3-carbonitrile; 1-(2,3-Difluoro-4-hydroxy-phenyl)-2-(2-ethyl-pyrrol-1-yl)-1H-indole-3-carbonitrile; 2-(2-Cyano-pyrrol-1-yl)-1-(2,3-difluoro-4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 1-(2,3-Difluoro-4-hydroxy-phenyl)-2-(2-methyl-pyrrol-1-yl)-1H-indole-3-carbonitrile; 1-(2-Fluoro-4-hydroxy-phenyl)-2-pyrrol-1-yl-1H-indole-3-carbonitrile; 1-(2,3-difluoro-4-hydroxyphenyl)-2-(3-methylbut-2-enyl)-1H-indole-3-carbonitrile; [1-(4-Hydroxy-phenyl)-2-phenyl-1H-indol-3-yl]-acetonitrile; [1-(4-Hydroxy-phenyl)-2-phenyl-1H-indol-3-yl]-acetic acid; 2-[1-(4-Hydroxy-phenyl)-2-phenyl-1H-indol-3-yl]-acetamide; 4-(3-Isopropenyl-2-phenyl-indol-1-yl)-phenol; 4-[3-(2-Methyl-2H-pyrazol-3-yl)-2-phenyl-indol-1-yl]-phenol; 4-(2-Phenyl-3-thiazol-4-yl-indol-1-yl)-phenol; 4-(2-Phenyl-3-prop-1-ynyl-indol-1-yl)-phenol; 1-(4-Hydroxy-phenyl)-2-((E)-propenyl)-1H-indole-3-carboxylic acid, amide; 1-(4-Hydroxy-phenyl)-2-(2-methyl-prop-1-enyl)-1H-indole-3-carboxylic acid, amide; 1-(4-Hydroxy-phenyl)-2-((Z)-1-methyl-propenyl)-1H-indole-3-carboxylic acid, amide; 4-(2-Phenyl-3-pyrazol-1-yl-indol-1-yl)-phenol; 4-(3-Imidazol-1-yl-2-phenyl-indol-1-yl)-phenol; 4-[3-(5-Methyl-pyrazol-1-yl)-2-phenyl-indol-1-yl]-phenol; 2-Bromo-1-(4-hydroxy-phenyl)-1H-indole-3-carboxylic acid, amide; 1-(4-Hydroxy-phenyl)-2-((Z)-3,3,3-trifluoro-propenyl)-1H-indole-3-carbonitrile; (Z)-2-bromo-N'-hydroxy-1-(4-hydroxyphenyl)-1H-indole-3-carboximidamide; (Z)-N'-hydroxy-1-(4-hydroxyphenyl)-2-(1H-pyrrol-1-yl)-1H-indole-3-carboximidamide; 2-(3,5-Dimethyl-isoxazol-4-yl)-1-(4-hydroxy-phenyl)-1H-indole-3-carboxylic acid, amide; (Z)-N'-hydroxy-1-(4-hydroxyphenyl)-2-(2-methylprop-1-enyl)-1H-indole-3-carboximidamide; 1-(4-Hydroxy-phenyl)-2-phenyl-1H-indole-3-carboxylic acid; hydroxyamide; (Z)-N'-hydroxy-1-(4-hydroxyphenyl)-2-phenyl-1H-indole-3-carboximidamide; 1-(4-Hydroxy-

phenyl)-2-pyrrol-1-yl-1H-indole-3-carboxylic acid, amide; [1-(4-Hydroxy-phenyl)-2-pyrrol-1-yl-1H-indol-3-yl]-carbamic acid tert-butyl ester; 2-(3,5-Dimethyl-isoxazol-4-yl)-1-(4-hydroxy-phenyl)-N-methyl-1H-indole-3-carboxamidine; Methyl 2-(3,5-dimethylisoxazol-4-yl)-1-(4-hydroxyphenyl)-1H-indole-3-carb imidate; N-((2-(3,5-dimethylisoxazol-4-yl)-1-(4-hydroxyphenyl)-1H-indol-3-yl)(imino)methyl)acetamide; 2-(5-ethyl-3-methylisoxazol-4-yl)-1-(4-hydroxyphenyl)-1H-indole-3-carboxamide; (Z)-2-(2-ethyl-1H-pyrrol-1-yl)-N-hydroxy-1-(4-hydroxyphenyl)-1H-indole-3-carboximidamide; (Z)—N'-hydroxy-1-(4-hydroxyphenyl)-2-(2-methyl-1H-pyrrol-1-yl)-1H-indole-3-carboximidamide; 1-(4-hydroxyphenyl)-2-(2-methyl-1H-pyrrol-1-yl)-1H-indole-3-carboxamide; 4-(3-chloro-2-(3,5-dimethylisoxazol-4-yl)-1H-indol-1-yl)phenol; (Z)-2-((Z)-but-2-en-2-yl)-N'-hydroxy-1-(4-hydroxyphenyl)-1H-indole-3-carboximidamide; (Z)—N'-hydroxy-1-(4-hydroxyphenyl)-2-(5-methyl-1H-pyrazol-1-yl)-1H-indole-3-carboximidamide; (Z)—N'-hydroxy-1-(4-hydroxyphenyl)-2-(4-methylthiophen-3-yl)-1H-indole-3-carboximidamide; (Z)-2-(2,5-dimethyl-1H-pyrrol-1-yl)-1H-hydroxy-1-(4-hydroxyphenyl)-1H-indole-3-carboximidamide; (Z)—N'-hydroxy-1-(4-hydroxyphenyl)-2-phenoxy-1H-indole-3-carboximidamide; 1-(4-Hydroxy-phenyl)-2-phenyl-1H-indole-3-carboxylic acid; 2-(3,5-Dimethyl-isoxazol-4-yl)-1-(3-fluoro-4-hydroxy-phenyl)-1H-indole-3-carboxylic acid; 2-(3,5-Dimethyl-isoxazol-4-yl)-1-(2-fluoro-4-hydroxy-phenyl)-1H-indole-3-carboxylic acid; 1-(2,3-Difluoro-4-hydroxy-phenyl)-2-(3,5-dimethyl-isoxazol-4-yl)-1H-indole-3-carboxylic acid; 1-(4-Hydroxy-phenyl)-2-((Z)-propenyl)-1H-indole-3-carboxylic acid; 1-(4-Hydroxy-phenyl)-2-((E)-propenyl)-1H-indole-3-carboxylic acid; 1-(4-Hydroxy-phenyl)-2-(2-methyl-prop-1-enyl)-1H-indole-3-carboxylic acid; 1-(4-Hydroxy-phenyl)-2-(2-methyl-allyl)-1H-indole-3-carboxylic acid; 1-(4-Hydroxy-phenyl)-2-((Z)-1-methyl-propenyl)-1H-indole-3-carboxylic acid; 1-(3-Fluoro-4-hydroxy-phenyl)-2-thiophen-3-yl-1H-indole-3-carboxylic acid; 1-(3-Fluoro-4-hydroxy-phenyl)-2-thiophen-2-yl-1H-indole-3-carboxylic acid; 1-(3-Fluoro-4-hydroxy-phenyl)-2-(1-methyl-1H-pyrrol-2-yl)-1H-indole-3-carboxylic acid; 1-(3-Fluoro-4-hydroxy-phenyl)-2-(3-methyl-thiophen-2-yl)-1H-indole-3-carboxylic acid; 2-Bromo-1-(4-hydroxy-phenyl)-1H-indole-3-carboxylic acid; 1-(4-Hydroxy-phenyl)-2-pyrrol-1-yl-1H-indole-3-carboxylic acid; 2,7-Dibromo-1-(2,5-difluoro-4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 2-Bromo-4-fluoro-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 4-Fluoro-1-(4-hydroxy-phenyl)-2-pyrrol-1-yl-1H-indole-3-carbonitrile; 4-Fluoro-1-(4-hydroxy-phenyl)-2-phenyl-1H-indole-3-carbonitrile; 2-(3,5-Dimethyl-isoxazol-4-yl)-4-fluoro-1-(4-hydroxy-phenyl)-1H-indole-

3-carbonitrile; 4-Fluoro-1-(4-hydroxy-phenyl)-2-(2-methyl-prop-1-enyl)-1H-indole-3-carbonitrile; 5-Fluoro-1-(4-hydroxy-phenyl)-2-phenyl-1H-indole-3-carbonitrile; 2-(3,5-Dimethyl-isoxazol-4-yl)-5-fluoro-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 5-Fluoro-1-(4-hydroxy-phenyl)-2-pyrrol-1-yl-1H-indole-3-carbonitrile; 5-Fluoro-1-(4-hydroxy-phenyl)-2-(2-methyl-prop-1-enyl)-1H-indole-3-carbonitrile; (Z)-2-(3,5-dimethylisoxazol-4-yl)-5-fluoro-N'-hydroxy-1-(4-hydroxyphenyl)-1H-indole-3-carboximidamide; (Z)-2-(3,5-dimethylisoxazol-4-yl)-4-fluoro-N'-hydroxy-1-(4-hydroxyphenyl)-1H-indole-3-carboximidamide; (Z)-5-fluoro-N'-hydroxy-1-(4-hydroxyphenyl)-2-(2-methylprop-1-enyl)-1H-indole-3-carboximidamide; 4-Chloro-2-(3,5-dimethyl-isoxazol-4-yl)-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 2-(3,5-dimethylisoxazol-4-yl)-4,5-difluoro-1-(4-hydroxyphenyl)-1H-indole-3-carbonitrile; 2-(4-cyano-1-methyl-1H-pyrazol-5-yl)-4-fluoro-1-(4-hydroxyphenyl)-1H-indole-3-carbonitrile; 2-(3,5-dimethylisoxazol-4-yl)-5-fluoro-1-(4-hydroxyphenyl)-1H-indole-3-carboximidamide; 2-(3,5-dimethylisoxazol-4-yl)-5-fluoro-1-(4-hydroxyphenyl)-1H-indole-3-carboxamide; 2-Bromo-7-fluoro-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 2-(3,5-Dimethyl-isoxazol-4-yl)-7-fluoro-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; 2-(3,5-Dimethyl-isoxazol-4-yl)-4,7-difluoro-1-(4-hydroxy-phenyl)-1H-indole-3-carbonitrile; (Z)-2-(3,5-dimethylisoxazol-4-yl)-4,7-difluoro-N'-hydroxy-1-(4-hydroxyphenyl)-1H-indole-3-carboximidamide; 1-(2,5-Difluoro-4-hydroxyphenyl)-2-(3,5-dimethyl-isoxazol-4-yl)-1H-indole-3-carbonitrile; 1-(3-bromo-4-hydroxyphenyl)-2-(2-methylprop-1-enyl)-1H-indole-3-carboxamide; (Z)-2-(3,5-dimethylisoxazol-4-yl)-1-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-1H-indole-3-carboximidamide; (Z)-1-(2,5-difluoro-4-hydroxyphenyl)-2-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-1H-indole-3-carboximidamide; (Z)-1-(3,5-difluoro-4-hydroxyphenyl)-2-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-1H-indole-3-carboximidamide; (Z)-2-(3,5-dimethylisoxazol-4-yl)-1-(3-fluoro-4-hydroxyphenyl)-N'-hydroxy-1H-indole-3-carboximidamide; (Z)-1-(3-chloro-4-hydroxyphenyl)-2-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-1H-indole-3-carboximidamide; 2-(3,5-dimethylisoxazol-4-yl)-1-(2-fluoro-4-hydroxyphenyl)-1H-indole-3-carboxamide; (Z)-1-(2,3-difluoro-4-hydroxyphenyl)-2-(3,5-dimethylisoxazol-4-yl)-N'-hydroxy-1H-indole-3-carboximidamide; 1-(2,3-difluoro-4-hydroxyphenyl)-2-(3,5-dimethylisoxazol-4-yl)-1H-indole-3-carboxamide; 1-(2-fluoro-4-hydroxyphenyl)-2-(3-methylthiophen-2-yl)-1H-indole-3-carbonitrile; 2-(3,5-dimethyl-1H-pyrazol-4-yl)-1-(2-fluoro-4-hydroxyphenyl)-1H-indole-3-carbonitrile; 1-(2-fluoro-4-hydroxyphenyl)-2-(1-methyl-1H-pyrazol-5-yl)-1H-indole-3-carbonitrile; 1-(2-fluoro-4-

hydroxyphenyl)-2-(1,3,5-trimethyl-1H-pyrazol-4-yl)-1H-indole-3-carbonitrile; 1-(2-fluoro-4-hydroxyphenyl)-2-(3-(trifluoromethyl)-1H-pyrazol-4-yl)-1H-indole-3-carbonitrile; (Z)-1-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-2-(3-methylthiophen-2-yl)-1H-indole-3-carboximidamide; (Z)-1-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-2-(1-methyl-1H-pyrazol-5-yl)-1H-indole-3-carboximidamide; (Z)-1-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-2-(1,3,5-trimethyl-1H-pyrazol-4-yl)-1H-indole-3-carboximidamide; (Z)-4-fluoro-1-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-2-(1-methyl-1H-pyrazol-5-yl)-1H-indole-3-carboximidamide; (Z)-4-fluoro-1-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-2-(1,3,5-trimethyl-1H-pyrazol-4-yl)-1H-indole-3-carboximidamide; (Z)-2-(3,5-dimethyl-1H-pyrazol-4-yl)-4-fluoro-1-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-1H-indole-3-carboximidamide; (Z)-4-fluoro-1-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-2-(3-methylthiophen-2-yl)-1H-indole-3-carboximidamide; (Z)-2-(3,5-dimethyl is oxazol-4-yl)-4-fluoro-1-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-1H-indole-3-carboximidamide; (Z)-1-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-2-(3-methylthiophen-2-yl)-1H-indole-3-carboximidamide; (Z)-1-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-2-(1-methyl-1H-pyrazol-5-yl)-1H-indole-3-carboximidamide; (Z)-1-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-2-(1,3,5-trimethyl-1H-pyrazol-4-yl)-1H-indole-3-carboximidamide; (Z)-2-(3,5-dimethyl-1H-pyrazol-4-yl)-1-(2-fluoro-4-hydroxyphenyl)-N'-hydroxy-1H-indole-3-carboximidamide; methyl 2-(3,5-dimethylisoxazol-4-yl)-1-(2-fluoro-4-hydroxyphenyl)-1H-indole-3-carbimide; 2-(3,5-dimethylisoxazol-4-yl)-1-(3-fluoro-4-hydroxyphenyl)-1H-indole-3-carboxamide; 1-(2,5-difluoro-4-hydroxyphenyl)-2-(3,5-dimethylisoxazol-4-yl)-1H-indole-3-carboximidamide; 2-(3,5-dimethylisoxazol-4-yl)-1-(3-fluoro-4-hydroxyphenyl)-1H-indole-3-carboximidamide; (Z)-1-(3-fluoro-4-hydroxyphenyl)-N'-hydroxy-2-(1H-pyrrol-1-yl)-1H-indole-3-carboximidamide; 1-(3-fluoro-4-hydroxyphenyl)-2-(1H-pyrrol-1-yl)-1H-indole-3-carboxamide; (Z)-1-(2,3-difluoro-4-hydroxyphenyl)-N'-hydroxy-2-(1H-pyrrol-1-yl)-1H-indole-3-carboximidamide; 1-(2,3-difluoro-4-hydroxyphenyl)-2-(1H-pyrrol-1-yl)-1H-indole-3-carboxamide; (Z)-2-(2,5-dimethyl-1H-pyrrol-1-yl)-1-(3-fluoro-4-hydroxyphenyl)-N'-hydroxy-1H-indole-3-carboximidamide; (Z)-1-(3-fluoro-4-hydroxyphenyl)-N'-hydroxy-2-(2-methyl-1H-pyrrol-1-yl)-1H-indole-3-carboximidamide; 1-(3,5-difluoro-4-hydroxyphenyl)-2-(3,5-dimethylisoxazol-4-yl)-1H-indole-3-carboxamide; or (Z)-2-(3,5-dimethylisoxazol-4-yl)-6-fluoro-N'-hydroxy-1-(4-hydroxyphenyl)-1H-indole-3-carboximidamide.

In some embodiments, the ER β ligand is a compound selected from the ER β ligands

disclosed in U.S. Patent No. 8,334,280, hereby incorporated by reference.

In some embodiments, the ER β ligand is 2-(3-fluoro-4-hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol (ERB-041; Wyeth). The ER β ligand may be a substituted benzoxazole, such as any of the compounds disclosed in U.S. Patent No. 6,794,403 or U.S. Patent Application
5 Publication No. 2011/0212923 (each of which is hereby incorporated by reference).

The ER β ligand may be 2-(5-hydroxy-1,3-benzoxazol-2-yl) benzene-1,4-diol; 3-(5-hydroxy-1,3-benzoxazol-2-yl)benzene-1,2-diol; 2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol; 2-(3-chloro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol; 2-(2-chloro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol; 2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-6-ol; 2-(3-tert-butyl-4-
10 hydroxyphenyl)-1,3-benzoxazol-6-ol; 2-(6-hydroxy-1,3-benzoxazol-2-yl)benzene-1,4-diol; 3-(6-hydroxy-1,3-benzoxazol-2-yl)benzene-1,2-diol; 4-(6-hydroxy-1,3-benzoxazol-2-yl)benzene-1,2-diol; 2-(3-chloro-4-hydroxyphenyl)-1,3-benzoxazol-6-ol; 4-(5-hydroxy-1,3-benzoxazol-2-yl)benzene-1,3-diol; 4-(6-hydroxy-1,3-benzoxazol-2-yl)benzene-1,3-diol; 6-chloro-2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol; 6-bromo-2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-
15 5-ol; 6-chloro-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol; 5-chloro-2-(4-hydroxyphenyl)-1,3-benzoxazol-6-ol; 7-bromo-2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol; 7-bromo-2-(2-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol; 7-bromo-2-(2,3-difluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol; 2-(4-hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol; 7-(1,2-dibromoethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol; 7-(1-bromovinyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-
20 ol; 7-ethynyl-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol; 2-(4-hydroxyphenyl)-7-propyl-1,3-benzoxazol-5-ol; 7-butyl-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol; 7-cyclopentyl-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol; ethyl 5-hydroxy-2-(4-hydroxyphenyl)-1,3-benzoxazole-7-carboxylate; 2-(4-hydroxyphenyl)-7-phenyl-1,3-benzoxazol-5-ol; 2-(4-hydroxyphenyl)-7-methoxy-1,3-benzoxazol-5-ol; 7-ethyl-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol; 7-ethyl-2-(2-ethyl-4-hydroxyphenyl)-1,3-benzoxazol-5-ol; 5-hydroxy-2-(4-hydroxyphenyl)-1,3-benzoxazole-7-carbaldehyde; 7-(hydroxymethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol; 7-(bromomethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol; [5-hydroxy-2-(4-hydroxyphenyl)-1,3-benzoxazol-7-yl]acetonitrile; 7-(1-hydroxy-1-methylethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol; 2-(4-hydroxyphenyl)-7-isopropenyl-1,3-benzoxazol-5-ol; 2-(4-hydroxyphenyl)-7-isopropyl-1,3-benzoxazol-5-ol; 7-bromo-2-(4-hydroxy-3-(trifluoromethyl)phenyl)-1,3-benzoxazol-5-ol; 7-(2-furyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol; 2-(3-fluoro-4-

hydroxyphenyl)-7-(2-furyl)-1,3-benzoxazol-5-ol; 2-(4-hydroxyphenyl)-7-thien-2-yl-1,3-benzoxazol-5-ol; 2-(4-hydroxyphenyl)-7-(1,3-thiazol-2-yl)-1,3-benzoxazol-5-ol; 2-(3-fluoro-4-hydroxyphenyl)-5-hydroxy-1,3-benzoxazole-7-carbonitrile; 4-bromo-2-(4-hydroxyphenyl)-7-methoxy-1,3-benzoxazol-5-ol; 4,6-bibromo-2-(4-hydroxyphenyl)-7-methoxy-1,3-benzoxazol-5-ol; or 7-bromo-2-(3,5-difluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol.

The ER β ligand may be 2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol, 2-(3-chloro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol, 2-(3-fluoro-4-hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol, 2-(2-chloro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol, 2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-6-ol, 2-(3-tert-butyl-4-hydroxyphenyl)-1,3-benzoxazol-6-ol, 2-(3-chloro-4-hydroxyphenyl)-1,3-benzoxazol-6-ol, 6-chloro-2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol, 6-bromo-2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol, 6-chloro-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol, 5-chloro-2-(4-hydroxyphenyl)-1,3-benzoxazol-6-ol, 7-bromo-2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol, 7-bromo-2-(2-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol, 7-bromo-2-(2,3-difluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol, 2-(4-hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol, 7-(1,2-dibromoethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol, 7-(1-bromovinyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol, 7-ethynyl-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol, 2-(4-hydroxyphenyl)-7-propyl-1,3-benzoxazol-5-ol, 7-butyl-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol, 7-cyclopentyl-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol, ethyl 5-hydroxy-2-(4-hydroxyphenyl)-1,3-benzoxazole-7-carboxylate, 2-(4-hydroxyphenyl)-7-phenyl-1,3-benzoxazol-5-ol, 2-(4-hydroxyphenyl)-7-methoxy-1,3-benzoxazol-5-ol, 7-ethyl-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol, 7-ethyl-2-(2-ethyl-4-hydroxyphenyl)-1,3-benzoxazol-5-ol, 5-hydroxy-2-(4-hydroxyphenyl)-1,3-benzoxazole-7-carbaldehyde, 7-(hydroxymethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol, 7-(bromomethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol, [5-hydroxy-2-(4-hydroxyphenyl)-1,3-benzoxazol-7-yl] acetonitrile, 7-(1-hydroxy-1-methylethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol, 2-(4-hydroxyphenyl)-7-isopropenyl-1,3-benzoxazol-5-ol, 2-(4-hydroxyphenyl)-7-isopropyl-1,3-benzoxazol-5-ol, 7-bromo-2-(4-hydroxy-3-(trifluoromethyl)phenyl)-1,3-benzoxazol-5-ol, 7-(2-furyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol, 2-(3-fluoro-4-hydroxyphenyl)-7-(2-furyl)-1,3-benzoxazol-5-ol, 2-(4-hydroxyphenyl)-7-thien-2-yl-1,3-benzoxazol-5-ol, 2-(4-hydroxyphenyl)-7-(1,3-thiazol-2-yl)-1,3-benzoxazol-5-ol, 2-(3-fluoro-4-hydroxyphenyl)-5-hydroxy-1,3-benzoxazole-7-carbonitrile, 4-bromo-2-(4-hydroxyphenyl)-7-methoxy-1,3-benzoxazol-5-ol, 4,6-dibromo-2-(4-hydroxyphenyl)-

7-methoxy-1,3-benzoxazol-5-ol, or 7-bromo-2-(3,5-difluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol.

In some embodiments, the ER β ligand is a compound selected from the ER β ligands disclosed in U.S. Patent Application Publication No. 2007/0021495 or 2013/0274344, each of which is hereby incorporated by reference.

In some embodiments, the ER β ligand is a halogen-substituted phenyl-2H-indazole, such as indazole chloride (*see, e.g.*, Moore, S. M. et al. Proc. Nat'l Acad. Sci. USA 111(5):18061-66 (2014), hereby incorporated by reference).

In some embodiments, the ER β ligand is not estriol. The ER β ligand may be a non-steroidal compound. In some embodiments, the ER β ligand is not a steroid hormone.

The long term administration of some estrogens requires the periodic co-administration of a progestogen to prevent harmful side effects. Some ER β ligand receptors, however, such as AC-186, KBRV1, and KBRV2, do not require the co-administration of a progestogen.

Accordingly, in some embodiments, the method does not comprise the co-administration of a progestogen, such as norethindrone, *e.g.*, either periodically or continuously. In some embodiments, the method does not comprise the co-administration of a gestagen or progestin. For example, a patient may not be taking any one of chlormadinone acetate, cyproterone acetate, desogestrel, dienogest, 5 α -dihydroprogesterone, drospirenone (Yasmin®), ethinodiol acetate, ethynodiol diacetate, etonogestrel (Nexplanon®), gestodene, 17-hydroxyprogesterone, levonorgestrel (Alesse®), medroxyprogesterone acetate (17 α -hydroxy-6 α -methylprogesterone acetate; Provera®), megestrol, megestrol acetate (17 α -acetoxy-6-dehydro-6-methylprogesterone), nestorone, nomegestrol acetate, norethindrone, norethindrone acetate (also known as norethisterone acetate), norethynodrel (Enovid®), norgestimate, norgestrel, progesterone, tanaproget, or trimegestone while receiving an ER β ligand.

A. ER β ligand dosing

The ER β ligand may be a compound having the structure of formula I, and it may be administered at a dose sufficient to achieve a mean blood concentration of the compound between 1 ng/ml and 1000 ng/ml. The compound may be administered at a dose sufficient to achieve a mean blood concentration of the compound between 1 ng/ml and 1000 ng/ml or about 1-100 mg/kg/day. For example, the compound may be administered at a dose sufficient to achieve a mean blood concentration of the compound between 10 ng/ml and 50 ng/ml, between

25 ng/ml and 75 ng/ml, between 50 ng/ml and 100 ng/ml, between 75 ng/ml and 125 ng/ml, between 100 ng/ml and 150 ng/ml, between 125 ng/ml and 175 ng/ml, between 100 ng/ml and 200 ng/ml, between 150 ng/ml and 250 ng/ml, between 200 ng/ml and 300 ng/ml, between 250 ng/ml and 350 ng/ml, between 300 ng/ml and 400 ng/ml, between 350 ng/ml and 450 ng/ml, between 400 ng/ml and 500 ng/ml, between 450 ng/ml and 650 ng/ml, between 550 ng/ml and 750 ng/ml, between 650 ng/ml and 850 ng/ml, between 750 ng/ml and 950 ng/ml, or between 850 ng/ml and 1050 ng/ml. In some embodiments, the compound may be administered at a dose sufficient to achieve a mean blood concentration of the compound between 100 ng/ml and 200 ng/ml. In other embodiments, the compound may be administered at a dose sufficient to achieve a mean blood concentration of the compound between 10 ng/ml and 20 ng/ml. In some
10 embodiments, the compound is administered at a dose sufficient to achieve a mean blood concentration of the compound between 10 ng/ml and 500 ng/ml.

The ER β ligand may be a compound having the structure of formula I, and it may be administered at a dose between 10 mg and 10 g per day, such as between 80 mg and 8000 mg per
15 day, or between 200 mg and 2000 mg per day. The ER β ligand may be a compound having the structure of formula I, and it may be administered at a dose of about 5 μ g/kg per day to about 100 mg/kg/day, such as about 50 μ g/kg per day to about 50 mg/kg/day or about 500 μ g/kg per day to about 5 mg/kg/day. The ER β ligand may be a compound having the structure of formula I, and it may be administered at a dose between 1 mg/kg and 100 mg/kg per day, such as between 4
20 mg/kg and 80 mg/kg per day, or between 10 mg/kg and 50 mg/kg per day.

The ER β ligand may be KBRV1 or KBRV2, and it may be administered at a dose between 1 mg and 10 g per day, such as between 5 mg and 1000 mg per day, or between 10 mg and 500 mg per day. The ER β ligand may be KBRV1 or KBRV2, and it may be administered at a dose between 0.1 mg/kg and 100 mg/kg per day, such as between 0.5 mg/kg and 100 mg/kg per
25 day, or between 1 mg/kg and 10 mg/kg per day.

An “effective amount,” as used herein, refers to an amount that is sufficient to achieve a desired biological effect. A “therapeutically effective amount,” as used herein refers to an amount that is sufficient to achieve a desired therapeutic effect. For example, a therapeutically effective amount can refer to an amount that is sufficient to improve at least one sign or
30 symptom of multiple sclerosis.

The dosage of the ER β ligand may be selected for an individual patient depending upon

the route of administration, severity of disease, age and weight of the patient, other medications the patient is taking and other factors normally considered by the attending physician, when determining the individual regimen and dosage level as the most appropriate for a particular patient. Furthermore, the exact individual dosages can be adjusted somewhat depending on a
5 variety of factors, including the specific combination of the agents being administered, the time of administration, the route of administration, the nature of the formulation, the rate of excretion, the particular disease being treated, the severity of the disorder, and the anatomical location of the disorder. Some variations in the dosage can be expected. *In vitro* or *in vivo* assays can be employed to help identify optimal dosage ranges.

10 The therapeutically effective dose of the ER β ligand included in the dosage form is selected at least by considering the type of ER β ligand selected and the mode of administration. The dosage form may include the ER β ligand in combination with other inert ingredients, including adjuvants and pharmaceutically acceptable carriers for the facilitation of dosage to the patient as known to those skilled in the pharmaceutical arts. The dosage form may be any form
15 suitable to cause the ER β ligand to enter into the tissues of the patient.

Pharmaceutically acceptable carriers can optionally comprise a suitable amount of a pharmaceutically acceptable excipient so as to provide the form for proper administration. Pharmaceutical excipients can be liquids, such as water and oils, including those of petroleum, animal, vegetable, or synthetic origin, including peanut oil, soybean oil, mineral oil, sesame oil
20 and the like. The pharmaceutical excipients can include, for example, saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea and the like. In addition, auxiliary, stabilizing, thickening, lubricating, and coloring agents can be used. In one embodiment, the pharmaceutically acceptable excipients are sterile when administered to a subject. Suitable pharmaceutical excipients also include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour,
25 chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol and the like. Any agent described herein, if desired, can also comprise minor amounts of wetting or emulsifying agents, or pH buffering agents.

In one embodiment, the dosage form of the ER β ligand is an oral preparation (liquid, tablet, capsule, caplet, or the like), which, when consumed results in elevated serum ER β ligand
30 levels. The oral preparation may comprise conventional carriers including diluents, binders, time-release agents, lubricants, and disintegrants.

In some embodiments, the dosage form of the ER β ligand is a sublingual preparation, which results in elevated serum ER β ligand levels when consumed.

In other embodiments of the invention, the dosage form of the ER β ligand may be provided in a topical preparation (lotion, cream, ointment, patch, or the like) for transdermal application.

Alternatively, the dosage form may be provided as a suppository or the like for transvaginal or transrectal application. In other embodiments, the dosage form may also allow for preparations to be applied subcutaneously, intravenously, intramuscularly, or via the respiratory system.

B. Treatment periods

The ER β ligand is preferably administered to the subject on a continuous basis, *e.g.*, for at least one treatment period. In certain embodiments a continuous basis is daily, *i.e.*, on consecutive days. The ER β ligand may be administered to the subject for multiple treatment periods (*e.g.*, multiple consecutive treatment periods), such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21, 22, 23, or 24 treatment periods.

As used herein, a “treatment period” refers to a period of time during which a subject is receiving an ER β ligand, on a continuous or daily basis, for the purpose of treating a neurodegenerative disease in the subject. In certain embodiments, each treatment period is at least 28 consecutive days, at least 56 consecutive days, at least 84 consecutive days, at least 112 consecutive days, at least 140 consecutive days, or at least 168 consecutive days. For example, each treatment period may be 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250,

251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, or 365 consecutive days.

In certain embodiments, each treatment period is at least 4 consecutive weeks, at least 8 consecutive weeks, at least 12 consecutive weeks, at least 16 consecutive weeks, at least 20 consecutive weeks, or at least 24 consecutive weeks. For example, each treatment period may be 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, or 52 consecutive weeks.

In certain embodiments, each treatment period is at least one month, at least two consecutive months, at least three consecutive months, at least four consecutive months, at least five consecutive months, or at least six consecutive months. For example, each treatment period may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 consecutive months.

C. Formulations

For oral administration, a given dose of each formulation can comprise one or more pills, tablets, capsules, or the like (*i.e.*, unit doses). For example, an 800 mg dose of an ER β ligand can be administered as four 200 mg capsules. For sublingual administration, a given dose of each formulation may comprise one or more tablets or lozenges (*i.e.*, unit doses) or a volume of liquid (*e.g.*, one or more drops) or a volume of spray (*e.g.*, one or more spray pumps).

When a given dose of any agent involves administration of more than a single unit dose, *e.g.*, four 200 mg capsules of an ER β ligand, the individual unit doses can be administered at essentially the same time, or they can be administered at different times on a given day, provided the entire daily dose is administered within a single day. For example, four 200 mg capsules of an ER β ligand can be taken together essentially once a day, or they may be taken two at a time twice a day, or they may be taken one at a time four times a day. Additional schedules are contemplated by the invention, again provided the entire daily dose is administered within a single day. While it may be preferable that the subject follow the same schedule from one day to

the next, such is not required, once again provided the entire daily dose is administered within a single day.

IV. SUBJECTS

The term “subject” as used herein refers to a living mammal and may be interchangeably used with the term “patient.” In certain embodiments, the subject is a human. Preferably, a human subject is female, such as a woman. In certain embodiments, the subject is a premenopausal or perimenopausal woman. In certain embodiments, the subject is a premenopausal woman. In certain embodiments, the subject is a perimenopausal woman. In certain embodiments, the subject is a postmenopausal woman.

The subject may have multiple sclerosis. In certain embodiments, the multiple sclerosis is relapsing-remitting multiple sclerosis. In certain embodiments, the multiple sclerosis is secondary-progressive multiple sclerosis. In certain embodiments, the multiple sclerosis is primary-progressive multiple sclerosis. In certain embodiments, the multiple sclerosis is progressive-relapsing multiple sclerosis. In certain embodiments, the subject has a mild form of any one of the foregoing subtypes of MS. In certain embodiments, the subject has a moderate form of any one of the foregoing subtypes of MS. In certain embodiments, the subject has an aggressive form of any one of the foregoing subtypes of MS.

In certain embodiments, the multiple sclerosis is, more accurately, so-called clinically isolated syndrome (CIS). An ER β ligand can be used, in accordance with the invention, to prevent or delay the onset of relapsing-remitting MS in subjects having CIS. In some embodiments, the subject has radiologically isolated syndrome.

Although the methods disclosed throughout the specification and claims are useful for treating multiple sclerosis in its various forms and stages, these methods can also be applied the treatment of other neurodegenerative diseases, such as, by way of illustration, Alzheimer’s disease, Parkinson’s disease, stroke, amyotrophic lateral sclerosis, cerebellar ataxia, frontotemporal dementia, prion disease, Huntington’s Disease, cerebral ischemia, idiopathic Morbus Parkinson, Parkinson syndrome, Morbus Alzheimers, cerebral dementia syndrome, infection-induced neurodegeneration disorders (*e.g.*, AIDS-encephalopathy, Creutzfeld-Jakob disease, encephalopathies induced by rubiola and herpes viruses and borrelioses), metabolic-toxic neurodegenerative disorders (such as hepatic-, alcoholic-, hypoxic-, hypo- or hyperglycemicallly-induced encephalopathies), encephalopathies induced by solvents or

pharmaceuticals, degenerative retina disorders, trauma-induced brain damage, trauma-induced bone marrow damage, cerebral hyperexcitability symptoms, cerebral hyperexcitability states (*e.g.*, of varying origin, such as after the addition of and/or withdrawal of medicaments, toxins, noxae, and drugs), neurodegenerative syndromes of the peripheral nervous system, peripheral nerve injury, and spinal cord injury. In certain preferred embodiments, the neurodegenerative disease is multiple sclerosis. In preferred embodiments, the patient is a woman. In some embodiments, the patient is a premenopausal or perimenopausal woman. In other embodiments, the patient is a postmenopausal woman.

The various methods disclosed herein can be methods for improving walking, vision, balance, cognition, or other symptoms in a subject, such as a subject with multiple sclerosis, and/or methods for improving multiple sclerosis functional composite (MSFC), EDSS, or MSSS scores in a subject, such as a subject with multiple sclerosis. Thus, in certain embodiments, the methods of treatment disclosed herein include methods for stabilizing or improving disability in a patient, whereby the patient's disability score (as measured by either of these tests or another suitable test) after six months, one year, or two years of therapy is at least about 10%, at least about 25%, at least about 40%, at least about 50%, or even at least about 60% higher relative to a control patient not receiving the ER β ligand therapy (but otherwise receiving the same treatment as the ER β ligand-treated patient). Alternatively, the patient's disability score (as measured by either of these tests or another suitable test) after six months, one year, or two years of therapy is within about 2% or within about 5% of an earlier assessment, or at least about 2%, at least about 5%, at least about at least about 10%, at least about 25%, at least about 40%, at least about 50%, or even at least about 60% higher than the earlier assessment.

For example, the progression of a walking disability can be tested using a walking test, *e.g.*, assessing the subject's performance on a 25-foot walk test at different points in time, such as at 0 months (baseline), 6 months, 1 year, and 2 years. The walking test may be a distance test (*e.g.*, a 25 foot walk test) or a timed test (*e.g.*, a 6 minute walk test), for example, or another multiple sclerosis walk scale may be employed. In certain embodiments, if there is documented worsening in walking (takes more seconds) by 20 percent as compared to baseline (optionally if this worsening is confirmed on a subsequent walk test (*e.g.*, 3 months later)), then the subject is deemed to have progressive worsening in walking. For such a patient not already receiving ER β ligand therapy, the subject demonstrating the progressive walking disability commences

treatment with ER β ligand. The walking test may be repeated (*e.g.*, at 1 year and/or 2 years from the start of ER β ligand treatment) to assess whether the ER β ligand treatment slowed or halted any further worsening in walking performance, *e.g.*, as measured by the walking test.

Improvements in cognition outcomes associated with MS therapy, whether slowing of cognitive decline, stabilization of cognitive decline, or improvement of cognitive function, can be assessed using the PASAT (*e.g.*, PASAT 2 or PASAT 3) or SDMT test, or alternatively the MS-COG test (see Erlanger et al., *J Neuro Sci* 340: 123-129 (2014)). Thus, in certain embodiments, the methods of treatment disclosed herein include methods for stabilizing or improving cognition in a patient, whereby the patient's cognition outcome after one year of therapy is at least about 2%, at least about 5%, at least about 10%, at least about 25%, at least about 40%, at least about 50%, or even at least about 60% higher relative to a control patient not receiving the ER β ligand therapy (but otherwise receiving the same treatment as the ER β ligand-treated patient), *e.g.*, as measured by any of the preceding tests. Alternatively, the patient's cognition outcome after six months, one year, or two years of therapy may be within about 2% or within about 5% of an earlier assessment, or at least about 2%, at least about 5%, at least about 10%, at least about 25%, at least about 40%, at least about 50%, or even at least about 60% higher than the earlier assessment, *e.g.*, as measured by any of the preceding tests at different times.

Methods of treatment disclosed herein include methods for stabilizing and/or improving fatigue and/or depression in a patient. The fatigue and/or depression of the patient after one year of therapy may be reduced by at least about 2%, at least about 5%, at least about 10%, at least about 25%, at least about 40%, at least about 50%, or even at least about 60% relative to a control patient not receiving the ER β ligand therapy (but otherwise receiving the same treatment as the ER β ligand-treated patient), *e.g.*, as measured by a Modified Fatigue Impact Scale (MFIS), Beck Depression Inventory, MS Quality of Life score, or Patient-Reported Outcomes Measurement Information System fatigue computer adaptive test.

For example, a subject who scores below 50 on PASAT (and optionally if such low score is verified upon a second subsequent test, such as within one week to one month of the first) may be deemed to have cognitive disability. For such a patient not already receiving ER β ligand therapy, the subject demonstrating the cognitive disability may commence treatment with ER β ligand. In certain embodiments, the cognitive test may be repeated (*e.g.*, at about six months

from the start of ER β ligand treatment) to assess whether the ER β ligand treatment slowed or halted any further worsening in cognitive performance, *e.g.*, as measured by the PASAT test. In certain such embodiments, the patient's score may increase by at least 3 points over the course of six to twelve months of ER β ligand therapy.

5 While the various methods disclosed herein are typically efficacious when administered without additional therapeutics, in certain embodiments, any of these methods further includes the step of administering to the subject an immunotherapeutic agent, wherein the immunotherapeutic agent is not an ER β ligand. That is, in certain embodiments the subject is administered, in addition to the ER β ligand, a second agent useful in the treatment of MS. Such
10 agents useful in the treatment of MS are, in general, immunotherapeutic agents. At least in connection with MS, such agents are sometimes referred to as disease-modifying therapies or disease-modifying therapeutics (DMTs).

ER β ligands may be used in patients who are at risk of developing a gynecological problem or cancer in which an estrogen receptor α agonist is contraindicated. For example, the
15 patient may present with a history of breast cancer, ovarian cancer, and/or uterine cancer. The subject may have a family history of breast cancer, ovarian cancer, and/or uterine cancer (*e.g.*, a parent, sister, grandparent, aunt, or cousin of the subject may have had breast cancer, ovarian cancer, or uterine cancer). The subject may be at risk of developing breast cancer, ovarian cancer, and/or uterine cancer.

20 V. IMMUNOTHERAPEUTIC AGENT

The term "immunotherapeutic agent" as used herein refers to a compound, other than an ER β ligand as defined herein, with an objectively measurable effect on at least one aspect of the immune system or an immune response. In certain embodiments, the immunotherapeutic agent is immunosuppressive, *i.e.*, it exerts an objectively measurable inhibitory effect on at least one
25 aspect of the immune system or an immune response. In certain embodiments, the immunotherapeutic agent is anti-inflammatory. In certain embodiments, the immunotherapeutic agent is a small molecule (molecular weight less than or equal to about 1.5 kDa) pharmaceutical compound or composition. In certain embodiments, the immunotherapeutic agent is a biological compound or composition, *e.g.*, an antibody, peptide, nucleic acid, etc.

30 In certain embodiments, the immunotherapeutic agent is selected from dimethyl fumarate (Tecfidera®; BG-12), fingolimod (Gilenya®), glatiramer acetate (Copaxone®, for example

“longer-lasting” 40 mg/ml or 20 mg/ml versions), interferon beta-1a (Avonex® or Rebif®), interferon beta-1b (Betaseron® or Extavia®), peginterferon beta-1a (Plegridy®), mitoxantrone (Novantrone®), natalizumab (Tysabri®), alemtuzumab (Lemtrada®), and teriflunomide (Aubagio®), mycophenolate mofetil, paclitaxel, cyclosporine, corticosteroids (*e.g.*, prednisone, methylprednisolone), azathioprine, cyclophosphamide, methotrexate, cladribine, 4-aminopyridine, and tizanidine. In certain embodiments, the immunotherapeutic agent is selected from dimethyl fumarate (Tecfidera®; BG-12), fingolimod (Gilenya®), glatiramer acetate (Copaxone®), interferon beta-1a (Avonex® or Rebif®), interferon beta-1b (Betaseron® or Extavia®), peginterferon beta-1a (Plegridy®), mitoxantrone (Novantrone®), natalizumab (Tysabri®), alemtuzumab (Lemtrada®), and teriflunomide (Aubagio®). In some embodiments, the immunotherapeutic agent is not mitoxantrone (Novantrone®). In some embodiments, the immunotherapeutic agent is not glatiramer acetate (Copaxone®).

In certain embodiments, the immunotherapeutic agent is dimethyl fumarate (Tecfidera®; BG-12). In certain embodiments, the immunotherapeutic agent is fingolimod (Gilenya®). In certain embodiments, the immunotherapeutic agent is glatiramer acetate (Copaxone®). In certain embodiments, the immunotherapeutic agent is interferon beta-1a (Avonex® or Rebif®). In certain embodiments, the immunotherapeutic agent is interferon beta-1b (Betaseron® or Extavia®). In certain embodiments, the immunotherapeutic agent is peginterferon beta-1a (Plegridy®). In certain embodiments, the immunotherapeutic agent is mitoxantrone (Novantrone®). In certain embodiments, the immunotherapeutic agent is natalizumab (Tysabri®). In certain embodiments, the immunotherapeutic agent is alemtuzumab (Lemtrada®). In certain embodiments, the immunotherapeutic agent is teriflunomide (Aubagio®).

In certain embodiments, the subject is receiving treatment with an immunotherapeutic agent. The method may comprise discontinuing treatment with the immunotherapeutic agent, *e.g.*, if the central nervous system (*i.e.*, brain) of the subject does not present with active lesions, *e.g.*, gadolinium-enhancing lesions. The method may comprise discontinuing treatment with the immunotherapeutic agent if no active lesions (*e.g.*, gadolinium-enhancing lesions) have been detected in the central nervous system (*i.e.*, brain) of the subject for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, or 52 weeks. The method may

comprise discontinuing treatment with the immunotherapeutic agent if no active lesions (*e.g.*, gadolinium-enhancing lesions) have been detected in the central nervous system (*i.e.*, brain) of the subject for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 months. The method may comprise discontinuing treatment with the immunotherapeutic agent if no active lesions (*e.g.*, gadolinium-enhancing lesions) have been detected in the central nervous system (*i.e.*, brain) of the subject for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 years.

In certain embodiments, the method comprises administering an ER β ligand to the subject without conjointly administering an immunotherapeutic agent to the subject. The method may comprise administering an ER β ligand to the subject without conjointly administering an immunotherapeutic agent to the subject, for example, if no active lesions (*e.g.*, gadolinium-enhancing lesions) have been detected in the central nervous system (*i.e.*, brain) of the subject for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, or 52 weeks. The method may comprise administering an ER β ligand to the subject without conjointly administering an immunotherapeutic agent to the subject, for example, if no active lesions (*e.g.*, gadolinium-enhancing lesions) have been detected in the central nervous system (*i.e.*, brain) of the subject for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 months. The method may comprise administering an ER β ligand to the subject without conjointly administering an immunotherapeutic agent to the subject, for example, if no active lesions (*e.g.*, gadolinium-enhancing lesions) have been detected in the central nervous system (*i.e.*, brain) of the subject for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 years.

Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included herewith for purposes of illustration only and are not intended to limit the invention.

EXEMPLIFICATION

Example 1-Use of Glatiramer Acetate (GA) and an ER β ligand for the Treatment of Multiple Sclerosis

This example describes a randomized, double-blind, placebo-controlled human clinical trial for the treatment of multiple sclerosis using glatiramer acetate (GA) and estriol.

Enrollment Criteria

Eligible patients were females, an age of 18-50 years, a diagnosis of relapsing remitting

multiple sclerosis as defined according to the McDonald criteria (Polman C. et al., Neurology 64:987 (200)), a baseline score of 0 to 4.5 on the Expanded Disability Status Scale (EDSS, which ranges from 0 to 10, with higher scores indicating more severe disability), and disease activity as evidenced by at least two documented relapses in the previous 24 months before screening or as evidenced by at least one documented relapse within 24 months before screening with a history of at least one gadolinium-enhancing lesion on a brain or cord magnetic resonance imaging (MRI) scan performed at least 3 months before or 3 months after the clinical relapse. Key exclusion criteria were progressive forms of multiple sclerosis, other clinically significant diseases, pre-specified laboratory test abnormalities, possible malignancy on mammogram or uterine ultrasound, exposure to glatiramer acetate for longer than 2 months before randomization, relapse or steroid use within 30 days prior to randomization, use of any interferon, adrenocorticotrophic hormone (ACTH), corticosteroids, intravenous immunoglobulins, or other listed MS treatments within 2 months before screening, those who were pregnant, breastfeeding, or trying to get pregnant, those not willing to discontinue other hormonal treatments, those who underwent surgical or natural menopause for longer than 1 or 3 years, respectively, with no hormone replacement therapy, and those who had ever been treated with a major immunosuppressive contraindicated treatment.

Table 1: Baseline Characteristics of the Intention-to-Treat Population.*

Patient Characteristics	Estriol + GA (N=82)	Placebo + GA (N=76)
Age-yr	37.7 ± 7.6	37.1 ± 7.3
Race – no. (%)†		
Caucasian	65 (79.3)	62 (81.6)
Black	9 (11.0)	7 (9.2)
Hispanic	7 (8.5)	6 (7.9)
Other	1 (1.2)	1 (1.2)
Time since diagnosis-yr	3.3 ± 4.6	2.9 ± 4.5
Number of previous relapses		
Within 1 yr before screening	1.5 ± 0.7	1.5 ± 0.7
Within 2 yr before screening	2.0 ± 0.7	2.3 ± 0.9
Prior GA treatment		
Never	25 (30.5)	27 (35.5)
Previously	17 (20.7)	6 (7.9)
During screening	40 (48.8)	43 (56.6)
Prior treatment with any interferon – no. (%) ‡		
No	59 (72.0)	50 (65.8)

Yes	23 (28.0)	26 (34.2)
Mean score on EDSS ¶	2.2 ± 1.2	2.1 ± 1.1
EDSS score at baseline- no. (%) ¶		
0	9 (11.0)	6 (7.9)
1.0 or 1.5	16 (19.5)	21 (27.6)
2.0 or 2.5	27 (32.9)	24 (31.6)
3.0 or 3.5	25 (30.5)	22 (29.0)
4.0	4 (4.9)	2 (2.6)
5.5	1 (1.2)	1 (1.3)
Gadolinium-enhancing lesions number	1.0 ± 2.3	0.9 ± 2.0
Active lesions on brain MRI – no. (%)		
No	55 (67.9)	53 (70.7)
Yes	26 (32.1)	22 (29.3)
Volume of lesions on T2 weighted Images – cm ³	6.8 ± 8.9	7.7 ± 11.2

* Plus-minus values are means ± SD. All patients were included as the intention-to-treat population who underwent randomization, except those with no data after randomization. There were no significant differences between baseline clinical or demographic characteristics between the study groups.

† Race was self-reported.

‡ Patients may have received more than one prior multiple sclerosis medication. Patients may have received other non-approved therapies for multiple sclerosis before enrollment in the study. The percentage of patients receiving medication for multiple sclerosis before study entry was balanced across treatment groups.

¶ Scores on the Expanded Disability Status Scale (EDSS) ranged from 0 to 10, with higher scores indicating a greater degree of disability. The baseline EDSS score was higher than inclusion criteria of 4.5 in two patients (EDSS = 5.5), one in each study group that were 4.5 at first screening visit, but 5.5 at baseline. One patient in the Estriol + GA group did not have a confirmed relapse within 24 months prior to randomization, with enrollment based on disease activity evidenced by MRI enhancing lesions.

Study Design

Sixteen sites randomized subjects 1:1 to oral estriol (8 mg daily) or oral placebo for 24 months (Figure 1). A four week taper commenced at month 24 for both estriol and placebo. To avoid taking unopposed estrogens, the Estriol + GA subjects also received a progestin (0.7 mg norethindrone) daily for two weeks duration every three months starting at month 6, and Placebo + GA received a second placebo for progestin. All started GA injections (20 mg/day per day) within 2 months of randomization. Randomization had one stratification factor, GA treatment during screening. Each study site had separate examining and treating neurologists unaware of

assignment. The examining neurologists performed neurologic assessments including EDSS, while treating neurologists managed patient care including treatment of relapses.

Efficacy Measures

Standardized neurologic assessments, including an EDSS assessment, were performed at months 0, 3, 6, 12, 18 and 24, and at the time of a suspected relapse (as an additional unscheduled visit). EDSS assessments were performed by physicians who were trained either by in-person training or online (www.Neurostatus.net). MRI scans were obtained at screening and at months 0, 3, 6, 12 and 24. Subjects were seen or contacted every 3 months for compliance assessments and for dispensing medications.

The primary efficacy end point was the annualized relapse rate. A relapse was defined as the appearance of new neurological symptoms or the worsening of pre-existing symptoms, lasting at least 48 hours in a subject who had been neurologically stable or improving in the previous 30 days, accompanied by an objective change in a neurological examination (*i.e.*, a worsening of 0.5 or more points on the EDSS or a worsening by 1.0 or more points on the pyramidal, cerebellar, brainstem or visual functional system scores, not due to fatigue alone and not associated with fever or infection). The treating physician made the decision concerning whether the relapse criteria had been met, incorporating whether a change in EDSS had been documented by the examining physician. Both treating and examining physicians were unaware of study group assignments. The standard treatment for relapse was a 3-5 day course of glucocorticoids at the discretion of the treating neurologist.

Secondary efficacy end points included the proportion of subjects with a relapse over all 24 months, the proportion of subjects with positive MRI scans for gadolinium enhancing lesions, a change in PASAT cognitive testing, a sustained improvement in PASAT cognitive testing (as defined by an increase of at least 3 points sustained over at least 6 months), a change in EDSS scores from baseline, disability progression (as defined by an increase in EDSS of at least 1.0 point in subjects with a baseline score of 1.0 or higher, or by an increase of at least a 1.5 points in subjects with a baseline score of 0, each sustained for at least 6 months). Tertiary end points included gray matter atrophy on MRI, and changes in results from baseline on questionnaires including the Modified Fatigue Impact Scale, Beck Depression Inventory, and MS Quality of Life.

Safety and Adverse Events

Safety assessments, including clinical, blood laboratory safety testing and assessments of estriol levels, occurred at months 0, 3, 6, 12, 18, and 24. On study blood tests included complete blood count (CBC) with differential and platelets; chemistry panel including sodium, potassium, creatinine, BUN, glucose, total protein, albumin, bilirubin (total), alkaline phosphatase, AST (SGOT), and ALT (SGPT), and lipid profile (HDL, LDL and triglycerides, cholesterol. Gynecologic exams were done at month 0, 6, 18 and at month 24 exit, with uterine ultrasounds at months 6, 18 and at month 24 exit. Mammograms were done in screening and at month 24 exit. Adverse event analysis was based on the percentage of patients who discontinued the study and the percentage of patients who discontinued the study possibly due to adverse events.

Statistical Analysis

The sample size was determined based on the primary end point of annualized relapse rate. A total sample of 150 eligible patients would provide approximately 80% power at a two-sided significance level of 0.10 for this phase II clinical trial to detect the difference in the annualized relapse rate of 0.76 versus 1.18 for Estriol plus GA group and the Placebo plus GA group in 2 years.

Intention-to-treat analyses were carried out for all end points. For the primary endpoint, a negative binomial regression model was used to compare both 12 months and 24 months annualized relapse rates between Estriol + GA versus Placebo + GA groups adjusted for covariates. To control the overall type I error, a sequential testing procedure was applied. A hierarchical statistical approach was used whereby results in the first 12 months of treatment would be assessed, and, if and only if, significance were met, results in the entire 24 months of treatment would be assessed. The earlier timepoint was compared first since GA requires time to reach full efficacy, potentially providing a greater window to detect efficacy 12 months after initiation of GA and study drug treatment. Consistent with a phase 2 study using a clinical outcome, a p-value < 0.10 was considered statistically significant.

For the time to first relapse analysis, Kaplan Meier curves and log-rank test were used to estimate and compare the relapse free probabilities of the two treatment groups. Cox proportional hazards model was used to compare the time to relapse free probabilities between two groups adjusting for covariates. The fixed effects include treatment groups (Estriol + GA vs Placebo + GA), baseline lesion number, age, and baseline EDSS score. The random effect of subject is included in the model to account for within subject correlation.

Mixed effects negative binomial regression model and linear mixed effects model were used to compare enhancing lesion volume (log-transformed) between treatment groups at all follow-ups, and mixed effects logistic model was used to compare the number of subjects positive for gadolinium enhancing lesions. Linear mixed effects model was carried out to compare the percent change in whole gray matter and cortical gray matter between treatment groups. For the exploratory endpoints of EDSS, PASAT, fatigue, depression, quality of life and brain volume measures, linear mixed effects model was used to compare treatment groups at 12 and 24 months.

Mixed effects models were used to assess the association among outcomes and estradiol levels at all follow-ups and using subjects in both treatment groups. Mixed effects logistic regression model was used to evaluate the association between the number of enhancing lesions and the occurrence of relapse at all follow-up intervals. Linear mixed effects model was carried out to evaluate the association between PASAT change and percent brain volume change, as well as between PASAT change and estradiol levels.

Multiple imputation on the missing data was also performed according to the pattern mixture model as a sensitivity analysis. The pattern mixture model provides the analysis with the possibility of non-random dropout. The missing data were sequentially imputed by the follow up time and the imputation model assumed that the treatment effect for patients after drop out is the same as taking placebo.

Additional Statistical Analysis

The statistical analysis was repeated using slightly different methods. These methods were used to obtain the statistics in Table 4 and Table 7; all other data reflects the statistical methods described above.

Since this trial was Phase 2 in size, significance level was set at $\alpha = 0.10$. For the time to first relapse analysis, Kaplan Meier curves and log-rank test were used to estimate and compare the relapse free probabilities of the two treatment groups. Cox proportional hazards model was used to compare the time to relapse free probabilities between two groups adjusting for covariates. Mixed effects models were used to analyze repeated measurement outcomes with the random effect of subject to account for within subject correlation. For the endpoints of EDSS, PASAT, fatigue, depression, quality of life, linear mixed effects model was used to compare treatment groups through 12 and 24 months. Mixed effects negative binomial regression model

and linear mixed effects model were used to compare enhancing lesion number and volume (log-transformed) between treatment groups at all follow-ups, and mixed effects logistic model was used to compare the number of subjects positive for gadolinium enhancing lesions. Linear mixed effects model was carried out to compare the percent change in brain volumes between treatment groups.

Mixed effects logistic regression model was used to evaluate the association between the number of enhancing lesions and the occurrence of relapse, estriol levels and occurrence of relapse, and estriol levels and presence of enhancing lesions. Linear mixed effects model was carried out to evaluate the association between PASAT change and percent brain volume change, between PASAT change and estriol levels and between compliance and estriol levels.

Intention-to-treat analyses were carried out for all end points, which included all patients who underwent randomization and for whom data existed after taking at least one dose of study drug. See Appendix for sensitivity analyses.

The sample size of 150 patients was used to provide approximately 80% power detect a one third reduction in relapse rates in Estriol + GA compared to Placebo + GA at a two-sided significance level of 0.10 for this phase 2 clinical trial to detect a difference in annualized relapse rates with an estimated rate of 0.75 versus 1.18 for Estriol + GA versus Placebo + GA, respectively, in 2 years.

RESULTS

PATIENTS

A total of 164 patients were randomized, of which 158 received study drug and had at least one visit thereafter (intention-to-treat population). Of the 158 patients, 82 were assigned to the Estriol + GA group and 76 to the Placebo + GA group (Figure 1). Baseline demographics and disease characteristics were well balance across both patient groups (Table 1).

The rate of discontinuation was similar between groups (Figure 1). A total of 60 patients (73.2%) in the Estriol plus GA group and 56 (73.7%) in the Placebo plus GA group completed the 24 month study treatment duration. Of the 158 patients, 15.8% discontinued the study during the first year (7.6% in the Estriol plus GA group and 8.2% in the Placebo plus GA group), and an additional 10.7% discontinued the study during the second year (6.3% and 4.4%, respectively).

Reasons for discontinuation did not differ between groups. The most common reasons for

discontinuation were lost to follow up or patient's decision based on family issues or time constraints.

EFFICACY

Estriol Levels

At month 3, serum estriol concentrations increased to a mid-pregnancy range in the Estriol plus GA treated group, while serum estriol concentrations did not exceed the estriol assay detection limit in the Placebo plus GA group (Figure 2A; Table 2).

Table 2: Estriol Levels					
Month	Estriol + GA Group			Placebo + GA Group	
	# pts	Mean $\pm$ SD, Median		# pts	Mean $\pm$ SD, Median
0	77	3.5 $\pm$ 6.0, 1.8		68	2.1 $\pm$ 2.3, 1.6
3	77	16.2 $\pm$ 25.3, 11.4		68	2.1 $\pm$ 1.6, 1.8
6	77	14.6 $\pm$ 14.8, 10.6		67	1.9 $\pm$ 1.4, 1.8
12	67	13.9 $\pm$ 19.3, 10.2		58	2.0 $\pm$ 1.4, 1.8
18	60	12.4 $\pm$ 9.8, 11.7		51	1.8 $\pm$ 1.2, 1.8
24	58	10.1 $\pm$ 6.9, 9.4		51	1.8 $\pm$ 1.1, 1.6

Serum total estriol levels are expressed as means $\pm$ SE in units of ng/mL. Free estriol levels were also measured and followed a similar pattern of change within individuals as total levels, with absolute free levels a fraction of the magnitude of the absolute total levels as expected.

Levels remained elevated through months 3, 6 and 12 in the Estriol plus GA group. However, by month 18, there was a trend for a decrease in estriol levels ($p = 0.065$), which reached

significance by month 24, with a drop of 38% from month 3 to 24 (16.2 ng/mL at month 3, 10.1 ng/mL at month 24, $p = 0.003$). Possible reasons for the significant drop in estriol levels at

month 24 in the Estriol plus GA group included drop out of those with relatively higher estriol levels prior to month 24 or poorer compliance in those who remained in the study at month 24.

To distinguish between these two possibilities, estriol levels were reexamined only in those who completed the study, and again estriol levels were again significantly decreased ($p = 0.0006$), thereby suggesting poorer compliance at month 24 in those who remained in the study.

Assessment of compliance using pill return counts showed that over 75% of those patients with a reduction in estriol levels by greater than 40% at month 24 did not have pill return counts showing compliance, while in those without such reductions in estriol levels, over 75% had pill return counts showing compliance at month 24. Compliance assessment revealed very strong correlations between and estriol levels and compliance in Estriol + GA ($P = 0.001$), but not in

Placebo + GA, with an equal rate of compliance at month 24 in Estriol + GA (0.88) and Placebo + GA (0.89).

Relapses

The primary outcome measure for efficacy was annualized relapse rate including all subjects on an intent-to-treat basis. The study was powered using alpha of 0.10 as recommended for Phase 2 trials. Since all subjects were starting GA treatment at the time of randomization to either Estriol or Placebo, and since GA treatment is known to take time to reach full potency in reducing disease activity, a hierarchical statistical approach was used whereby results in the first 12 months of treatment would be assessed, and if significance were met, results in the entire 24 months of treatment would be assessed. In the first 12 months of treatment, the relapse rate was reduced by 47% ($P = 0.021$) in the Estriol plus GA group as compared to the Placebo plus GA group (Figure 2B; Table 3). In the entire 24 months of treatment, the relapse rate was reduced by 32% ($P = 0.098$) in the Estriol plus GA group as compared to the Placebo plus GA group.

Regarding temporal patterns, relapse rates remained low and unchanged from month 12 (0.25) to month 24 (0.25) with Estriol + GA, while relapse rates decreased gradually from month 12 (0.48) to month 24 (0.37) with Placebo + GA. A more rapid onset of efficacy with Estriol + GA was also observed when examining the proportion of subjects relapse free over 24 months, with differences beginning at 6-12 months, favoring Estriol + GA, $P = 0.096$, (Fig. 2C).

The more rapid onset of efficacy with Estriol + GA was also observed for white matter gadolinium enhancing lesions on brain MRI. In Placebo + GA, the number of subjects with enhancing lesion positive MRIs gradually decreased from baseline to month 12 to month 24, while in Estriol + GA, the number was reduced markedly by month 12, with levels remaining low and stable at month 24 (Table 3). Also, enhancing lesion volumes were decreased at month 12 by 45% with Placebo + GA and by 67% with Estriol + GA. The earlier reduction in enhancing lesion activity with Estriol + GA was consistent with the earlier reduction in relapse rates with Estriol + GA, and a significant association between relapses and the presence of enhancing lesions was found ($P = 0.04$).

Table 3: Clinical and MRI End Points*

End Point	Estriol + GA (n=82)	Placebo + GA (n=76)
Annualized relapse rate in 12 months		
Rate (95% CI) †	0.25 (0.16 – 0.40)	0.48 (0.33 – 0.69)
Adjusted rate ratio E+GA vs. P+GA (95% CI) §	0.51 (0.29 – 0.90)* ¹	
Annualized relapse rate in 24 months		
Rate (95% CI) †	0.25 (0.17 – 0.37)	0.37 (0.25 – 0.53)
Adjusted rate ratio E+GA vs. P+GA (95% CI) §	0.65 (0.39 – 1.08) * ²	
Time to first confirmed relapse		
Proportion of pts with relapse at 12 months % (95% CI) ‡	22.8 (15.0 – 33.7)	33.1 (23.5 – 45.2)
Proportion of pts with relapse at 24 months % (95% CI) ‡	33.3 (23.8 – 45.4)	42.9 (32.1 – 55.5)
Adjusted hazard ratio E+GA vs. P+GA (95% CI)	0.63 (0.36 – 1.09) * ³	
¶ Time to disability progression		
Proportion of pts with progression at 24 months % (95% CI) ‡	11.4 (5.9 – 21.7)	15.8 (8.8 – 27.6)
Adjusted hazard ratio E+GA vs. P+GA (95% CI)	0.81 (0.32 – 2.07)	
¶ EDSS score reduction from baseline to Month 24		
Mean ± SD, Median	0.29 ± 0.98, 0.5	0.05 ± 1.13, 0.0
Lesion activity on brain MRI		
Percentage patients with enhancing lesions % (95% CI)		
Baseline	32.1 (21.9 – 42.3)	29.3 (19.0 – 39.6)
Month 12	14.5 (6.2 – 22.8)#	21.0 (10.8 – 31.1)
Month 24	14.6 (5.2 – 23.9)	14.6 (5.2 – 23.9)
Enhancing lesion volume (mean ± SD, median)		
Baseline	79.7 ± 220.0, 0	49.9 ± 121.2, 0
Month 12	26.0 ± 153.5, 0	27.4 ± 147.2, 0
Month 24	33.2 ± 115.9, 0	17.9 ± 71.1, 0

* Plus-minus values are means ± SD. CI denotes confidence interval, E+GA for Estriol+GA, and P+GA for Placebo+GA.

† Annualized relapse rates were calculated based on negative binomial regression.

§ Relapse rate ratio was estimated using negative binomial regression with adjustment for age, baseline EDSS (<2 vs. ≥2), number of relapse 12 months prior study entry (0-1 vs. >1), MS duration (<1 vs. ≥1 year), prior GA treatment (never vs. past/current), and prior interferon treatment (yes vs. no).

- ‡ Values were calculated using the Kaplan-Meier product-limit method. Progression defined as EDSS increase of at least 1.0 point in subjects with baseline score of 1.0 or higher or increase of at least 1.5 points with baseline score of 0, each sustained for at least 6 months.
- ¶ Hazard ratio was estimated using Cox proportional hazard regression. For relapse, age, baseline EDSS (<2 vs. ≥2), number of relapse 12 months prior study entry (0-1 vs. >1), MS duration (<1 vs. ≥1 year), prior GA treatment (never vs. past/current), and prior interferon treatment (yes vs. no) were adjusted; for EDSS progression, age and baseline EDSS (<2 vs. ≥2) were adjusted.
- *¹ P = 0.021; *² P = 0.098; *³ P = 0.096
- # P = 0.14 comparing the difference between the two groups at Month 12 using mixed effect logistic model adjusted for age and baseline number of gadolinium enhancing lesions.

Disabilities

Exploratory disability outcomes revealed promising trends for improvement in the Estriol plus GA group. The Expanded Disability Status Scale (EDSS) is a standard composite disability score used extensively in MS trials. Higher scores indicate worse disability. The probability of disability worsening or EDSS progression (as defined by an increase in EDSS of 1 point for over 6 months) was 15.8% for the Placebo plus GA group, and 11.4% for the Estriol plus GA group (Table 3). EDSS scores were then assessed for possible improvement with combination treatment. While EDSS scores in the Placebo plus GA group were stable and unchanged over the entire 24 month treatment duration, the Estriol plus GA group showed a significant improvement in EDSS scores by the end of study, month 24, with a median change in EDSS of a half step (EDSS absolute median change = -0.5, $p = 0.03$); however, group differences in EDSS improvement were not powered for significance (Figure 3A).

Exploratory clinical outcomes showed beneficial trends in the Estriol plus GA group. The Modified Fatigue Impact Scale (MFIS) total scores revealed significant improvement by end of study month 24 in the Estriol plus GA group ($p = 0.01$), with no change in the Placebo plus GA group ($p = \text{NS}$), with a significant between group difference ($p = 0.03$) (Figure 3B). Beck Depression Inventory (BDI) total scores and MS Quality of Life (MSQOL) scores also showed beneficial trends (Figure 4C and Table 4).

Table 4: Disabilities

End Point	Estriol + GA (N=82)	Placebo + GA (N=76)	Comparing the two study groups §	
Proportion of pts with disability progression over 24 Months as % (95% CI) †	11.4 (5.9 – 21.7)	15.8 (8.8 – 27.6)		
E+GA vs. P+GA			0.81(0.32 – 2.07)	p=0.664
Adjusted hazard ratio (95% CI) ‡				
EDSS Score[§]				
Baseline (Mean ± SD, Median)	2.22 ± 1.16, 2.3	2.13 ± 1.11, 2.0		
Change from baseline at Mon 12	N=69	N=63		
Mean ± SD, Median	-0.13 ± 1.06, 0	-0.06 ± 1.11, 0	-0.14 (-0.47 – 0.19)	p=0.404
Change from baseline at Mon 24	N=56	N=56		
Mean ± SD, Median	-0.29 ± 0.98, -0.5	-0.05 ± 1.13, 0.0	-0.22, (-0.57 – 0.12)	p=0.198
Fatigue score[§]				
Baseline (Mean ± SD, Median)	33.7 ± 19.4, 32	31.1 ± 19.0, 28.5		
Change from baseline at Mon 12	N=70	N=62		
Mean ± SD, Median	-3.4 ± 13.5, -3	-0.6 ± 15.1, -0.5	-2.6 (-6.7 – 1.5)	p=0.218
Change from baseline at Mon 24	N=58	N=56		
Mean ± SD, Median	-5.0 ± 14.0, -3	0.5 ± 12.8, 1	-5.7 (-10.0 – -1.4)	p=0.009
Depression Score[§]				
Baseline (Mean ± SD, Median)	11.3 ± 9.2, 10	10.9 ± 10.0, 9		
Change from baseline at Mon 12	N=70	N=62		
Mean ± SD, Median	-1.6 ± 7.1, -2	-0.6 ± 9.4, -0.5	-0.6 (-2.9 – 1.6)	p=0.583
Change from baseline at Mon 24	N=59	N=56		
Mean ± SD, Median	-2.6 ± 6.0, -3	-1.2 ± 6.8, -2	-1.1 (-3.5 – 1.3)	p=0.368
MS QOL – Physical score[§]				
Baseline (Mean ± SD, Median)	64.3 ± 19.3, 64.2	66.3 ± 19.0, 69.2		
Change from baseline at Mon 12	N=69	N=62		
Mean ± SD, Median	4.6 ± 12.2, 3.2	1.5 ± 16.2, 2.4	2.4 (-2.0 – 6.8)	p=0.279
Change from baseline at Mon 24	N=58	N=56		
Mean ± SD, Median	5.1 ± 16.7, 3.9	3.1 ± 13.8, 4.6	2.2, (-2.3 – 6.8)	p=0.338
MS QOL – Mental Score[§]				
Baseline (Mean ± SD, Median)	80.7 ± 24.1, 86.3	83.5 ± 24.5, 92.9		
Change from baseline at Mon 12	N=69	N=62		
Mean ± SD, Median	3.0 ± 20.3, -0.8	-0.5 ± 23.6, 0.2	2.1 (-4.3 – 8.5)	p=0.525
Change from baseline at Mon 24	N=58	N=56		
Mean ± SD, Median	4.6 ± 20.2, 4.6	0.8 ± 20.7, 2.4	2.8 (-3.9 – 9.5)	p=0.419
MSFC Score[§]				
Baseline (Mean ± SD, Median)	-0.04 ± 0.69, 0.05	0.06 ± 0.79, 0.24		
Change from baseline at Mon 12	N=70	N=58		
Mean ± SD, Median	0.12 ± 0.37, 0.09	0.06 ± 0.38, 0.06	0.07 (-0.03 – 0.20)	p=0.262
Change from baseline at Mon 24	N=60	N=54		
Mean ± SD, Median	0.10 ± 0.35, 0.09	0.08 ± 0.43, 0.10	0.03 (-0.10 – 0.16)	p=0.629
PASAT3 Score[§]				
Baseline (Mean ± SD, Median)	51.0 ± 8.9, 55	52.3 ± 9.1, 56		
All patients				
Change from baseline at Mon 12	N=70	N=61		
Mean ± SD, Median	1.93 ± 5.59, 1.0	0.13 ± 4.46, 0	1.62 (-0.03 – 3.27)	p=0.034
Change from baseline at Mon 24	N=60	N=55		
Mean ± SD, Median	1.07 ± 4.04, 1.0	1.11 ± 4.29, 0	-0.11 (-1.81 – 1.60)	p=0.902
Pts with baseline PASAT3<55				
Change from baseline at Mon 12	N=33	N=25		
Mean ± SD, Median	4.70 ± 6.56, 4.0	1.60 ± 5.99, 1.0	3.00 (0.68 – 5.32)	p=0.011

Plus-minus values are means $\pm$ SD. CI denotes confidence interval, E+GA indicates Estriol+GA, and P+GA indicates Placebo+GA.

a Change from baseline negative value indicates improvement.

b Change from baseline positive value indicates improvement.

5 § Linear mixed effect model was developed using all follow-up data to estimate the difference of score change from baseline between the two study groups at Months 24 and 12 while baseline score was adjusted.

In order to estimate the difference of PASAT3 score change between the two study groups for patients with baseline <55 and $\geq$ 55, dichotomized baseline PASAT3 score (<55 vs $\geq$ 55) and its interaction
10 terms with treatment and month were included in the model and all patients follow-up data were used. The data in this column are: mean difference (95% Confidence interval of the mean difference) and p-value.

‡ Values were calculated using the Kaplan-Meier product-limit method. Progression defined as EDSS increase of at least 1.0 point in subjects with baseline score of 1.0 or higher or increase of at least 1.5
15 points with baseline score of 0, each sustained for at least 6 months.

¶ Hazard ratio was estimated using Cox proportional hazard regression. Age and baseline EDSS (<2 vs. $\geq$ 2) were adjusted.

There were no significant differences between groups in the Multiple Sclerosis
20 Functional Composite (MSFC), which reflects a composite of scores including the Paced Auditory Serial Addition Test (PASAT) for cognition, the 9 hole peg test and the 25 foot walk test (Table 5). However, an interesting effect of combination treatment was observed on cognitive disability. A perfect PASAT score is 60, with scores lower than 55 serving as a continuous variable for disability. By 12 months of treatment, PASAT scores improved
25 significantly as compared with scores at baseline, among patients receiving Estriol plus GA, while no significant improvement was observed in those receiving Placebo plus GA, ($p = 0.04$ between group difference, all adjusted for covariates of age, education and baseline scores). Subgroup analysis showed that this improvement in PASAT scores in the Estriol plus GA group at month 12 was due to improvements in those with more cognitive disability at baseline (Figure
30 5). This beneficial effect on PASAT scores at 12 months in the Estriol plus GA group could not be attributed to practice effects of repeated testing since the comparison was with the Placebo plus GA group tested at identical time points.

Table 5: Multiple Sclerosis Functional Composite (MSFC)

End Point	Estriol + GA (N=82)	Placebo + GA (N=76)
MSFC Score ^a		
Baseline score (Mean ± SD, Median)	-0.04 ± 0.69, 0.05	0.06 ± 0.79, 0.24
Change from baseline at Month 12	N=70	N=58
Mean ± SD, Median	0.13 ± 0.37	0.06 ± 0.38
Change from baseline at Month 24	N=60	N=54
Mean ± SD, Median	0.10 ± 0.35	0.09 ± 0.43
PASAT3 score – All Patients ^a		
Baseline score (Mean ± SD, Median)	51.0 ± 8.9, 55	52.3 ± 9.1, 56
change from baseline at Month 12	N=70	N=61
Mean ± SD, Median	1.9 ± 5.6, 1.0 **	0.1 ± 4.5, 0
change from baseline at Month 24	N=60	N=55
Mean ± SD, Median	1.1 ± 4.0, 1.0	1.1 ± 4.3, 0
% change from baseline at Month 12	N=70	N=61
Mean ± SD, Median (%)	5.5 ± 15.6, 1.8 **	0.8 ± 9.9, 0
% change from baseline at Month 24	N=60	N=55
Mean ± SD, Median (%)	2.9 ± 10.6, 1.7	2.7 ± 10.3, 0
PASAT3 score – Patients with baseline score < 55 ^a		
Baseline score (Mean ± SD, Median)	43.7 ± 7.6, 45	44.7 ± 9.1, 49
change from baseline at Month 12	N=33	N=25
Mean ± SD, Median	4.7 ± 6.6, 4.0 *	1.6 ± 6.0, 1.0
change from baseline at Month 24	N=26	N=23
Mean ± SD, Median	2.3 ± 5.2, 3.5	3.0 ± 5.9, 4.0
% change from baseline at Month 12	N=33	N=25
Mean ± SD, Median (%)	12.6 ± 19.9, 7.6 *	4.0 ± 14.0, 3.8
% change from baseline at Month 24	N=26	N=23
Mean ± SD, Median (%)	6.4 ± 14.8, 6.8	6.9 ± 14.7, 9.1
9-Hole Peg Test ^b		
Baseline value (Mean ± SD, Median)	19.7 ± 3.8, 19.0	19.1 ± 2.7, 18.8
Change from baseline at Month 12	N=70	N=63
Mean ± SD, Median	-0.6 ± 1.5, -0.6	-0.1 ± 1.8, -0.4
Change from baseline at Month 24	N=60	N=56
Mean ± SD, Median	-0.1 ± 3.5, -0.6	-0.4 ± 1.5, -0.5
25-foot Walk Time ^b		
Baseline value (Mean ± SD, Median)	4.9 ± 1.0, 4.7	4.9 ± 1.5, 4.5
Change from baseline at Month 24	N=70	N=60
Mean ± SD, Median	0.1 ± 0.8, 0.1	0.0 ± 0.9, 0.0
Change from baseline at Month 24	N=60	N=55
Mean ± SD, Median	-0.1 ± 0.6, -0.0	0.1 ± 1.1, 0.1

a Change from baseline positive value indicate improvement for MSFC and PASAT3

b Change from baseline positive value indicate worsening for 9-Hole Peg test and 25-Foot Walk Time.

** P < 0.05, student t-test comparing the means of the two study groups

* P < 0.10, student t-test comparing the means of the two study groups

§ Values were calculated using the Kaplan-Meier product-limit method.

In contrast to month 12 observations, absolute PASAT scores were no different at month 24 in the Estriol plus GA group compared to the Placebo plus GA group (Figure 3C; Tables 4 and 5). This was due to both a trend for improvement in the Placebo plus GA group as well as a trend for worsening in the Estriol plus GA group. To address whether a trend for worsening in the Estriol plus GA group at month 24 might be related to the decrease in estriol levels at month 24 (Figure 2A), correlations between estriol levels and improvement in PASAT scores were assessed. Indeed, higher estriol levels correlated with greater improvement in PASAT scores ($p = 0.03$ for all patients; $p = 0.07$ for Estriol + GA patients only). Further, when serum estriol levels were dichotomized to greater than or less than 6 ng/mL, estriol levels greater than or equal to 6 ng/mL correlated strongly with improvement in PASAT scores (All patients, $p = 0.009$; Estriol plus GA group, $p = 0.006$).

Gray matter volumes, specifically cortical gray matter volumes, have previously been associated with cognitive test scores. There was less cortical gray matter atrophy (45%) and whole gray matter atrophy (30%) at month 12 in the Estriol plus GA group compared to the Placebo plus GA group (cortical gray matter: Estriol + GA = -0.41, Placebo + GA = -0.74, $p = 0.079$; whole gray matter: Estriol + GA = -0.47, Placebo + GA = -0.68, $p = 0.139$) (Figure 3D & 3E). This gray matter sparing was independently confirmed using voxel-based morphometry (VBM), the latter revealing which gray matter regions were preserved with Estriol + GA compared to Placebo + GA (Figures 3 and 6). Subgroup analysis showed that this gray matter sparing was present in the group of patients that were enhancing lesion negative (cortical gray matter 52%: Estriol + GA = -0.36, Placebo + GA = -0.76, $p = 0.048$; whole gray matter 39%: Estriol + GA = -0.44, Placebo + GA = -0.70, $p = 0.097$), while absent in the group that was enhancing lesion positive. Similar to effects on PASAT scores, beneficial effects on gray matter sparing in the Estriol + GA group were no longer present at month 24. Indeed, correlations between PASAT improvement and gray matter sparing were found (cortical gray matter, $p = 0.0327$; whole gray matter, $p = 0.0359$), which was present in the Estriol + GA group (cortical gray matter, $p = 0.0159$; whole gray matter, $p = 0.0093$) and absent in the control Placebo + GA group. In contrast, the Estriol + GA group compared to the Placebo + GA group had more white matter atrophy, but this occurred only in patients who were enhancing lesion positive, with no differences in those who were enhancing lesion negative. Greater white matter atrophy occurring only in the enhancing lesion positive patients with Estriol + GA treatment was

consistent with pseudoatrophy due to anti-inflammatory effects of Estriol + GA in white matter, which in turn was consistent with both the greater reduction in MRI enhancing lesions in white matter and greater reductions in clinical relapse rates in the Estriol + GA group (Figures 2B & 2C).

Table 6: MRI Volumes

End point §	Estriol + GA (N=82)	Placebo + GA (N=76)
Baseline Volume (cc) §§		
Whole brain	1604 ± 62	1602 ± 51
Whole gray matter	954 ± 51	926 ± 52
Cortical gray matter	754 ± 46	761 ± 42
White matter	650 ± 35	640 ± 32
% change -- Baseline to 12 months		
All patients		
Whole brain	-0.48 ± 0.69, -0.45	-0.49 ± 0.63, -0.43
Whole gray matter	-0.47 ± 0.82, -0.49	-0.68 ± 0.71, -0.66
Cortical gray matter	-0.41 ± 0.94, -0.49 *	-0.74 ± 0.81, -0.69
White matter	-0.50 ± 1.06, -0.11	-0.21 ± 0.82, -0.15
Patients without enhancing lesions at baseline		
Whole gray matter	-0.44 ± 0.76, -0.45 *	-0.70 ± 0.74, -0.66
Cortical gray matter	-0.36 ± 0.89, -0.42 **	-0.76 ± 0.85, -0.73
White matter	-0.18 ± 0.94, 0.07	-0.15 ± 0.71, -0.15
Patients with enhancing lesions at baseline		
Whole gray matter	-0.61 ± 0.84, -0.54	-0.65 ± 0.64, -0.64
Cortical gray matter	-0.60 ± 0.92, -0.57	-0.67 ± 0.72, -0.69
White matter	-1.08 ± 1.03, -0.92 **	-0.36 ± 1.06, -0.14
% change -- Baseline to 24 months		
All patients		
Whole brain	-0.87 ± 0.82, -0.99	-0.77 ± 0.72, -0.88
Whole gray matter	-0.95 ± 0.75, -0.93	-0.95 ± 0.75, -0.93
Cortical gray matter	-0.94 ± 0.87, -0.96	-1.05 ± 0.86, -0.94
White matter	-0.75 ± 1.28, -0.64	-0.52 ± 1.12, -0.54
Patients without enhancing lesions at baseline		
Whole gray matter	-0.92 ± 0.80, -0.89	-0.98 ± 0.77, -0.92
Cortical gray matter	-0.93 ± 0.89, -0.97	-1.12 ± 0.88, -0.94
White matter	-0.38 ± 1.21, -0.35	-0.46 ± 1.17, -0.48
Patients with enhancing lesions at baseline		
Whole gray matter	-0.99 ± 0.68, -0.98	-0.82 ± 0.70, -0.92
Cortical gray matter	-0.95 ± 0.85, -0.93	-0.82 ± 0.80, -0.93
White matter	-1.32 ± 1.31, -1.20 **	-0.62 ± 0.97, -0.84

§ Data presented as Mean ± SE, median; negative values indicate volume loss.

§§ No significant difference between the two study groups for baseline volumes. Wilcoxon rank sum test.

** P < 0.05, linear mixed effects model for the difference of the two groups means, adjusted for baseline volume and enhancing lesions present or absent.

* P < 0.10, linear mixed effects model for the difference of the two groups means, adjusted

for baseline volume and enhancing lesions present or absent.

Table 7: MRI		Estriol + GA (N=82)	Placebo + GA (N=76)	Comparing the two study groups §
Enhancing Lesion Volume [§]				
Baseline (Mean ± SD, Median)		79.7 ± 228, 0	54.2 ± 126, 0	
Change from baseline at Mon 12		N=69	N=62	
Mean ± SD, Median		+51.2 ± 202, 0	+18.7 ± 134, 0	+12.5 (-69.0 ~ 44.1) p=0.665
Change from baseline at Mon 24		N=55	N=55	
Mean ± SD, Median		+39.3 ± 196, 0	+34.0 ± 120, 0	+1.6 (-62.6 ~ 59.5) p=0.960
Number of Enhancing Lesions [§]				
Baseline (Mean ± SD, Median)		1.0 ± 2.3, 0	0.9 ± 2.1, 0	
Change from baseline to Mon 12		N=68	N=62	
Mean ± SD, Median		-0.9 ± 2.2, 0	-0.5 ± 1.8, 0	0.89, (0.54 ~ 1.45) p=0.631
Change from baseline to Mon 24		N=55	N=55	
Mean ± SD, Median		-0.9 ± 2.5, 0	-0.5 ± 2.4, 0	0.89, (0.54 ~ 1.48) p=0.655
Lesion activity on Brain MRI ~ proportion of patients with enhancing lesion				
Baseline	% (95% CI)	32.1 (21.9 ~ 42.3)	29.3 (19.0 ~ 39.6)	
Month 12	% (95% CI)	14.5 (6.2 ~ 22.8)	21.0 (10.8 ~ 31.3)	0.30 (0.07 ~ 1.31) p=0.110
Month 24	% (95% CI)	14.6 (5.2 ~ 23.9)	14.6 (5.2 ~ 23.9)	0.66 (0.13 ~ 3.40) p=0.616
Brain Volume ~ All Patients				
Baseline (Mean ± SD, Median)				
Whole brain		1604 ± 62, 1607	1602 ± 51, 1602	
Whole gray matter		954 ± 51, 957	926 ± 52, 967	
Cortical gray matter		754 ± 46, 755	761 ± 42, 762	
White matter		650 ± 35, 650	640 ± 37, 635	
% change from baseline (Mean ± SD, Median) [§]				
Whole Brain	at Mon 12	-0.50 ± 0.70, -0.49	-0.50 ± 0.64, -0.43	-0.00 (-0.23 ~ 0.23) p=0.988
	at Mon 24	-0.89 ± 0.82, -1.6	-0.78 ± 0.73, -0.91	-0.62 (-0.26 ~ 0.22) p=0.877
Whole gray matter	at Mon 12	-0.48 ± 0.82, -0.50	-0.69 ± 0.71, -0.68	0.21 (-0.05 ~ 0.47) p=0.108
	at Mon 24	-0.96 ± 0.75, -0.94	-0.95 ± 0.76, -0.93	0.12 (-0.16 ~ 0.39) p=0.411
Cortical gray matter	at Mon 12	-0.44 ± 0.92, -0.51	-0.72 ± 0.80, -0.67	0.29 (-0.01 ~ 0.58) p=0.056
	at Mon 24	-0.96 ± 0.86, -0.97	-1.04 ± 0.87, -0.93	0.23 (-0.09 ~ 0.54) p=0.156
White matter	at Mon 12	-0.51 ± 1.06, -0.16	-0.20 ± 0.83, -0.11	-0.29 (-0.63 ~ 0.04) p=0.090
	at Mon 24	-0.77 ± 1.28, -0.66	-0.54 ± 1.12, -0.56	-0.20 (-0.56 ~ 0.15) p=0.261
Brain Volume ~ Patients without enhancing lesions at baseline				
% change from baseline (Mean ± SD, Median) [§]				
Whole brain	at Mon 12	-0.35 ± 0.58, -0.41	-0.49 ± 0.63, -0.43	0.10 (-0.16 ~ 0.36) p=0.420
	at Mon 24	-0.72 ± 0.80, -0.57	-0.80 ± 0.76, -0.99	0.08 (-0.19 ~ 0.36) p=0.540
Whole gray matter	at Mon 12	-0.45 ± 0.77, -0.48	-0.71 ± 0.74, -0.68	0.24 (-0.05 ~ 0.53) p=0.106
	at Mon 24	-0.94 ± 0.81, -0.91	-0.99 ± 0.78, -0.93	0.14 (-0.16 ~ 0.45) p=0.358
Cortical gray matter	at Mon 12	-0.39 ± 0.89, -0.43	-0.74 ± 0.84, -0.64	0.34 (0.01 ~ 0.67) p=0.043
	at Mon 24	-0.96 ± 0.89, -0.95	-1.11 ± 0.89, -0.93	0.29 (-0.06 ~ 0.63) p=0.106
White matter	at Mon 12	-0.19 ± 0.95, 0.06	-0.14 ± 0.72, -0.11	-0.10 (-0.48 ~ 0.28) p=0.620
	at Mon 24	-0.41 ± 1.13, -0.35	-0.51 ± 1.18, -0.51	-0.00 (-0.40 ~ 0.39) p=0.988
Brain Volume ~ Patients with enhancing lesions at baseline				
% change from baseline (Mean ± SD, Median) [§]				
Whole brain	at Mon 12	-0.81 ± 0.77, -0.65	-0.53 ± 0.67, -0.49	-0.23 (-0.58 ~ 0.12) p=0.206
	at Mon 24	-1.13 ± 0.82, -1.04	-0.74 ± 0.67, -0.66	-0.25 (-0.61 ~ 0.11) p=0.182
Whole gray matter	at Mon 12	-0.61 ± 0.84, -0.54	-0.65 ± 0.64, -0.64	0.08 (-0.31 ~ 0.47) p=0.698
	at Mon 24	-1.00 ± 0.68, -0.98	-0.83 ± 0.71, -0.92	-0.02 (-0.42 ~ 0.38) p=0.923
Cortical gray matter	at Mon 12	-0.60 ± 0.92, -0.57	-0.67 ± 0.72, -0.69	0.08 (-0.37 ~ 0.53) p=0.722
	at Mon 24	-0.95 ± 0.85, -0.93	-0.82 ± 0.80, -0.93	0.02 (-0.44 ~ 0.48) p=0.919
White matter	at Mon 12	-1.09 ± 1.03, -0.92	-0.36 ± 1.08, -0.13	-0.67 (-1.19 ~ -0.15) p=0.012
	at Mon 24	-1.32 ± 1.31, -1.20	-0.62 ± 0.97, -0.84	-0.58 (-1.11 ~ -0.04) p=0.034

Plus-minus values are means $\pm$ SD. CI denotes confidence interval, E+GA indicates Estriol+GA, and P+GA indicates Placebo+GA.

a Change from baseline negative value indicates improvement.

b Change from baseline negative value indicates worsening.

5 § For Volume of Enhancing Lesions and Brain Volume (whole brain, gray matter, cortical gray matter and white matter, respectively), linear mixed effect model was developed using all follow-up data to estimate the difference of the value change from baseline between the two study groups at Months 24 and 12 while baseline value was adjusted. In order to estimate the difference of brain volume change between the two study groups for patients with and without enhancing lesion at baseline, baseline enhancing lesion number (present /absent) was included in the model and all patients follow-up data were used. The data in this column are: mean difference (95% Confidence interval of the mean difference) and p-value.

For Number of Enhancing Lesions, the data in this column are E+GA vs. P+GA mean of lesions number ratio (95% CI) and p-value based on mixed effect negative binomial regression model.

15 For Lesion Activity on Brain MRI, the data in this column are E+GA vs. P+GA odds ratio (95% CI) and p-value based on mixed effect logistic regression model.

SAFETY

20 Estriol plus GA was found to be safe and well tolerated with regard to adverse events including gynecological outcomes (Table 8). Regarding adverse events, irregular menses occurred more with Estriol + GA ($P < 0.001$), while vaginal infections occurred more with Placebo + GA ($P < 0.05$), with no increase in discontinuations due to either.

Table 8: Adverse Events and Serious Adverse Events

Adverse Events †	Estriol+GA (N=82)	Placebo+GA (N=76)
Any adverse event - no. of events, [no of pts, % of pts]	480 [76, 93%]	392 [67, 87%]
Most frequent events- no. of events [no of pts, % of pts]		
Copaxone injection area abnormalities	51 [26, 32%]*	30 [14, 18%]
Upper respiratory infection	33 [22, 27%]	38 [26, 34%]
Irregular menses / spotting	26 [19, 23%]	4 [3, 4%]

Urinary tract infection	23 [15, 18%]	16 [10, 13%]
Fatigue	15 [13, 16%]	10 [8, 10%]
Depression /anxiety	14 [12, 15%]	10 [9, 12%]

Menstrual flow amount increased	12 [11, 13%]	8 [6, 8%]
Headache	11 [9, 11%]	12 [11, 14%]
Nausea/vomiting	9 [7, 9%]	5 [5, 6%]
Sinusitis	6 [6, 7%]	14 [10, 13%]*
Arm/leg numbness, tingling	7 [6, 7%]	10 [7, 9%]
Gastroenteritis	7 [5, 6%]	4 [3, 4%]
Dizziness	5 [4, 5%]	10 [7, 9%]
Vision problem (blurry, double)	6 [4, 5%]	7 [7, 9%]
Back pain	5 [4, 5%]	5 [5, 6%]
Menstrual cramp	4 [4, 5%]	5 [4, 5%]
Insomnia	4 [4, 5%]	4 [4, 5%]
Heart palpitation	2 [2, 2%]	4 [4, 5%]
Shingles	2 [2, 2%]	4 [4, 5%]
Vaginal infection	1 [1, 1%]	9 [8, 10%]**
Adverse events leading to discontinuation – no. (%)	5 (6%)	5 (6%)
Severe adverse events †	9 [8, 10%]	12 [10, 13%]
MS relapse	2 [2, 2%]¶	6 [5, 6%]¶
Pregnancy termination	2 [2, 2%]	0
UTI	1 [1, 1%]	1 [1, 1%]
Migraine headache related eye pain	1 [1, 1%]	0
Heart failure	1 [1, 1%]	0
Pace maker implantation	1 [1, 1%]	0
Pyelonephritis	1 [1, 1%]	0
Systolic heart failure	1 [1, 1%]	0
Accidentally took other's drug	0	1 [1, 1%]
Acute appendicitis	0	1 [1, 1%]
B-cell lymphoma §	0	1 [1, 1%]
Car accident related body numbness	0	1 [1, 1%]
Right knee replacement	0	1 [1, 1%]
Other safety events monitored		
Uterus		
Endometrial thickness > 8mm (ultrasound) – no. (%)	24 (29)	27 (36)
Endometrial biopsies performed §§ – no. (%)	9 (11)	6 (8)
Fibroids (ultrasound) – no. (%)	8 (10)	8 (11)
Abnormal proliferation on biopsy – no. (%)	0	0
Breast		
Fibrocystic disease on clinical exam	5 (6)	4 (5)
Mammogram with malignancy	0	0

† All patients who took at least one dose of study drug were included. However, among the 6 patients who dropped shortly after baseline visit, five did not have safety evaluation data and were excluded from the safety analysis. The listed events reported by % were rounded up to nearest integer. The events are listed by decreasing incidence in the Estriol + GA group, within each category.

- * AE significantly higher in one treatment group compared to the other; *** indicating $P < 0.001$, ** indicating $P < 0.05$, and * indicating $P < 0.10$.
- ‡ SAE patients were all hospitalized, but none had severe or immediately life-threatening condition.
- § This patient, in the placebo group, discontinued the study at the time of B-cell lymphoma diagnosis when was on study for 12 months and died 17 months later.
- ¶ In Estriol+GA group, both patients discontinued the study: one before and one after Month 12. In Placebo+GA group, 3 patients discontinued the study: 1 before and 2 after Month 12.
- §§ Four patients had multiple uterine endometrial biopsies: two patients had two biopsies each in the Estriol+GA group and two patients had three biopsies each in the Placebo+GA group. No abnormal proliferation was found.
- Note No laboratory abnormalities occurred significantly more frequently in either treatment group.

Sensitivity analysis

Original analyses included all subjects on an intention-to-treat basis. 73.6% of the subjects completed the entire 24 month treatment duration, with a total of 22 drop outs in the GA plus Estriol group and 20 drop outs in the GA plus Placebo group (Figure 1; Table 9). This drop out rate was expected considering the unique nature of this study whereby the study only provided estriol and placebo treatments, while patients provided their own injectable GA treatment. The reason for drop out did not differ between treatment groups (Figure 1). Sensitivity analyses for both the primary endpoint of relapse rate, as well as for other exploratory outcomes of brain volume loss and PASAT scores, each confirmed the robustness of the original analyses.

Table 9: Drop Outs

Subjects who completed month 24 (M24) versus subjects who dropped after month 12 (M12)

Study status	Estriol + GA	Placebo + GA	Total
Completion	60	56	116
Dropped before M12*	12	13	25
Dropped after M12**	10	7	17
Total	82	76	158

*Among the 25 subjects, 8 had M3 visit and 17 (8 in Estriol group and 9 in placebo group) had M6 visit.

**Among the 17 subjects, 5 (4 in Estriol group and 1 in placebo group) had M18 visit.

Between month 12 and 24, there were 10 drop outs in the Estriol plus GA group (10/70 = 14.2%) and 7 drop outs in the Placebo plus GA group (7/56 = 12.5%). Adverse events did not differ by group when split into those before or after 12 months (Table 10). Regarding the primary outcome measure of relapse rate at end of study (month 24), the primary endpoint of a reduction in relapse rate by one third in the Estriol plus GA group as compared to the Placebo plus GA group was reached. The observation that relapse rates were reduced more dramatically, by nearly half, at the earlier time point of month 12 could have been due in part to drop outs between month 12 and 24. Thus, the effect of drop outs on the primary outcome measure of relapse rates was formally analyzed by imputing missing data due to dropout based on the pattern mixture model. This analysis sought to address the possibility of missing data not being random. Similar results were obtained in analyses with and without imputation, supporting the robustness of the results for the primary endpoint (Table 11).

Table 10: Adverse events split by drop out before or after 12 months

Body system	Estriol + GA		Placebo + GA	
	Completed (N=60)	Discontinued (N=22)	Completed (N=56)	Discontinued (N=20)
Breast	11 [10]		5 [5]	
Cancer				1 [1]
Cardiovascular	7 [5]	2 [2]	8 [8]	
GA related?	53 [20]	18 [8]	22 [11]	10 [4]
Endocrinology	1 [1]			
Extremity	41 [24]	13 [8]	43 [21]	4 [2]
General	50 [28]	11 [6]	48 [26]	9 [6]
GI	25 [14]	6 [5]	19 [15]	3 [3]
GU	21 [14]	3 [2]	17 [10]	3 [3]
GYN	16 [15]	4 [2]	18 [15]	3 [3]
Head/Neck	33 [20]	5 [3]	42 [26]	9 [4]
Lymph			1 [1]	1 [1]
Menses	36 [26]	8 [5]	14 [10]	3 [2]
Mental	17 [14]	6 [5]	14 [10]	5 [5]
MRI related			3 [2]	
Skeleton	3 [3]	1 [1]	2 [1]	
Neurology	17 [12]	2 [2]	13 [9]	1 [1]
Respiratory	42 [25]	5 [4]	47 [26]	7 [3]
Skin	19 [15]	4 [4]	15 [10]	2 [2]
Total	392 [58]	88 [18]	331 [53]	61 [13]

Data was presented as: Number of events [# of patients]

Sensitivity analysis relapse rate

For the primary endpoint, the main analysis seeks to compare the relapse event rate between treatment groups based on the negative binomial regression. As a sensitivity analysis, recurrent events analysis was performed based on Andersen Gill model to compare the relapse hazard rate between treatment groups. Both analyses showed similar results, and significant and meaningful reduction in relapse rates was found in the Estriol plus GA group as compared to the Placebo plus GA group.

Table 11: Sensitivity analysis for the primary end point – Annualized Relapse Rate				
Analysis	Method*	Missing Data Imputed	Results	
			0-12 Months	0 – 24 Months
Original analysis	Negative binomial regression model	No	Relapse rate ratio 0.51 95% CI: 0.29 – 0.90 P = 0.021	Relapse rate ratio: 0.65 95% CI: 0.39 – 1.08 P = 0.098
Sensitivity analysis	Negative binomial regression model	Yes, multiple imputation based on the pattern mixture model.	Relapse rate ratio 0.56 95% CI: 0.31 – 1.03 P = 0.063	Relapse rate ratio: 0.63 95% CI: 0.37 – 1.08 P = 0.096
Sensitivity analysis	Andersen Gill model; relapse was treated as recurrent event.	No	Hazard ratio 0.49 95% CI: 0.28 – 0.87 P = 0.015	Hazard ratio: 0.63 95% CI: 0.38 – 1.03 P = 0.067

*Age, baseline EDSS, number of relapse 12 months prior study entry, MS duration, prior GA treatment and prior interferon treatment were included in the model as covariates.

Missing data due to dropouts

The missing data due to study dropouts complicate the statistical analysis because certain assumptions must be made about the missingness mechanism and unobserved values to deal with incomplete observations. For the primary analysis of longitudinal endpoints were based on the assumption that missing data are ignorable, which means that missingness is independent of the unobserved outcomes after accounting for the appropriate observed data in the model. Similarly, for the time to event endpoints, the Kaplan-Meier curves and proportional hazard model were based on the assumption that dropout time is non-informative and independent of the event time.

Longitudinal endpoints

Sensitivity analyses regarding missing data were performed to demonstrate the robustness of study conclusion. For this, multiple imputation analysis were performed on the missing data according to the pattern mixture model as a sensitivity analysis to address the possibility of data being non-ignorable or missing not at random (MNAR) (Little, R. & L. Yau, Biometrics 52:1324 (1996)). The missing data were sequentially imputed by the follow up time, and the imputation model assumed that the treatment effect for patients after dropout is the same as taking placebo (Ratitch, B. & M. O'Kelly, Proc. Pharm. Industry SAS User Group, Nashville (2011)). The analyses results were compared for relapse rate with assumptions of ignorable and non-ignorable missing data. The results are similar with and without imputation, and significant and meaningful reduction in relapse rates was observed in the Estriol plus GA group as compared to the Placebo plus GA group.

Similar imputation analyses were carried out for the endpoints of brain volume change in gray matter, in cortical gray matter, and PASAT3 to evaluate the possible impact of study dropout. Table 12 compares analyses of brain volume change for gray matter and cortical gray matter. Longitudinal analyses results for PASAT3 with and without imputation were also done. The imputation analysis showed no significant improvement in the Placebo plus GA group at both 12 and 24 months.

Table 12:

Missing data analysis for some secondary end points – Comparing the difference of means between the two study groups at Months 12 and 24

End Point	Month	Original Data (Ignorable missing data)	Multiple imputation Data (Non-ignorable missing data)
		Estimate difference (95% CI)	Estimate difference (95% CI)
Whole gray matter volume (% change from baseline) §	12	0.19 (-0.06, 0.45), P = 0.14	0.19 (-0.06, 0.45), P = 0.14
	24	0.10 (-0.17, 0.37), P = 0.48	0.13 (-0.13, 0.40), P = 0.32
Cortical gray matter volume (% change from baseline) §	12	0.26 (-0.03, 0.55), P = 0.08	0.26 (-0.03, 0.55), P = 0.08
	24	0.21 (-0.10, 0.51), P = 0.19	0.23 (-0.07, 0.54), P = 0.13
PASAT3 score (Change from baseline) ¶	12	1.5 (-0.1, 4.3), P = 0.06	1.6 (0.0, 3.2), P = 0.05
	24	-0.3 (-1.9, 1.4), P = 0.74	0.3 (-1.5, 2.0), P = 0.77

§ Values were calculated based on linear mixed effect model adjusted for baseline volume and enhancing lesions (present vs. absent).

¶ Values were calculated based on linear mixed effect model adjusted for baseline PASAT3 score.

§ Values were calculated based on linear mixed effect model adjusted for baseline volume and enhancing lesions (present vs. absent).

¶ Values were calculated based on linear mixed effect model adjusted for baseline PASAT3 score.

5

MRI Methodologies:

MRI scans were performed at 0, 3, 6, 12 and 24 months using a standardized protocol implemented at each site that consisted of the following: T1-weighted 3D volume, pre and post contrast: TR2200, TE3.4, TI 900, 176 slices, 1mm³. Dual-echo fast spin echo: TR10000, TE12/95, 50 slices, 1x1x3mm. Fluid attenuated inversion recovery (FLAIR): TR10380, TE88, TI88, TI2500, 50 slices, 1x1x3mm. Minor changes were allowed to accommodate different platforms and field strengths at each site. MRI data in Dicom format were fully anonymized prior to transfer and then uploaded to the central MRI reading center database. Prior to study onset, each site provided a dummy scan utilizing the standardized sequences for review by the central MRI reading center to verify scan quality and fidelity. Quality control was maintained at each site using standard procedures for clinical scanners (daily phantoms, stability testing). Quarterly phantoms were collected from 12 of the 15 sites, most using the standard American College of Radiology (ACR) phantom. One site upgraded from a Siemens 1.5T to a 3.0T in November 2013, resulting in the acquisition of one month 24 scan on the new scanner. One site upgraded from a Phillips Achieva 3.0T to a Phillips Intera 3.0T after the first subject completed month 24. All subsequent studies were performed on the Intera.

Scans underwent a standard review locally by a radiologist blind to study details to assess for any new or unusual findings as a safety measure. Incoming imaging data was reviewed for completeness and fidelity to study pulse sequences by the imaging core investigators. Local radiologists and imaging core investigators were all blind to randomization assignment. All MRI investigators remained blinded to treatment assignment until the end of the study.

Analysis of gadolinium enhancing lesions and T2 lesions were performed as described by Sicotte et al. (Ann. Neurology 52:421 (2002)). Briefly, MRI data was coded by study site and randomization number. The number and volume of enhancing lesions were quantified on the post contrast T1 weighted scans by an experienced investigator who was blind to treatment group using a semi-automated threshold-based algorithm. To assess T2 lesions, all FLAIR images were RF corrected, then intensity normalized and registered into a common space

defined by the baseline scan for each individual. All subsequent scans were registered to the baseline exam for spatial normalization using a rigid body model. T2 lesion areas were determined using a semi-automated intensity based segmentation procedure by a trained, experienced researcher verified by a single investigator (NLS).

5 MRI brain, whole gray matter, whole white matter and cortical gray matter volumes were determined using a pairwise Jacobian integration (PJI) method. Pre-processing for structural T1-weighted images included 1) N3 non-uniformity correction, 2) histogram-based intensity normalization, 3) linear standard space registration using ICBM 2009c nonlinear symmetric template, 4) patch-based brain extraction, and 5) lesion-inpainting. Inputs to PJI were a pair of
10 baseline and follow-up pre-processed structural T1-weighted images. The PJI consisted of 1) linear skull-constrained symmetric registration, 2) halfway transformation and resampling, 3) nonlinear symmetric registration using ANTS, and 4) voxelwise Jacobian determinant calculation on the warp field. Whole gray matter and whole white matter tissue masks were generated by SPM8 Segment function. Additional nonlocal means denoising was applied. For
15 whole brain tissue masks, the whole gray matter and whole white matter masks were combined. For cortical gray matter mask, a standard cortical mask was nonlinearly transformed and merged with gray matter mask. The standard template was the ICBM (ICBM 2009c nonlinear symmetric version), and the nonlinear registration was performed by ANTS. Finally, the Jacobian determinants were averaged within the masks for percent volume change in cortical
20 gray matter, whole gray matter, whole white matter, and whole brain.

Voxel-based morphometry (VBM) analyses were performed as described by Kurth et al. (Neuroimage Clin. 4:454 (2014)). All subjects included in the VBM cohort were required to have at least reached month 12 of the study, and all images had to pass quality control before and after image preprocessing to be included in the VBM cohort. Using this criteria, the VBM
25 cohort consisted of 111 subjects (62 in the estriol + GA, and 49 in the placebo + GA group) from 13 sites for month 12 analyses, and 86 of these subjects (45 in the estriol + GA, and 41 in the placebo + GA group) for 24 month analyses.

Brain images were preprocessed utilizing SPM8 and the VBM8 toolbox. White matter lesions were in-painted to minimize their impact based on manual delineations that were used for
30 the analysis of new T2 lesions. For this purpose, these manually delineated lesion masks were coregistered to the T1-weighted images, corrected if necessary, and used for lesion in-painting as

described by Chard et al. (J. Magn. Reson. Imaging 34:223 (2010)). The lesion in-painted images were subsequently realigned for each subject using halfway-registrations and corrected for bias-field inhomogeneities. The realigned, bias corrected images were then tissue-classified into gray matter, white matter, and cerebrospinal fluid and registered to MNI space through linear and non-linearly transformations (see <http://dbm.neuro.uni-jena.de/vbm8/VBM8-Manual.pdf>). More specifically, the tissue classification was based on maximum a posteriori segmentations, accounted for partial volume effects, and was refined by applying a spatially adaptive non-local means denoising filter as well as a hidden Markov random field model. These methods made the tissue-classification independent of tissue probability maps and thus additionally minimized the influence of misclassifications, lesions, and altered geometry. Using DARTEL, the gray matter segments were then spatially normalized to the DARTEL Template supplied with the VBM8 Toolbox (see <http://dbm.neuro.uni-jena.de/vbm>), resulting in a voxel-wise comparability between subjects and time-points. Finally, the gray matter segments were smoothed with a Gaussian kernel (8 mm full width at half maximum). These smoothed gray matter segments constituted the input for the statistical analysis. For visualization, a mean template was created from the normalized brain images of all subjects, allowing the results from the statistical analysis to be related to the underlying mean anatomy of the subject sample.

VBM Statistical Analyses.

For the statistical analysis, a general linear model was applied that used the smoothed gray matter segments as the dependent and group x time as the independent variable. Subject and scan site were added as variables of no interest, thus effectively controlling for inter-individual differences (*e.g.* individual anatomy, age, disease duration, etc.) as well as the potentially confounding impact of different scanners. Non-sphericity was modeled and accounted for as described previously and implemented in SPM8. Applying this model, the interaction between group and time was calculated using T-tests to investigate group differences in local gray matter changes between month 0 and month 12 (month 0 and month 24, respectively). In addition, the gray matter loss within each group was investigated by calculating T-tests for month 0 > month 12 (month 0 > month 24, respectively) for each group separately. All results were corrected for multiple comparisons by controlling the false discovery rate (FDR) using a threshold of $P \leq 0.05$. Corrected results were rendered on the mean template of all

subjects in Figure 3. In addition, significant findings were visualized using maximum intensity projections as shown in Figure 6.

Example 2-Use of ER β ligand /AC-186 in a Mouse Model of Multiple Sclerosis

Materials and Methods

Animals. C57BL/6 and NOD mice 8 weeks old were purchased from Jackson Laboratories (Bar Harbor, ME). Animals were maintained under environmentally controlled conditions in a 12-hour light/dark cycle with access to food and water *ad libitum*. All procedures were done in accordance with the guidelines of the National Institutes of Health and the Chancellor's Animal Research Committee of the University of California, Los Angeles Office for the Protection of Research Subjects.

Reagents. The estrogen receptor β ligand AC-186 was dissolved in either Miglyol 812N liquid oil (Sasol North America) or sesame oil (Sigma Aldrich) as following concentration; 1.5 mg/mL for 3 mg/kg group, 5 mg/mL for 10 mg/kg group, and 15 mg/mL for 30 mg/kg group. The estrogen receptor β ligand diarylpropionitrile (DPN, Tocris Biosciences) was dissolved in 10% molecular-grade ethanol and diluted with 90% of either Miglyol 812N liquid oil or sesame oil.

EAE and Treatments. Animals were injected subcutaneously with Myelin Oligodendrocyte Glycoprotein (MOG), amino acids 35–55 (200 μ g/animal, American Peptides), emulsified in complete Freund's adjuvant (CFA) and supplemented with *Mycobacterium Tuberculosis H37ra* (300 μ g/animal, Difco Laboratories), over two sites drained by left inguinal and auxiliary lymph nodes in a total volume of 0.1 ml/mouse. One week later, a booster immunization was delivered over contra lateral lymph nodes. Pertussis toxin (500 ng/mouse) (List Biological Laboratories, Inc.) was injected intraperitoneally on days 0 and 2. Animals were monitored daily for EAE signs based on a standard EAE 0–5 scale scoring system: 0—healthy, 1—complete loss of tail tonicity, 2—loss of righting reflex, 3—partial paralysis, 4—complete paralysis of one or both hind limbs, and 5—moribund. Animals received 0.05 ml of either of 1.5, 5, 15 mg/mL AC-186, vehicle (sesame oil or Miglyol 812N liquid oil), or DPN (8mg/kg/day) via subcutaneous injections every other day. Animals were treated with AC-186 or vehicle after EAE induction. Specifically, treatment was initiated at the first clear signs of clinical disease (EAE grade 2 at day 13 – 15), and continued to the endpoint of the experiment.

Rotarod Testing. Motor behavior was tested up to two times per week for each mouse using a rotarod apparatus (Med Associates Inc., St. Albans, VT). Briefly, animals were placed on a

rotating horizontal cylinder for a maximum of 200 seconds. The amount of time the mouse remained walking on the cylinder, without falling, was recorded. Each mouse was tested on a speed of 3-30 rpm and given three trials for any given day. The three trials were averaged to report a single value for an individual mouse, and then averages were calculated for all animals within a given treatment group.

Histological Preparation. Mice were exposed to a lethal dose of isoflurane and perfused transcardially with ice-cold 1× PBS for 8–15 min, followed by 10% formalin for 10-15 min. Spinal cords and brains were dissected and submerged in 10% formalin overnight at 4°C, followed by 30% sucrose in PBS for 24 h. Tissues were embedded in 75% gelatin/15% sucrose solution for cryostat sectioning then post-fixed overnight in 10% formalin and cryoprotected in 30% sucrose. The embedded tissues were stored in –80 °C after flash frozen in dry ice. 40 µm thick free-floating spinal cord cross-sections, and sagittal brain sections were obtained with a microtome b cryostat (model HM505E) at –20 °C. Tissues were collected serially and stored in 1× PBS with 1% sodium azide in 4°C until immunohistochemistry.

Immunofluorescence. Prior to histological staining, 40-mm thick free-floating sections were thoroughly washed with 1X PBS to remove residual sodium azide. In the case of anti-MBP labeling, tissue sections were processed with an additional 1 h incubation with 5% glacial acetic acid in 100-proof ethanol at room temperature (RT). After washing tissue sections were permeabilized with 0.3% TritonX-100 and 2% normal goat serum in 1X PBS for 30 min at RT and blocked with 10% normal goat serum in 1X PBS for 1 hr. Tissues were then incubated with primary antibodies overnight in 4 °C. The following primary antibody (Ab) were used: Rat anti-MBP (Millipore) at 1:1000 dilutions, Rabbit anti-NF200 (Sigma Aldrich) at 1:750 dilutions, Rabbit anti-beta-APP (Life Technologies) at 1:200 dilutions, Mouse anti-NeuN at 1:1000 dilutions (Millipore), Rabbit anti-PSD95, and Rabbit anti-Synapsin1 at 1:500 dilutions (Millipore), and Rat anti-CD45 at 1:1500 dilutions (Millipore). The next day tissues were washed and incubated with secondary antibodies conjugated to Cy5 or Cy3 (Millipore) for 1 hr at RT. A nuclear stain DAPI (2 ng/mL; Molecular Probes) was added 10 min prior to final washes after secondary Ab incubation. Sections were mounted on slides, allowed to semi-dry, and cover slipped in fluoromount G (Fisher Scientific). IgG-control experiments were performed for all primary Ab, and only non-immunoreactive tissues under these conditions were analyzed.

Chromagen Immunohistochemistry. Tissue sections were thoroughly washed with 1x PBS to remove residual sodium azide and treated with 3% hydrogen peroxide for 30 min at RT and then simultaneously blocked with 10% NGS and permeabilized with 0.3% Triton X-100 in 1x PBS for 1 h at room temperature. Tissues were then incubated with primary antibodies overnight in 4 °C. The following primary antibodies were used: Rat anti-CD3 at 1:2000 dilutions (BD Pharmingen), anti-Calbindin D28K at 1:1000 dilutions (Millipore), and Rabbit anti-Iba1 at 1:10000 dilutions (Wako Chemicals), were added for 2 hour at RT, and then placed in 4°C overnight. Tissue sections then followed with secondary Ab labeling at 1:1000 dilutions (Vector labs) for 1 h at room temperature and then with Avidin-Biotin Conjugation solution (Vector Labs) for 1 hour at RT. Tissue sections were treated with DAB peroxidase substrate (Vector labs) according to manufacturer instructions. IgG-control experiments were performed for all primary Ab, and only non-immunoreactive tissues under these conditions were analyzed.

Microscopy and Quantification. Three spinal cord cross-sections at the C5-12 level from each mouse (n=3-6) were captured under microscope at 10x magnification. To quantify demyelination in the spinal cord and cerebellum, white matter was manually delineated on the basis of DAPI staining, and MBP staining intensity was calculated and reported in the sampled area. Axonal damage was assessed by counting beta-APP⁺ cells in a confocal 10x microscope in spinal cord. Axonal densities were calculated by counting the number of NF200⁺ or NeuN⁺ neuronal cells in a 10x confocal image in the sampled area of the captured tissue section. Cerebellar Purkinje (Calbindin⁺) cells were manually counted using a brightfield 10x microscope over the entire sagittal cerebellum. PSD-95 and Synapsin1 density was measured and reported as a percentage of the sampled area. CD45⁺, CD3⁺, and Iba1⁺ cells in spinal cord cross-sections were manually quantified under either of a confocal 10x microscope for CD45⁺ cells or a brightfield 10x microscope for CD3⁺ and Iba1⁺ cells.

MRI Acquisition. Mice were anesthetized with isofluorane and their heads secured with bite and ear bars. Respiration rate was monitored and the mice were maintained at 37° C using a circulating water pump. In vivo magnetic resonance imaging was performed using a 200 mm horizontal bore 7.0 T Bruker imaging spectrometer with a micro-imaging gradient insert with a maximum gradient strength of 100 G/cm and 30 mm birdcage RF coil (Bruker Instruments, Billerica, MA). An actively decoupled quadrature surface coil array was used for signal reception and a 72-mm birdcage coil was used for transmission. Images were acquired and

reconstructed using ParaVision 5.1 software. Imaging parameters were as follows: rapid-acquisition with relaxation enhancement (RARE) sequence, matrix dimensions = $256 \times 128 \times 64$; field of view = $3.84 \text{ cm} \times 1.92 \text{ cm} \times 0.96 \text{ cm}$; repetition time (TR) = 3500 ms; apparent time to echo (apparent TE) = 32 ms; echo train length = 16; total scan time = 37 mins. Spatial resolution was $150 \mu\text{m}^3$ per voxel.

MRI Analysis. Images were skull-stripped using the Brain Surface Extractor (BSE) and residual non-brain signal was removed by a single operator manually editing the masks using BrainSuite 11a and bias-field inhomogeneities removed using the N3 correction. After inhomogeneity correction, a minimum deformation atlas (MDA) was produced. Images were spatially and intensity normalized to the MDA using a rigid-body transformation and an intensity rescaling cost function in Alignlinear (AIR). This process permits the comparison of images in a standard space correcting for both gross positional and intensity differences, yet preserving anatomically significant local changes. Following creation of this atlas, cerebral cortices and cerebella were manually labeled on the atlas. The labels were then warped onto the individual spatially normalized images to produce standardized estimates of gray matter volumes in individual subjects. All automated image processing was performed using the LONI Pipeline Processing Environment on an 8-processor core Mac Pro computer (Apple, Cupertino, CA).

Cerebral cortex and cerebellum labels were based on the Mouse Atlas Project 2003 mouse brain atlas. For clarity and consistency, the cerebral cortex label was bounded ventrally by the plane inferior to the most anterior point of the corpus callosum at midline. Importantly, this label contained the somatosensory regions (primary and secondary) and the motor cortex (primary and secondary). Additional anatomical information was obtained from the Franklin and Paxinos mouse brain atlas (Franklin and Paxinos, 2008).

Statistics. Global and regional brain volume changes in EAE mice and control animals were compared with repeated measures ANOVAs using SPSS 22 (IBM, Armonk, NY). If Mauchly's test indicated that the assumption of sphericity had been violated ($p < 0.05$), then the degrees of freedom were corrected using the Huynh-Feldt estimates of sphericity. Regression analysis and Welch's t-tests were performed in Excel 2011 (Microsoft, Redmond, WA). All results are presented as mean $\pm$ standard deviation.

ER β ligands protect cerebellar function

Three doses of the AC-186 compound were tested in C57BL/6 female mice: low (3mg/kg, every other day), medium (10 mg/kg, every other day), and high (30 mg/kg, every other day), each injected subcutaneously in a sesame oil vehicle. The ER β ligand diarylpropionitrile (DPN) was tested as a positive control because DPN can partially ameliorate EAE and because the treatment effects in EAE experiments can vary based on the level of disease severity within an experiment. Direct comparisons between treatments were only made between treatment groups within a single EAE experiment and not between treatment groups of different EAE experiments. DPN, however, was difficult to dissolve in sesame oil, and thus, DPN was first dissolved in ethanol and added to sesame oil at a concentration of 10% ethanol in sesame oil. The AC-186 compound dissolved in sesame oil after five minutes on a nutating shaker. Thus, figure 7 shows graphs in which the vehicle consisted of sesame oil for AC-186 (all doses) and vehicle, while the DPN vehicle was 10% ethanol in sesame oil. The low (3 mg/kg) dose of AC-186 had no effect on the EAE score, the medium dose (10mg/kg) displayed a trend toward improvement, and the high (30 mg/kg) dose displayed a significant effect in ameliorating standard EAE clinical scores ($p=0.0299$) (figures 7 and 8). The difference in improvement between the 30 mg/kg AC-186 group and all other groups increased with time.

To assess whether a more novel EAE outcome might be affected as well by the high (30 mg/kg) dose, rotarod was also performed, as shown in figure 9. Rotarod performance is likely more aligned with coordination and cerebellar function than standard EAE scores, which reflect principally walking and spinal cord pathology. No significant effect was observed for mice receiving 30 mg/kg AC-186 in sesame oil relative to those receiving vehicle only; however, very late in disease, at the time when ER β ligands are known to start working, the performance curves trended toward divergence, with AC-186 treated mice trending toward improved performance. Further, the rotarod test is insensitive in detecting differences when the vehicle group performs well, and the vehicle group performed well in this case, staying on the rotarod for approximately 150 seconds. Additionally, during the final two time-points, on days 48 and 50, the AC-186 group performed perfectly during the 200 second test, and thus, a significant effect may have been masked by the experimental design.

DPN protects against EAE better when administered in a Miglyol vehicle rather than in a sesame oil vehicle, and thus, the effect of the choice of vehicle on the efficacy of AC-186 was assessed. Accordingly, the AC-186 compound was assessed in C57BL/6 males using sesame oil

and miglyol as vehicles. Male mice receiving AC-186 administered at 30 mg/kg in miglyol performed significantly better than mice receiving miglyol alone as assessed by both EAE score (figure 11) and rotarod performance (figure 12). In contrast, male mice receiving AC-186 administered at 30 mg/kg in sesame oil did not perform significantly better than mice receiving sesame oil alone as assessed by EAE score (figure 11) and rotarod performance (figure 12).
5 Notably, the AC-186 solution dissolved more rapidly in miglyol than sesame oil, and miglyol could dissolve AC-186 by merely pipetting for 30 seconds. In contrast, AC-186 required mixing/nutating for 5 minutes to dissolve the compound in sesame oil.

The 30 mg/kg dose of AC-186 dissolved in sesame oil did not display efficacy in male
10 C57BL/6 mice, in contrast with the results obtained for female C57BL/6 mice as described above.

Sesame oil has been shown previously to have some nonspecific immunostimulatory effects, and thus is not most commonly used as a vehicle in EAE. As shown in figure 13, the different vehicle type does not affect EAE differentially when given without an ER β ligand.
15 Rather, the different vehicle type likely affects the ability of a given ER β ligand to protect in EAE, with Miglyol enabling better EAE protection than sesame oil when either DPN or AC-186 are administered.

The effect of AC-186 was assessed in female NOD mice using a MOG-induced EAE model. The efficacy of AC-186 was tested in miglyol and sesame oil vehicles. In these models,
20 AC-186 improved EAE scores relative to vehicle only for mice receiving 10 mg/kg or 30 mg/kg in miglyol ($p < 0.001$), and the 30mg/kg group trended toward increased efficacy relative to the 10 mg/kg group (figure 14). In comparison, the positive control DPN, which had not previously been tested in the NOD EAE model, also significantly ameliorated EAE, appearing similar to the disease reduction observed with the 30 mg/kg dose of AC-186. In contrast, AC-186 in sesame
25 oil did not ameliorate EAE scores, consistent with data above in the C57BL/6 male EAE experiment where AC-186 in miglyol ameliorated EAE while AC-186 in sesame oil did not.

Rotarod testing had never before been done in the NOD EAE model, and rotarod scores were surprisingly poor with most mice staying on the rotarod for only 0-100 seconds and none staying on for 125-200 seconds. These results were surprising in part because the EAE walking scores
30 were reasonably, although not dramatically, severe in the moderate range of 2-3. These results may suggest that EAE in the NOD model preferentially affects cerebellar or other balance related

pathways as compared to EAE in the C57BL/6 model. The intervention produced no significant improvement in rotarod scores (figure 15).

The effect of AC-186 was assessed in male NOD mice using a MOG-induced EAE model. The efficacy of AC-186 was tested in miglyol vehicle only. AC-186 improved EAE scores relative to vehicle only for mice receiving 30 mg/kg in miglyol ($p < 0.0001$) (figure 16). Mice treated with AC-186 displayed no significant improvement in performance in the rotarod experiment relative to mice treated with vehicle only (figure 17).

Two doses of the AC-186 compound were tested in C57BL/6 female mice: medium (10 mg/kg) and high (30 mg/kg), each in a miglyol vehicle. Both the medium dose (10mg/kg) and high dose (30 mg/kg) displayed significant efficacy in ameliorating the standard EAE clinical scores relative to the miglyol vehicle alone ($p=0.0001$) (figure 18). Additionally, the high dose (30 mg/kg) displayed significant efficacy in the rotarod experiment relative to the miglyol vehicle alone ($p=0.0005$) (figure 19).

Table 13: Summary of standard EAE and rotarod scores

Mouse Strain	Sex	AC186	Vehicle	Results	
				Standard EAE Score	Rotarod Score
C57BL/6	F	30 mg/kg	<u>miglyol</u>	****	***
C57BL/6	M	30 mg/kg	<u>miglyol</u>	****	****
NOD	F	30 mg/kg	<u>miglyol</u>	****	<u>n.i.</u>
NOD	M	30 mg/kg	<u>miglyol</u>	****	<u>n.i.</u>
C57BL/6	F	10 mg/kg	<u>miglyol</u>	**** (less robust than 30 mg/kg)	<u>n.s.</u>
C57BL/6	M	10 mg/kg	<u>miglyol</u>	N/A	N/A
NOD	F	10 mg/kg	<u>miglyol</u>	****	<u>n.i.</u>
NOD	M	10 mg/kg	<u>miglyol</u>	<u>n.s.</u>	<u>n.i.</u>
C57BL/6	F	30 mg/kg	sesame	*	<u>n.s.</u>
C57BL/6	M	30 mg/kg	sesame	<u>n.s.</u>	<u>n.s.</u>
NOD	F	30 mg/kg	sesame	<u>n.s.</u>	<u>n.i.</u>
NOD	M	30 mg/kg	sesame	N/A	N/A
C57BL/6	F	10 mg/kg	sesame	<u>n.s.</u>	<u>n.s.</u>
C57BL/6	F	3 mg/kg	sesame	<u>n.s.</u>	<u>n.s.</u>

n.s.= not significant; N/A = not applicable, not done; * $p<0.05$, *** $p=0.0004$, **** $p<0.0001$

n.i. = not informative; NOD females and NOD males have very poor rotarod performance, and thus the rotarod experiment is not sensitive in the NOD strain.

Histological markers suggest that ER β ligands are neuroprotective

Neuroprotective effects of AC-186 were observed by NF200 and beta-APP staining, with

each of the 30 mg/kg and the 10 mg/kg doses (figure 20). C57BL/6 female mice were treated with AC-186 at 30 mg/kg/every other day in miglyol vehicle, 10 mg/kg/every other day in miglyol vehicle or with miglyol vehicle alone then underwent immunohistochemistry for axonal and myelin integrity using antibody staining for NF200, beta-APP and MBP. NF200 indicated axonal integrity with decreases indicating axonal loss, beta-APP also indicated axonal integrity with increases indicating axonal damage, and MBP staining indicated myelin integrity with decreases indicating demyelination during EAE. AC-186 30 mg/kg/every other day treatment in EAE significantly preserved axon numbers (NF200) and reduced axonal damage (beta-APP), with a trend for sparing myelin (MBP). These beneficial effects on axons in spinal cord are consistent with protective effects on clinical scores as assessed by EAE standard scores and rotarod performance above.

Neuropathology of spinal cords focusing on immune cells is shown in Figure 21. Regarding the assessment of immune cells by neuropathology, in contrast to previous experiments with DPN, which showed no significant effect on CD45 staining in the CNS, AC-186 treatment at both the 10 mg/kg and the 30 mg/kg doses reduced CD45 staining. To determine which immune cell population was reduced, Iba-1 stained cells with globoid morphology were used to assess macrophages and CD3 staining was used to assess T lymphocytes. AC-186 treatment at both the 30 mg/kg and the 10 mg/kg dose each reduced Iba-1 globoid cell staining, while neither AC-186 dose affected levels of CD3 staining.

These data are consistent with protective effects on clinical EAE scores. Effects on myelin staining were much less striking as compared to effects on axons as has been previously observed. Surprisingly, in contrast to previous experiments with DPN, which showed no effect on CD45 or Iba-1 globoid cell staining, AC-186 treatment reduced CD45 and Iba-1 globoid cell staining. However, neither dose of AC-186 affected levels of CD3 staining (Figure 21). Whether the effect of AC-186 on reducing Iba-1 globoid cells was due to a reduction in macrophage infiltration into the CNS or due to a reduction in the transition of CNS resident cells to the globoid morphology as a reaction to less axonal damage during AC-186 treatment remains unknown. The observation that T lymphocyte levels were unaffected by AC-186 treatment suggests that the adaptive immune response is not affected by treatment like the innate immune response is. Taken together, these results suggest that ER β ligands differ in their effect on CD45 and other immune marker staining, which can have therapeutic and mechanistic implications for

the treatment of MS subtypes.

Classic immunohistochemistry of spinal cord white matter showed axonal loss (by reduced NF200 staining) $p < 0.005$, axonal damage (by increased beta-APP staining) $p < 0.05$, and demyelination (by decreased MBP staining) $p < 0.005$, in vehicle treated EAE compared to healthy controls. ER beta ligand treatment of EAE mice after disease onset preserved axons and myelin (ER beta ligand EAE vs, vehicle EAE: axons $p < 0.05$; axonal damage $p < 0.05$; myelin $p < 0.005$). Assessment of spinal cord white matter inflammation in vehicle treated EAE compared to healthy controls showed increased inflammation (by CD45 staining) $p < 0.0001$, increased macrophages/activated microglia (by Iba-1 staining with globoid morphology) $p < 0.005$, and increased T lymphocytes (by CD3 staining) $p < 0.05$. ER beta ligand treatment of EAE partially reduced inflammation as detected by CD45 staining, $p < 0.05$, with this driven by the reduction in macrophages/activated microglia, $p < 0.05$, not T lymphocytes.

MRI analysis suggest that ER β ligands are neuroprotective

Female C57BL/6 mice treated with AC-186 at 30 mg/kg underwent *in vivo*, longitudinal MRI scanning at day 0, 30, and 60 after EAE induction. The mice treated with AC-displayed beneficial effects for both standard EAE clinical scores and for rotarod times, similar to the effects observed in above (Figure 22). Whole brain, cerebral cortex and cerebellar volumes were determined at each EAE time point in female C57Bl/6 mice that were treated with either AC-186 (AC-186) or vehicle (EAE), as well as in age-and sex-matched healthy control mice (NOR).

Mice underwent *in vivo*, longitudinal MRI scanning at day 0, 30, and 60 after EAE induction. Whole brain, cerebral cortex and cerebellar volumes were determined at each EAE time point in female C57Bl/6 mice that were treated with either AC-186 (AC-186, 30mg/kg/every other day) or vehicle (EAE), as well as in age-and sex-matched healthy control mice (NOR).

NORMAL vs. EAE

Whole brain volumes of both healthy and EAE vehicle mice were plotted against the disease duration (starting with the scans prior to disease induction) (Figure 23A). In order to quantify the significance of the decreases in whole brain volume observed in individual animals, a repeated-measures ANOVA was performed to assess the effect of time on whole brain volume. Brain volume remained stable over time in the healthy group, but showed a gradual decrease in the vehicle treated EAE group (time x group interaction $p = 1.1 \times 10^{-7}$). The mean volume of the

whole brain of mice sixty days after disease induction was $490 \text{ mm}^3 \pm 1.7 \text{ mm}^3$ (mean $\pm$ SEM) in healthy mice and $478 \text{ mm}^3 \pm 1.2 \text{ mm}^3$ in EAE mice, indicating a decrease in volume ($p = 5.3 \times 10^{-6}$).

Cerebral cortex volumes of healthy and EAE mice were plotted against disease duration and a similar pattern was observed (Figure 23B). Cerebral cortex volumes were stable in the healthy group, while gradually decreasing in the EAE group (time x group interaction $p = 4.0 \times 10^{-6}$). The volume of the cerebral cortex at sixty days after disease induction (d60) was 72.3 mm^3 (1.4 mm^3) in healthy mice and 68.9 mm^3 (1.8 mm^3) in EAE mice, a 4.7% decrease ($p = 1.5 \times 10^{-4}$) in volume.

Similarly, a progressive loss of cerebellar volume during EAE was observed in EAE mice compared to healthy mice (time x group interaction $p = 1.1 \times 10^{-5}$) (figure 23C). The volume of the whole cerebellum at sixty days after disease induction was 53.2 mm^3 (1.1 mm^3) in healthy mice and 50.0 mm^3 (1.1 mm^3) in EAE mice, a 6.0% decrease ($p = 3.1 \times 10^{-6}$) in volume.

These results demonstrated that whole brain, cerebral cortex and cerebellum volume decreases over time in mice with EAE.

AC-186 EAE vs. Vehicle EAE.

A decrease in the rate of atrophy was observed in AC-186 treated mice compared to vehicle treated EAE mice when whole brain volumes were plotted against the disease duration (starting with the scans prior to disease induction) (Figure 23A). In order to quantify the effect of AC-186 treatment on EAE mice, a repeated-measures ANOVA was performed. Brain volume decreased in both EAE and AC-186 mice, however this brain volume decrease was significantly smaller in AC-186 treated EAE mice (time x group interaction $p = 0.0013$). The volume of the whole brain sixty days after disease induction (d60) was 483 mm^3 (7.0 mm^3) in AC-186 treated EAE mice and 478 mm^3 (3.7 mm^3) in vehicle treated EAE mice, indicating a 2.7% difference ($p = 0.021$) in volume.

Cerebral cortex volumes of AC-186 treated EAE mice and vehicle treated EAE mice were plotted against disease duration and a similar pattern was observed (Figure 23B). Cerebral cortex atrophy rates were decreased in AC-186 treated EAE mice compared to vehicle treated EAE mice (time x group interaction $p = 0.0035$). The volume of the cerebral cortex at sixty days after disease induction (d60) was $70.8 \text{ mm}^3 \pm 0.5 \text{ mm}^3$ in AC-186 treated EAE mice and 68.9 mm^3 in vehicle treated EAE mice ($p = 0.014$).

When cerebellar volume was plotted against disease duration in AC-186 treated EAE mice and vehicle treated EAE mice, volume decreases over time were again observed (figure 23C). However, AC-186 treatment decreased the amount of volume loss compared to vehicle treatment (time x group interaction $p = 9.5 \times 10^{-4}$). The volume of the whole cerebellum at sixty days after disease induction (d60) was $53.2 \text{ mm}^3 \pm 0.3 \text{ mm}^3$ in AC-186 treated EAE mice and $50.0 \text{ mm}^3 \pm 0.4 \text{ mm}^3$ in vehicle treated EAE mice ($p = 3.1 \times 10^{-6}$).

Cerebral and cerebellar neuropathology revealed that AC-186 treatment prevented neuronal cell (NeuN) and synaptic (PSD-95) loss in cerebral cortex gray matter (Figure 24 top panel) and Purkinje neuronal cell (Calbindin) and synaptic (PSD-95) loss in the cerebellar cortex gray matter (Figure 24 bottom panel).

Spinal Cord Neuropathy

Neuropathology was repeated on spinal cord to confirm the histology results described above. The results depicted in figures 25 and 26 largely reproduce the results in figures 20 and 21, showing a protective effect on axons. However, a beneficial effect of AC-186 as compared to vehicle with regard to increased MBP staining, that was observed as a trend in the histological analyses described above (Figure 20), was observed as significant (Figure 25). Together, these data indicate that given a large enough sample size, AC-186 treatment does indeed have a beneficial effect on preserving myelin staining in the CNS. Thus, AC-186 treatment on EAE resulted in sparing of axons and myelin in spinal cords. Notably, the effect of AC-186 on lowering CD45 and Iba-1, but not CD3, which was observed during the histological analyses, was confirmed (Figure 26). Lower CD45 and Iba-1 staining in the CNS during AC-186 treatment is consistent with a beneficial effect of AC-186 treatment on clinical scores since these cells are thought to contribute to the pathogenesis of disease.

Cerebellar and Cerebral White Matter Neuropathy

As an extension of effects of AC-186 treatment on spinal cord white matter neuropathology, AC-186 treatment on cerebral and cerebellar white matter was assessed. AC-186 administered at 30 mg/kg/every other day had a protective effect on cerebellar and cerebral axons (NF200) and myelin (MBP) (Figures 27 and 28). These findings were consistent with the finding that AC-186 protected spinal cord white matter. Together, these data show a beneficial effect for AC-186 treatment initiated after disease onset, which reduced myelin and axonal loss in the white matter of the cerebellum and cerebrum during EAE.

Cerebellar and Cerebral Gray Matter Neuropathy

As an extension of effects of AC-186 treatment on cerebellar and cerebral white matter neuropathology, the effects of AC-186 treatment on cerebral and cerebellar gray matter was assessed. As shown in figure 29, AC-186 displayed a protective effect at a dose of 30 mg/kg/every other day for cerebellar cells (Calbindin+ Purkinje cells) and synapses (PSD-95 and Synapsin 1). Further, AC-186 displayed a protective effect at a dose of AC-186 30 mg/kg/every other day for cerebral cells (NeuN+ neurons) and synapses (PSD-95) (figure 30). No effect was observed for Synapsin 1 in cerebral gray matter, in contrast to cerebellar gray matter. These results are consistent with the previous findings on the differential effect of EAE on pre (Synapsin 1) versus post (PSD-95) synaptic protein expression (Du et al., Proceedings of the National Academy of Sciences USA, 111:2806-2806, 2014). Together, these data show a beneficial effect of AC-186 treatment, initiated after disease onset, in halting cellular and synaptic loss in cerebellar and cerebral gray matter during EAE.

INCORPORATION BY REFERENCE

All patents, published patent applications, and other publications mentioned in the description above are incorporated by reference herein in their entirety.

EQUIVALENTS

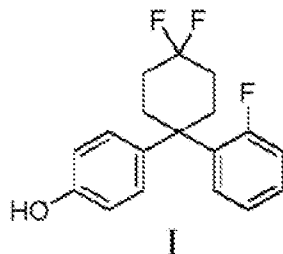
Having now fully described the present invention in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious to one of ordinary skill in the art that the same can be performed by modifying or changing the invention within a wide and equivalent range of conditions, formulations and other parameters without affecting the scope of the invention or any specific embodiment thereof, and that such modifications or changes are intended to be encompassed within the scope of the appended claims.

CLAIMS

1. A method for treating multiple sclerosis in a subject who does not present with active lesions (*e.g.*, gadolinium-enhancing lesions), comprising administering an estrogen receptor β ligand (ER β ligand) to the subject without conjointly administering a second immunotherapeutic agent for treating multiple sclerosis.
2. A method for treating multiple sclerosis in a subject receiving treatment with an immunotherapeutic agent who does not present with active lesions (*e.g.*, gadolinium-enhancing lesions), comprising:
 - administering an ER β ligand to the subject; and
 - discontinuing treatment with the immunotherapeutic agent.
3. A method for treating multiple sclerosis in a subject, comprising:
 - determining whether the brain of the subject presents with active lesions (*e.g.*, gadolinium-enhancing lesions);
 - administering an ER β ligand to the subject; and
 - discontinuing any second immunotherapeutic agent that the subject is receiving if the subject lacks active lesions.
4. The method of any one of the preceding claims, wherein the subject does not present with gadolinium-enhancing lesions.
5. The method of any one of the preceding claims, wherein the subject does not present with new or enlarging T2 lesions (*e.g.*, as assessed by comparing two MRI scans obtained at an interval of 6 months or more).
6. The method of any one of the preceding claims, wherein the immunotherapeutic agent is selected from interferon-beta 1a, interferon-beta 1b, glatiramer acetate, natalizumab, fingolimod, teriflunomide, dimethyl fumarate, mycophenolate mofetil, paclitaxel, cyclosporine, corticosteroids, azathioprine, cyclophosphamide, methotrexate, cladribine, 4-aminopyridine, and tizanidine.

7. The method of any one of the preceding claims, wherein active lesions are assessed by a gadolinium-enhanced magnetic resonance imaging (MRI) scan.
8. The method of any one of the preceding claims, wherein no active lesions have been detected in the subject for at least 6 months.
9. The method of claim 8, wherein no active lesions have been detected in the brain of the subject as assessed by gadolinium-enhanced MRI for at least 6 months.
10. The method of claim 8 or 9, wherein no new or enlarging T2 lesions have been detected in the brain of the subject for at least 6 months.
11. The method of any one of the preceding claims, wherein no active lesions have been detected in the subject for at least 12 months.
12. The method of any one of the preceding claims, wherein the subject has relapsing-remitting multiple sclerosis.
13. The method of any one of claims 1 to 11, wherein the subject has secondary progressive multiple sclerosis.
14. The method of any one of claims 1 to 11, wherein the subject has primary progressive multiple sclerosis,
15. The method of any one of claims 1 to 11, wherein the subject has progressive-relapsing multiple sclerosis.
16. The method of any one of claims 1 to 11, wherein the subject has clinically isolated syndrome.
17. The method of any one of claims 1 to 11, wherein the subject has radiologically isolated syndrome.
18. The method of any one of the preceding claims, wherein the subject has a cognitive deficit.

19. The method of any one of the preceding claims, wherein the subject has progressive walking disability.
20. A method for treating multiple sclerosis in a subject, comprising:
administering an ER β ligand to the subject;
determining whether the brain of the subject presents with active lesions; and
conjointly administering to the subject a second immunotherapeutic agent if the brain of the subject presents with active lesions.
21. The method of any one of the preceding claims, wherein the ER β ligand is a compound having the structure of formula I:



22. The method of claim 21, wherein the compound is administered at a dose of about 5 μ g/kg per day to about 100 mg/kg/day.
23. The method of claim 22, wherein the compound is administered at a dose of about 50 μ g/kg per day to about 50 mg/kg/day.
24. The method of claim 23, wherein the compound is administered at a dose of about 500 μ g/kg per day to about 5 mg/kg/day.
25. The method of any one of claims 1 to 20, wherein the ER β ligand is compound KBRV1 or KBRV2.
26. The method of claim 25, wherein the compound is administered at a dose of about 1 mg and 5 g per day.
27. The method of claim 26, wherein the compound is administered at a dose of about 5 mg and 1000 mg per day.

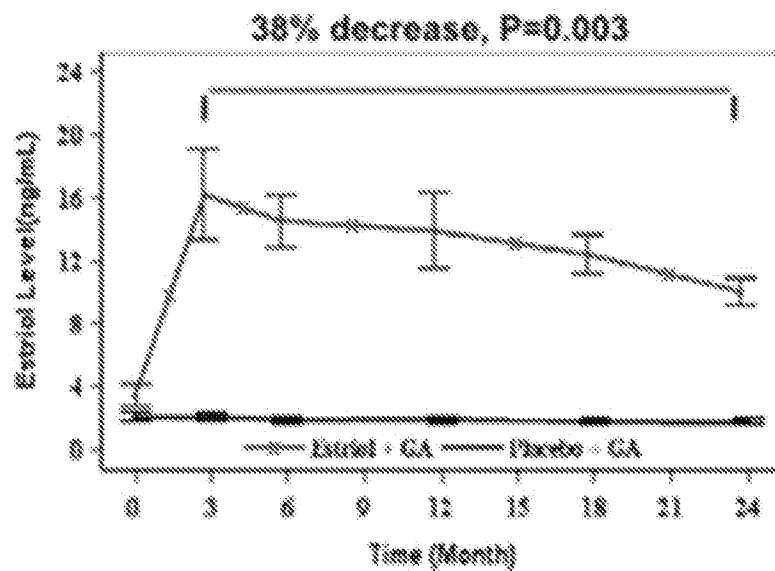
28. The method of claim 27, wherein the compound is administered at a dose of about 10 mg and 500 mg per day.
29. The method of any one of claims 21 to 28, wherein the compound is administered at a dose sufficient to achieve a mean blood concentration of the compound between 1 ng/ml and 1000 ng/ml.
30. The method of claim 29, wherein the compound is administered at a dose sufficient to achieve a mean blood concentration of the compound between 10 ng/ml and 500 ng/ml.
31. The method of claim 30, wherein the compound is administered at a dose sufficient to achieve a mean blood concentration of the compound between 100 ng/ml and 200 ng/ml.
32. The method of any one of the preceding claims, wherein administering an ER β ligand to the subject comprises administering the ER β ligand to the subject on a continuous basis.
33. The method of claim 32, wherein the continuous basis is daily.
34. The method of any one of the preceding claims, wherein a progestogen is not conjointly administered with the ER β ligand.
35. The method of any one of the preceding claims, wherein the subject displays evidence of cognitive decline.
36. The method of claim 35, wherein the evidence of cognitive decline is worsening performance on the Paced Auditory Serial Addition Test.
37. The method of any one of the preceding claims, wherein the subject displays evidence of brain atrophy.
38. The method of claim 37, wherein the evidence of brain atrophy is determined by MRI.
39. The method of claim 37 or 38, wherein the brain atrophy is cortical gray matter atrophy.
40. The method of any one of claims 37 to 39, wherein the brain atrophy is a decrease in whole brain volume.

41. The method of any one of the preceding claims, wherein the subject presents with a cognitive disability.
42. The method of claim 41, wherein the evidence of cognitive disability is determined by performance on a Paced Auditory Serial Addition Test or on a Symbol Digit Modalities Test.
43. The method of any one of the preceding claims, wherein the subject presents with a walking disability.
44. The method of claim 43, wherein the evidence of walking disability is determined by performance during a 25 foot walk test or during a 6 minute walk.
45. The method of any one of the preceding claims, wherein the subject scores 2 or higher on an Expanded Disability Status Scale.
46. The method of any one of the preceding claims, wherein the subject presents with fatigue.
47. The method of claim 46, wherein the evidence of fatigue is determined by performance on a Modified Fatigue Impact Scale test or on a Patient-Reported Outcomes Measurement Information System fatigue computer adaptive test.
48. The method of any one of the preceding claims, wherein:
- the method improves the cognitive ability of the subject, *e.g.*, as determined by performance on a Paced Auditory Serial Addition Test or on a Symbol Digit Modalities Test;
 - the method improves the walking ability of the subject, *e.g.*, as determined by performance during a walking test, such as a 25 foot walk test or a 6 minute walk test;
 - the method reduces the disability of the subject, *e.g.*, as measured on an Expanded Disability Status Scale;
 - the method reduces the fatigue of the subject, *e.g.*, as determined by performance on a Modified Fatigue Impact Scale test or on a Patient-Reported Outcomes Measurement Information System fatigue computer adaptive test; and/or
 - the method reduces depression in the subject, *e.g.*, as determined by a Multiple Sclerosis Quality of Life survey or on a Beck Depression Inventory test.

49. The method of any one of the preceding claims, wherein the subject presents with a history of breast cancer, ovarian cancer, and/or uterine cancer.
50. The method of any one of the preceding claims, wherein the subject has a family history of breast cancer, ovarian cancer, and/or uterine cancer.
51. The method of any one of the preceding claims, wherein the subject is at risk of developing breast cancer, ovarian cancer, and/or uterine cancer.

Figure 2

A



B

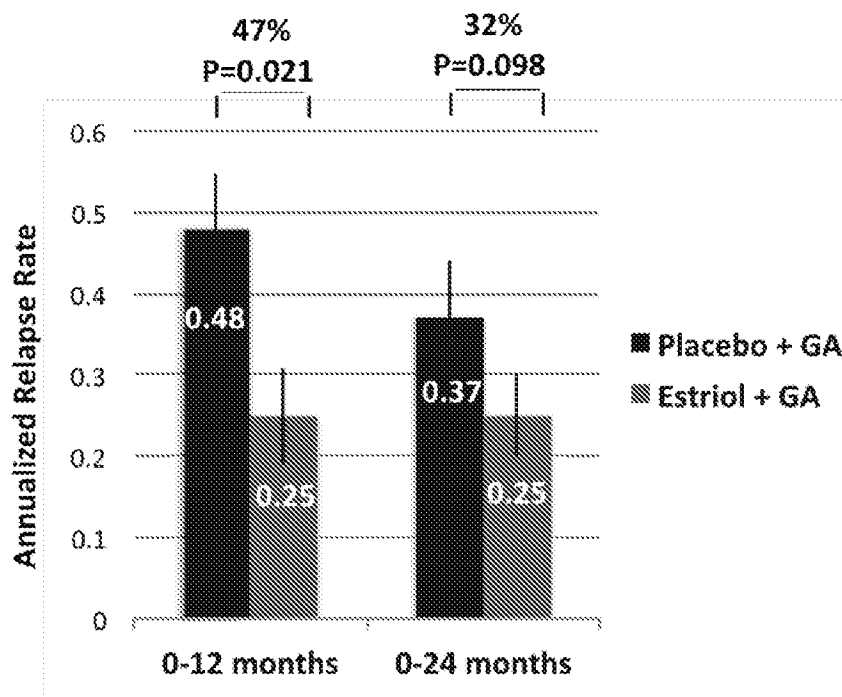


Figure 2 (continued)

C

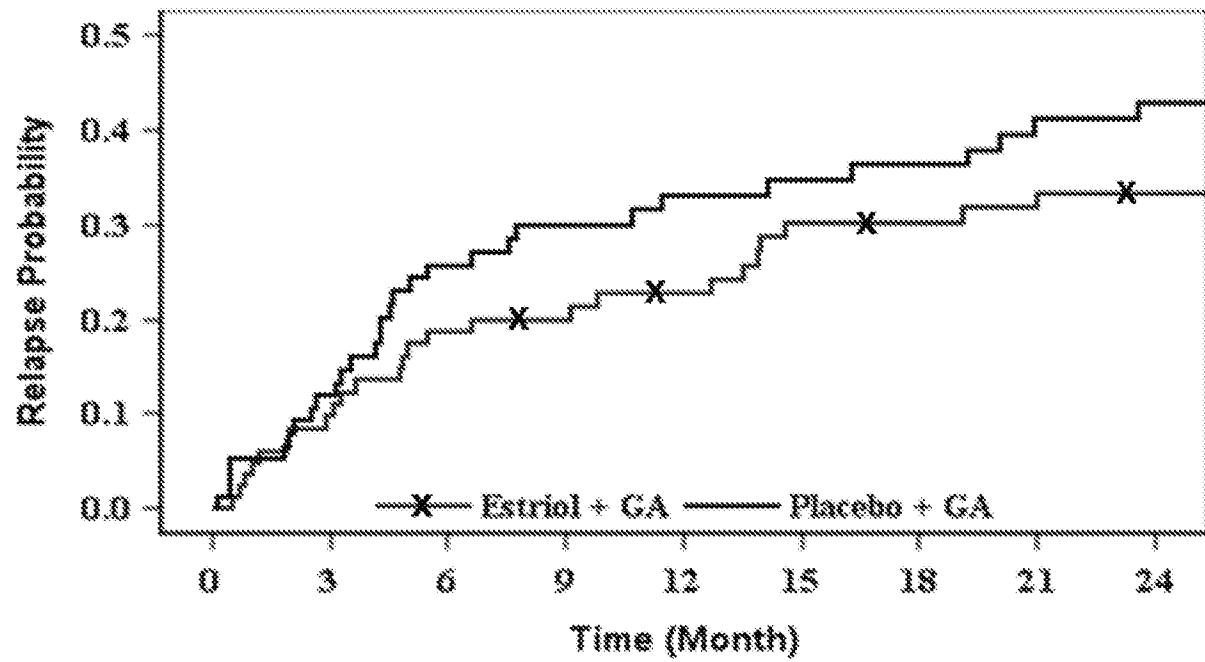


Figure 3

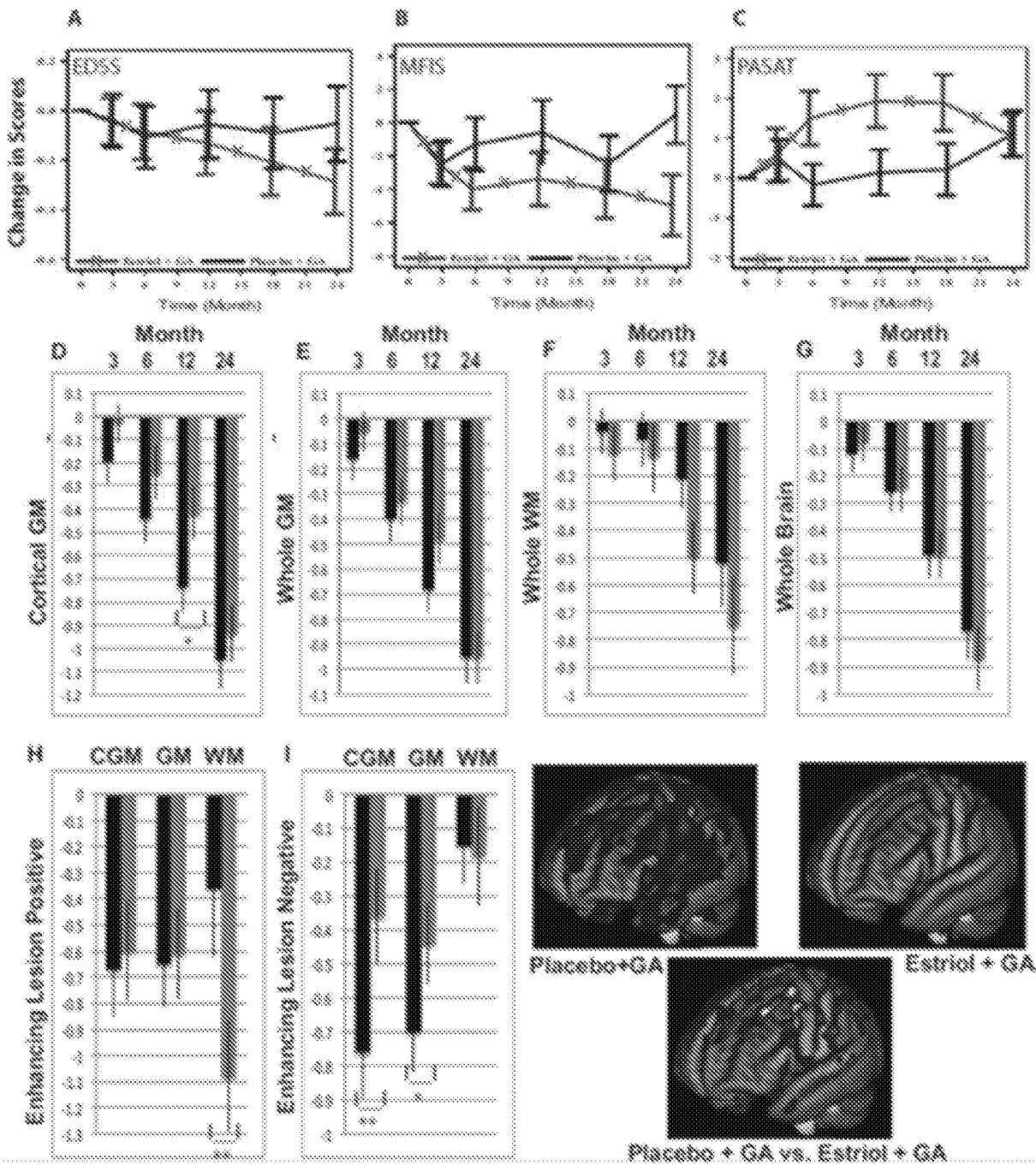
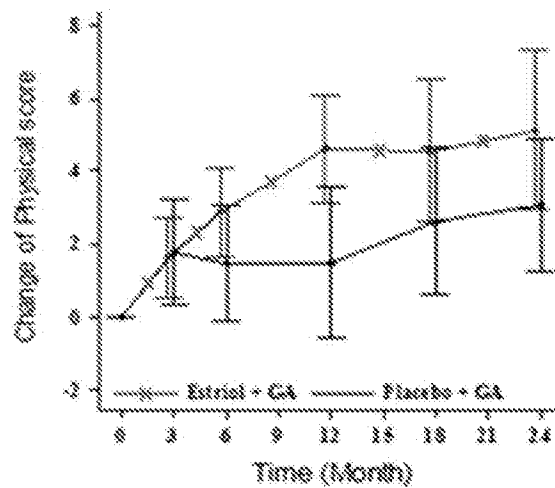
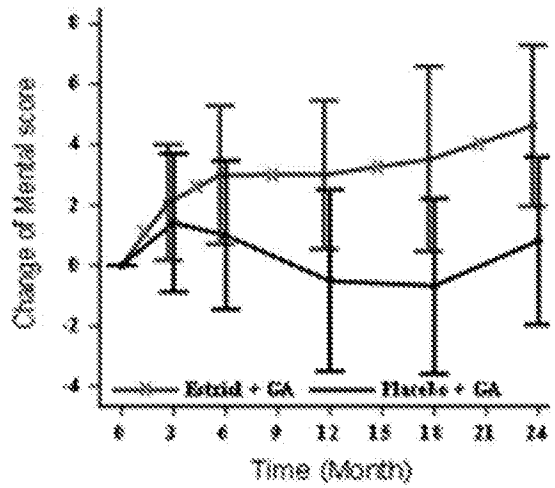


Figure 4

A



B



C

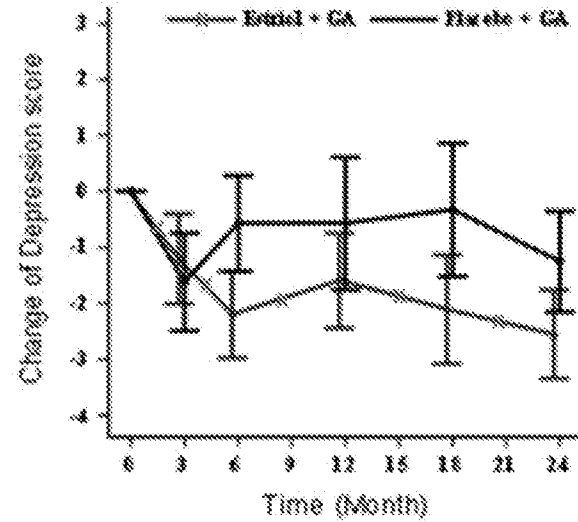


Figure 5

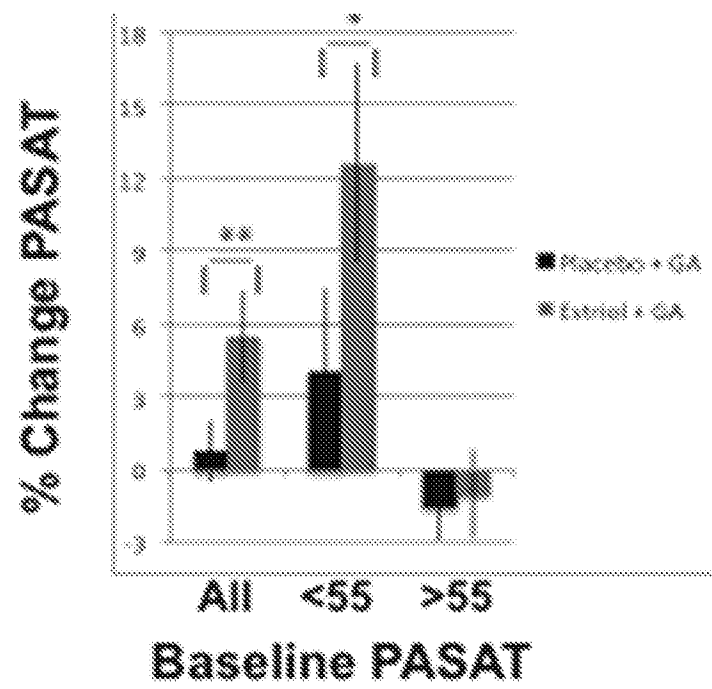
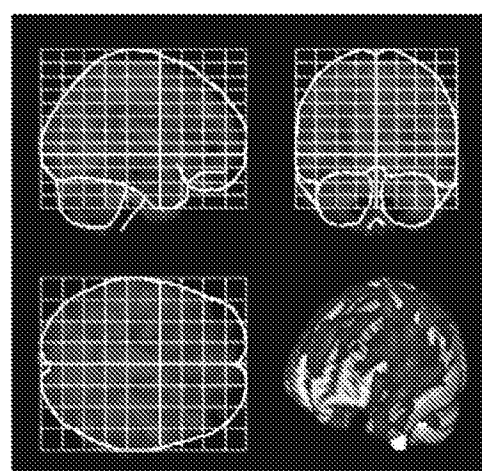
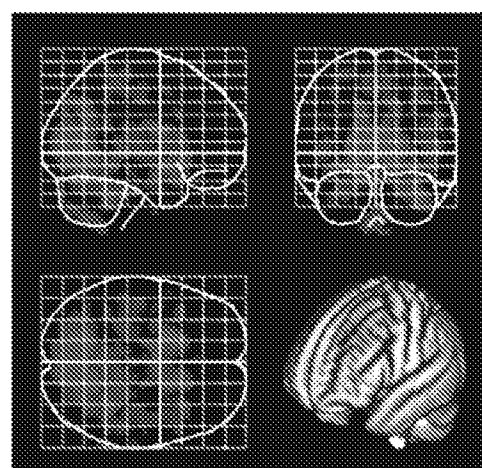


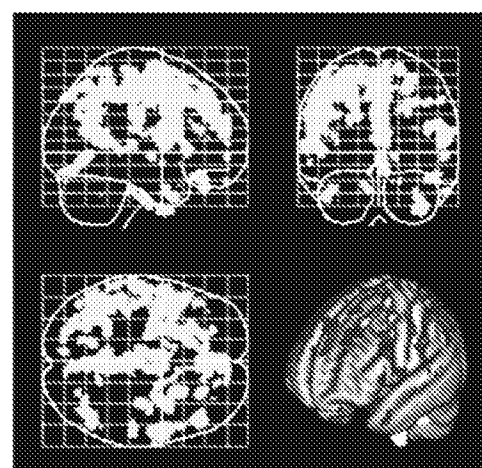
Figure 6



Placebo + GA



Estriol + GA



Placebo + GA vs. Estriol + GA

Figure 7

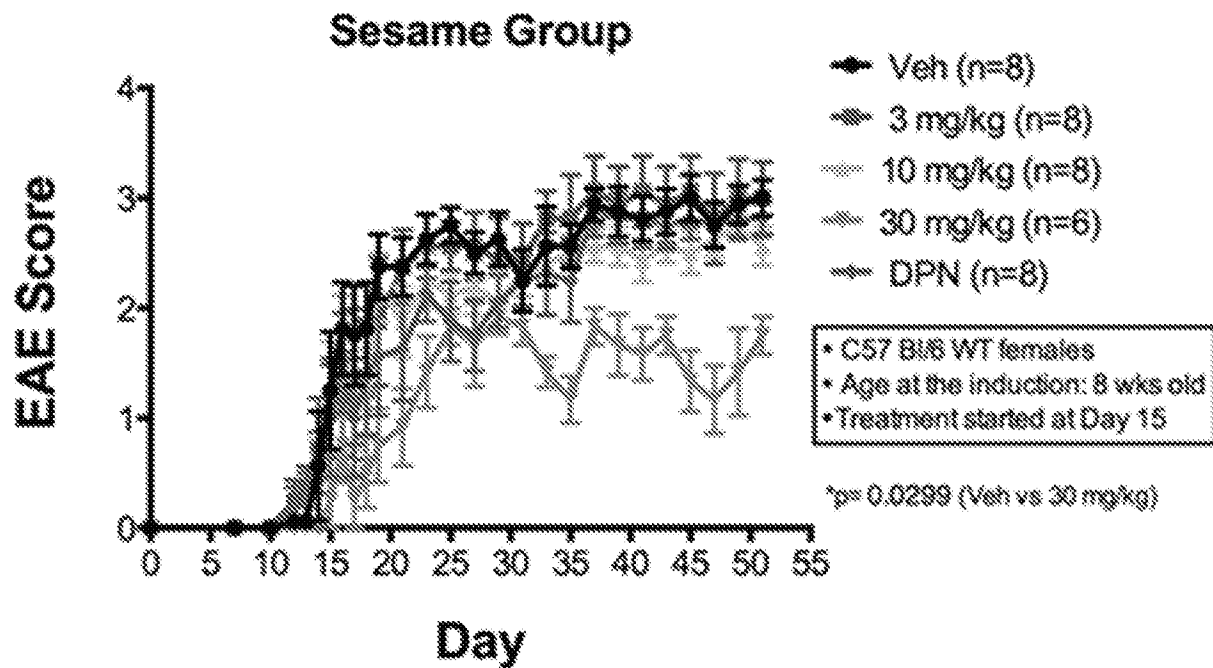


Figure 8

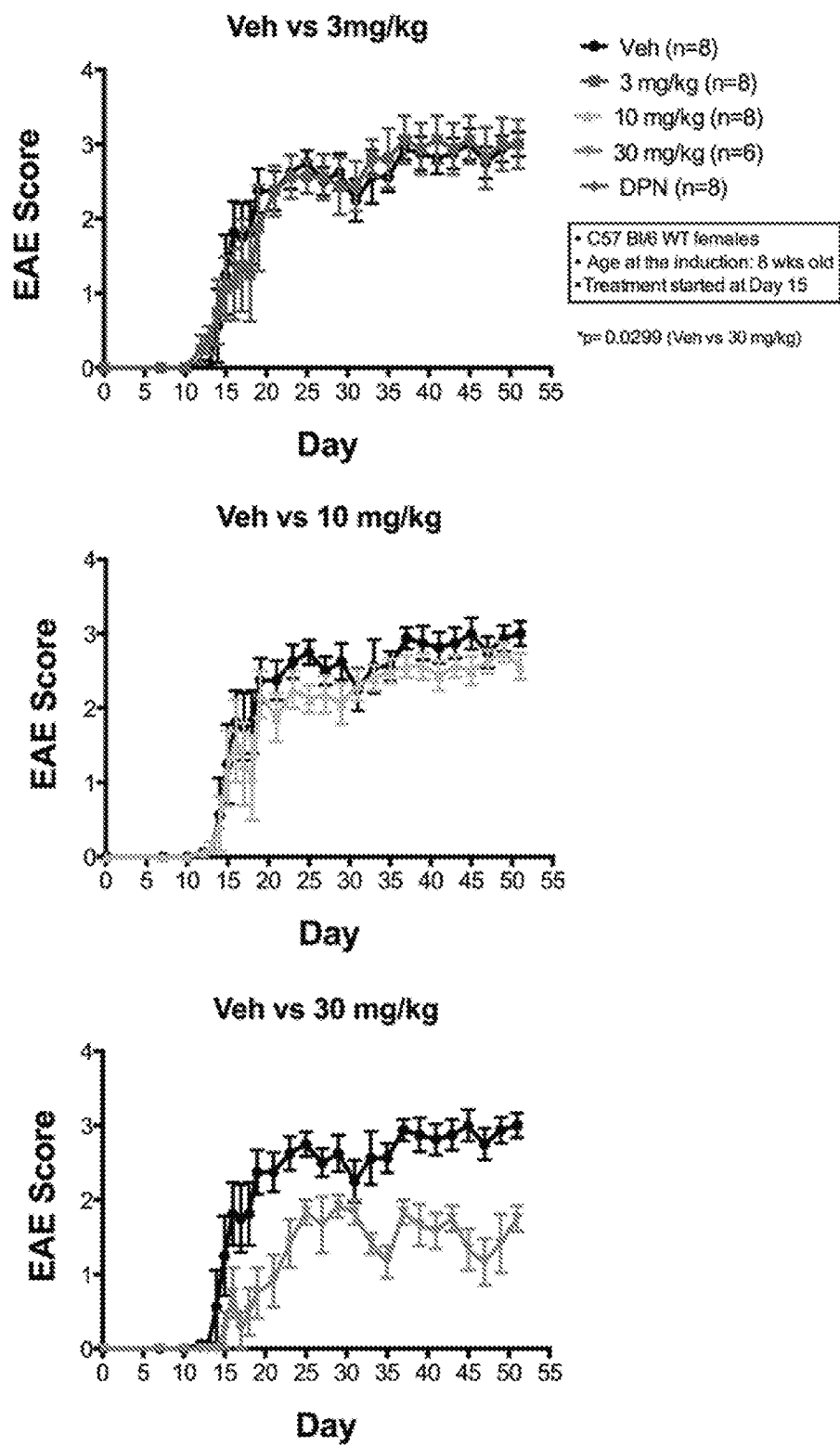


Figure 9

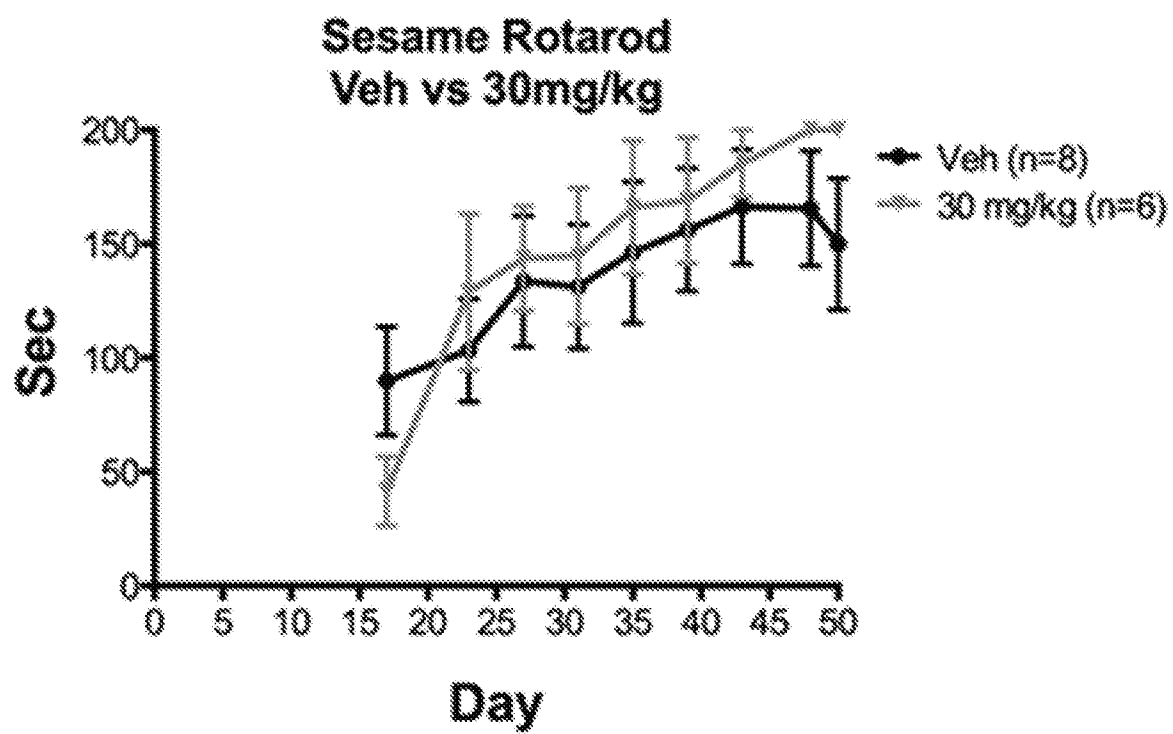


Figure 10

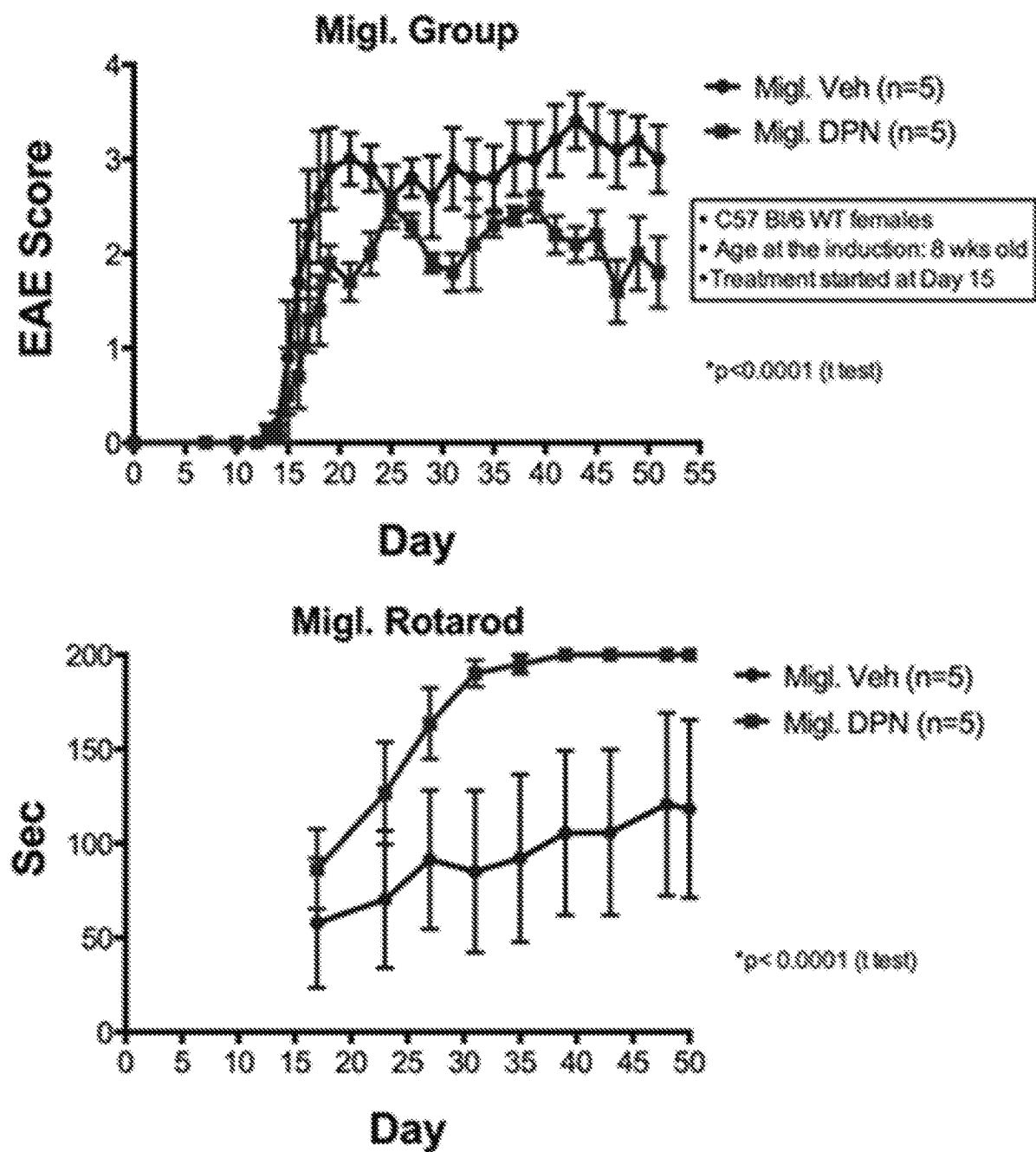


Figure 11

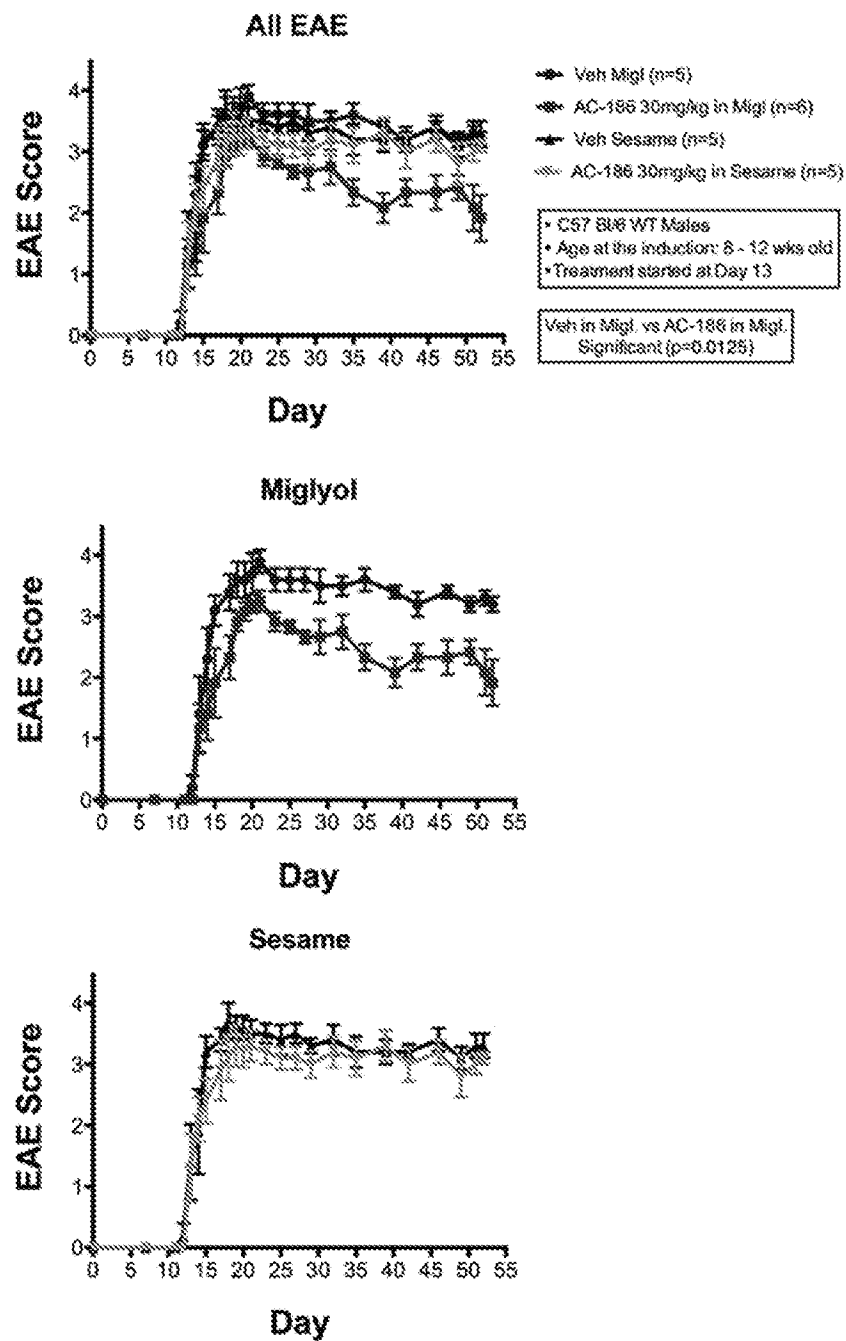


Figure 12

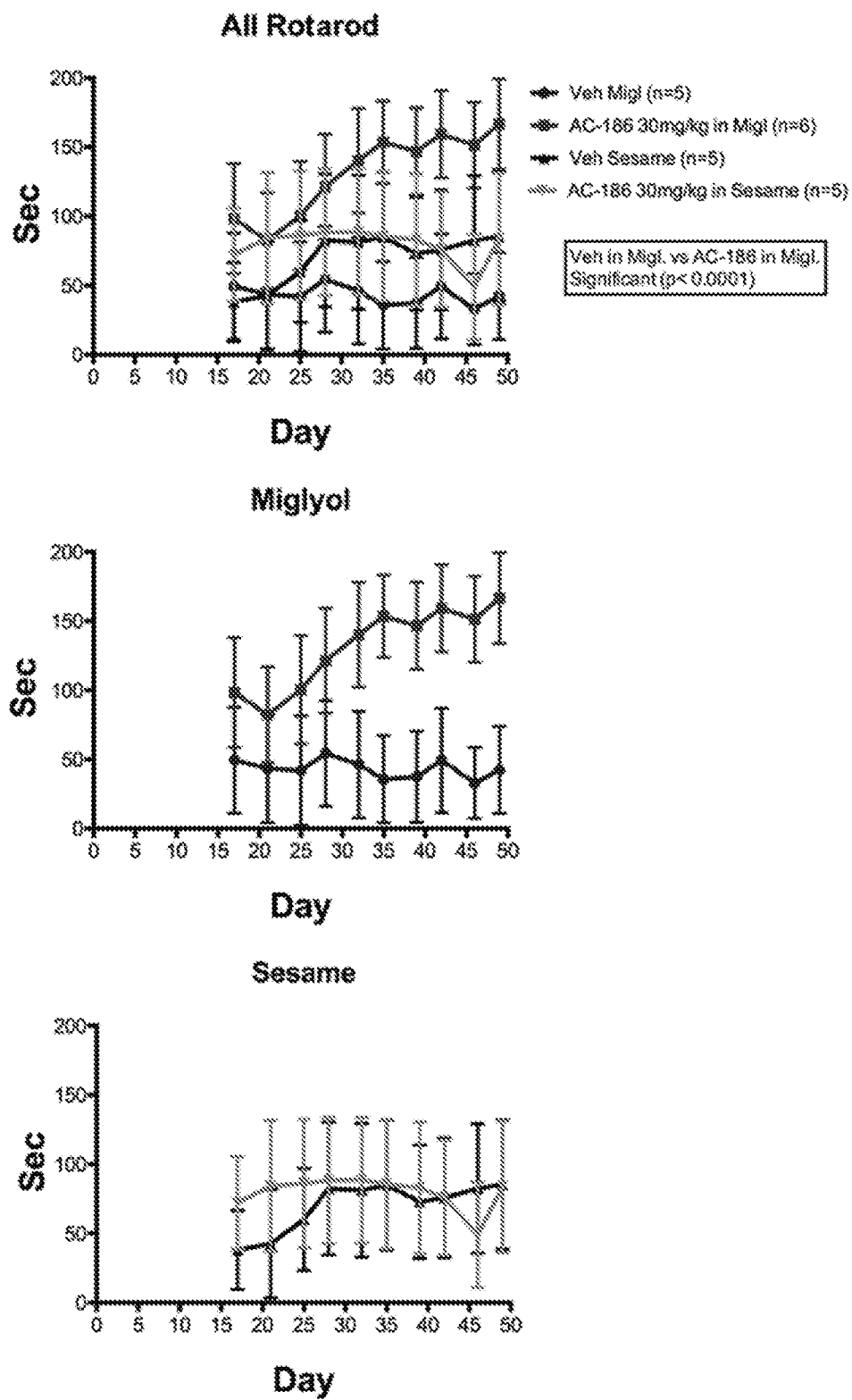


Figure 13

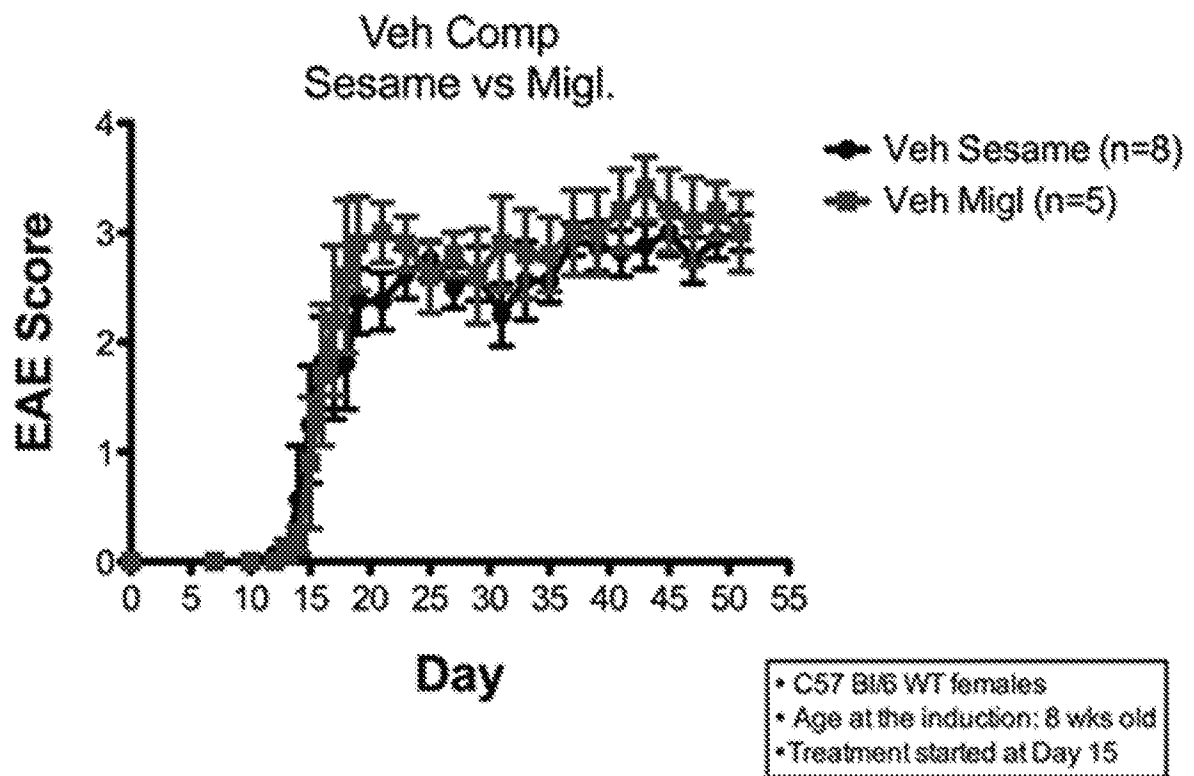


Figure 14

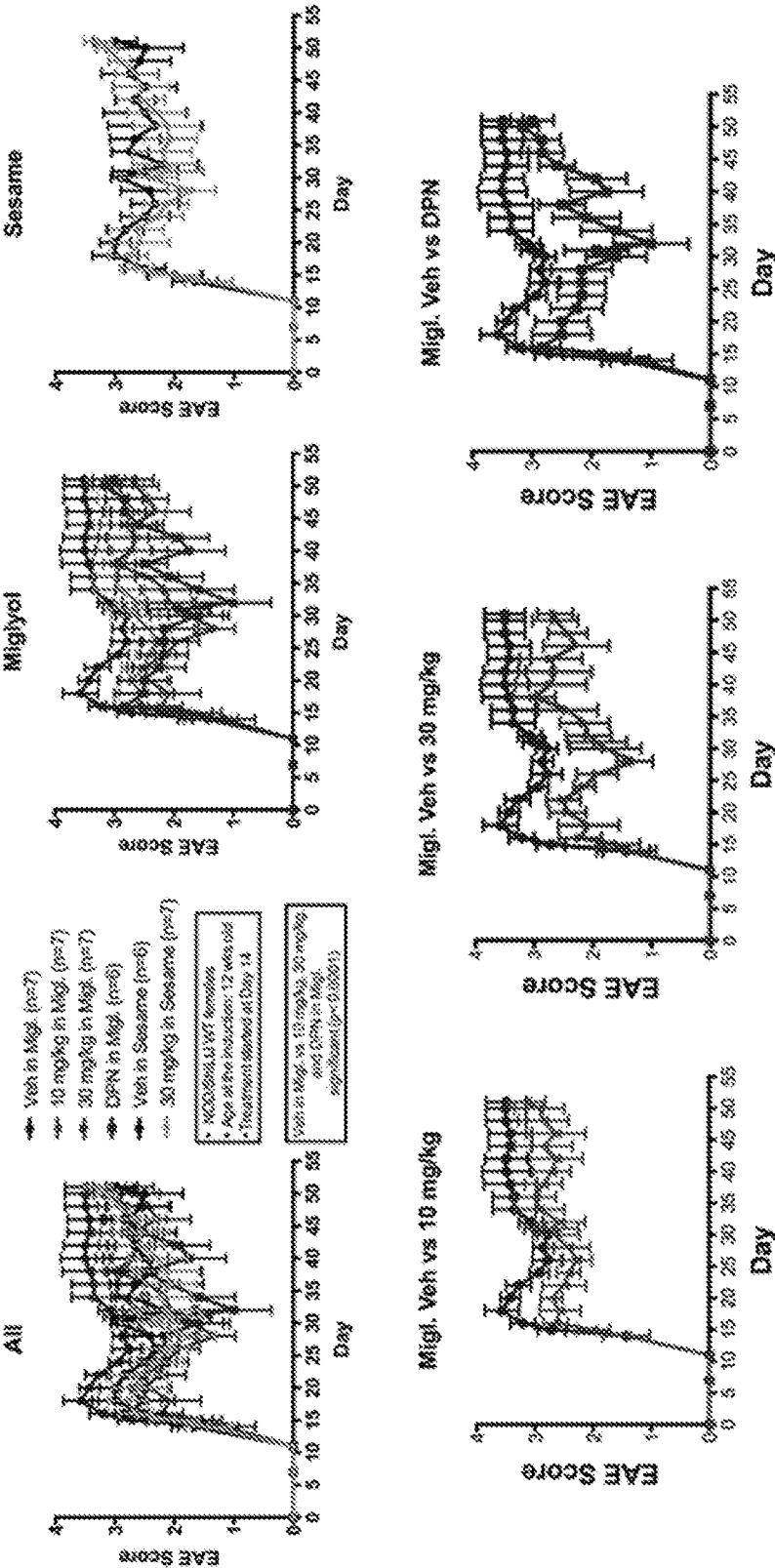


Figure 15

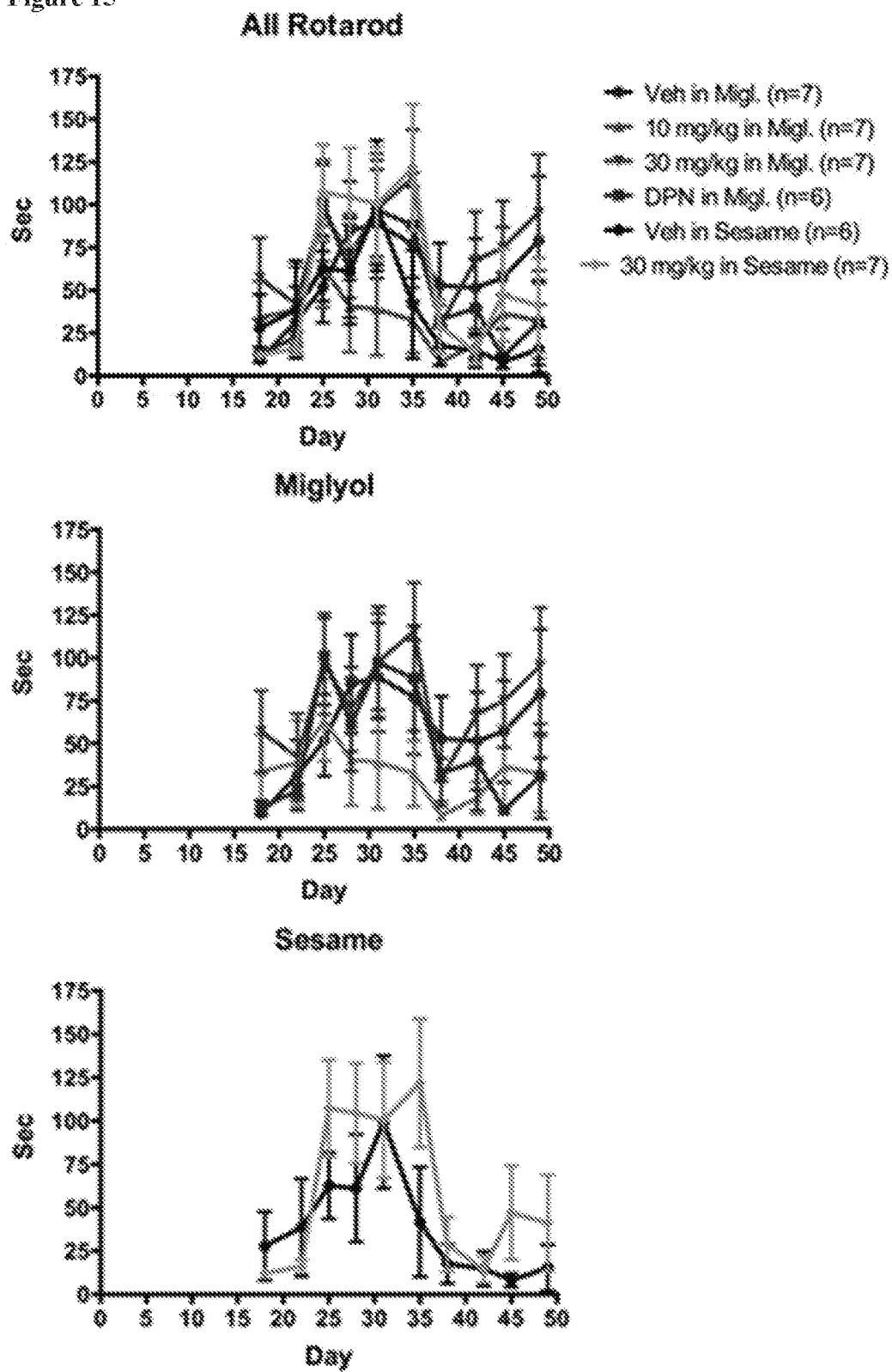


Figure 16

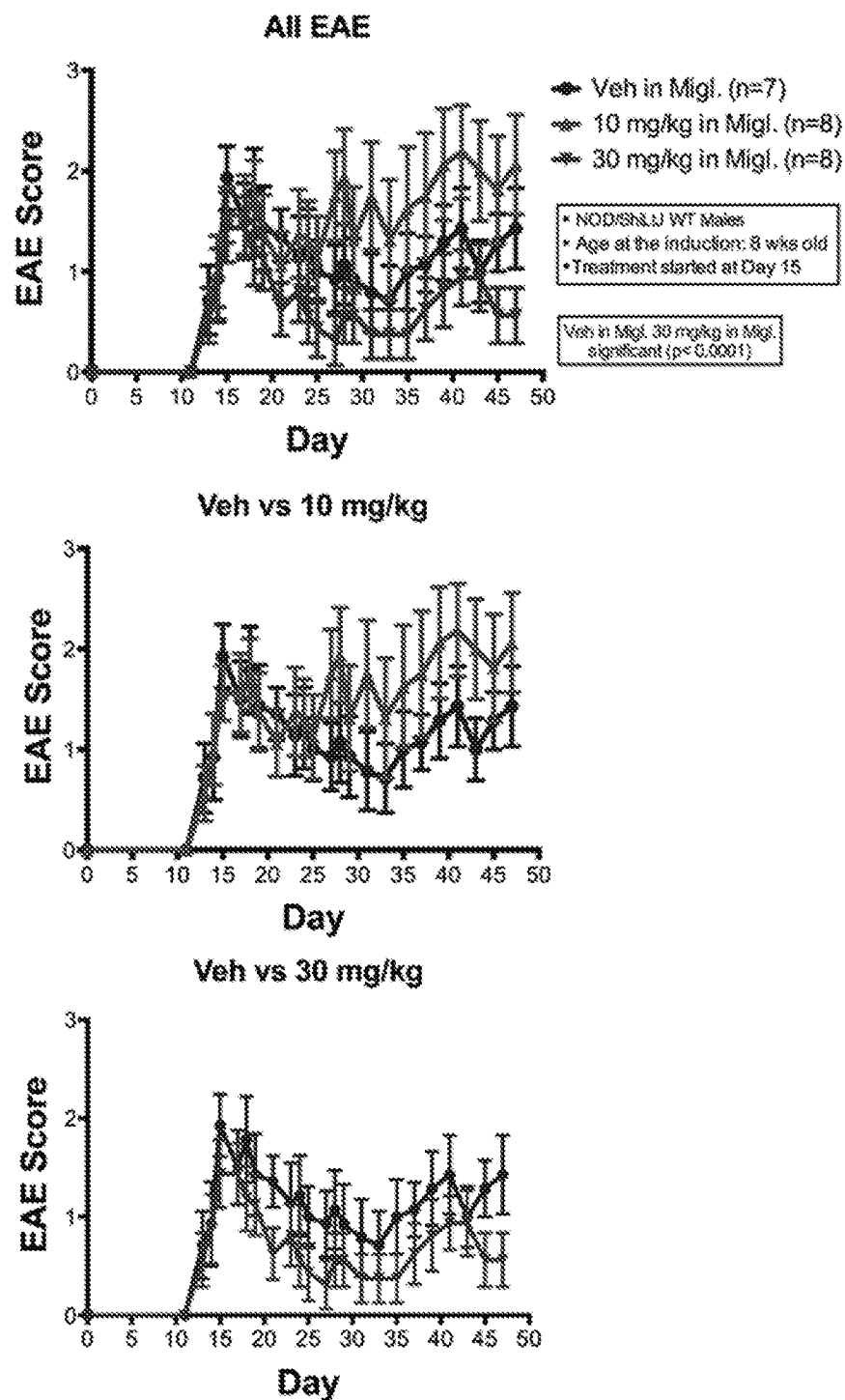


Figure 17

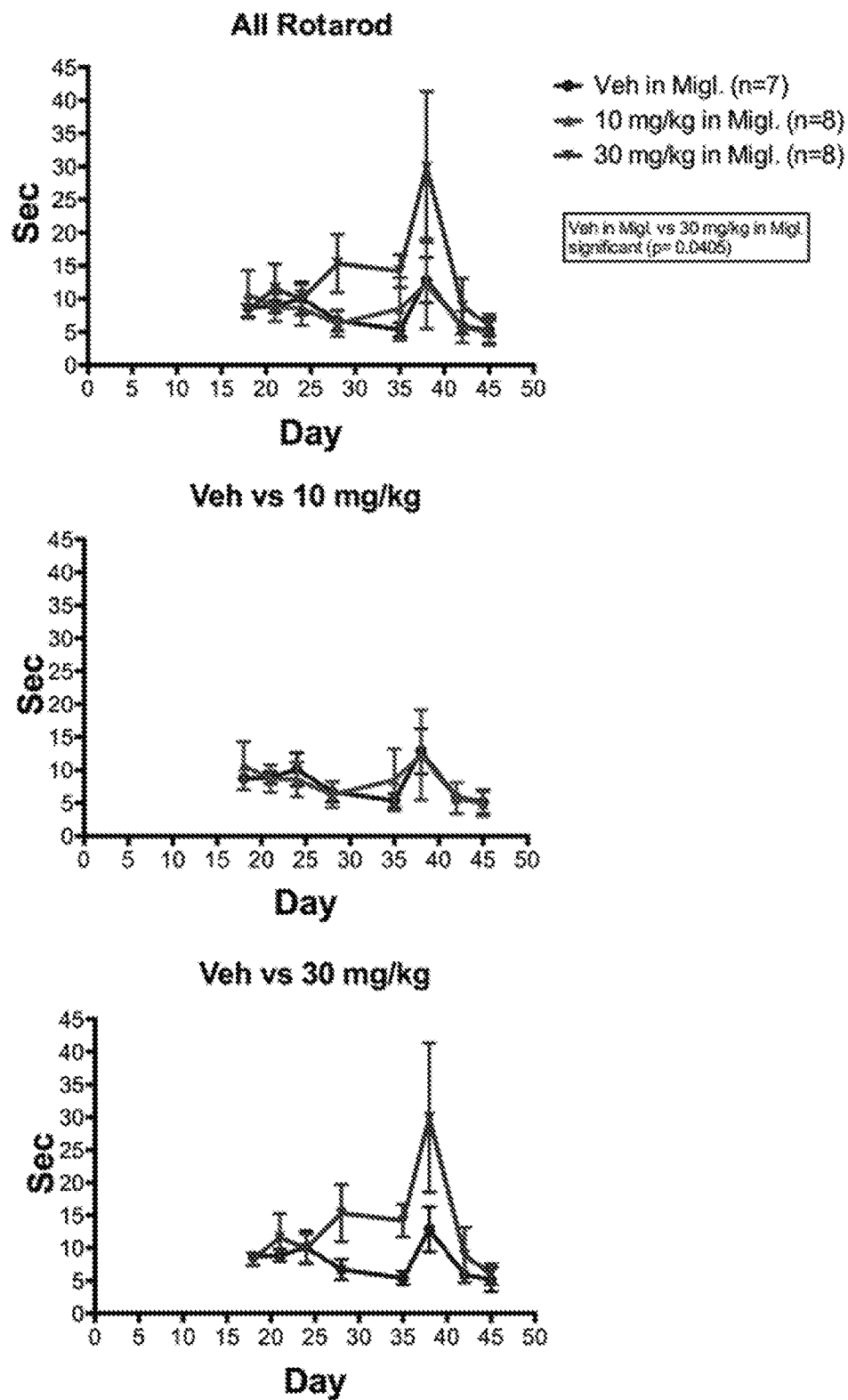


Figure 18

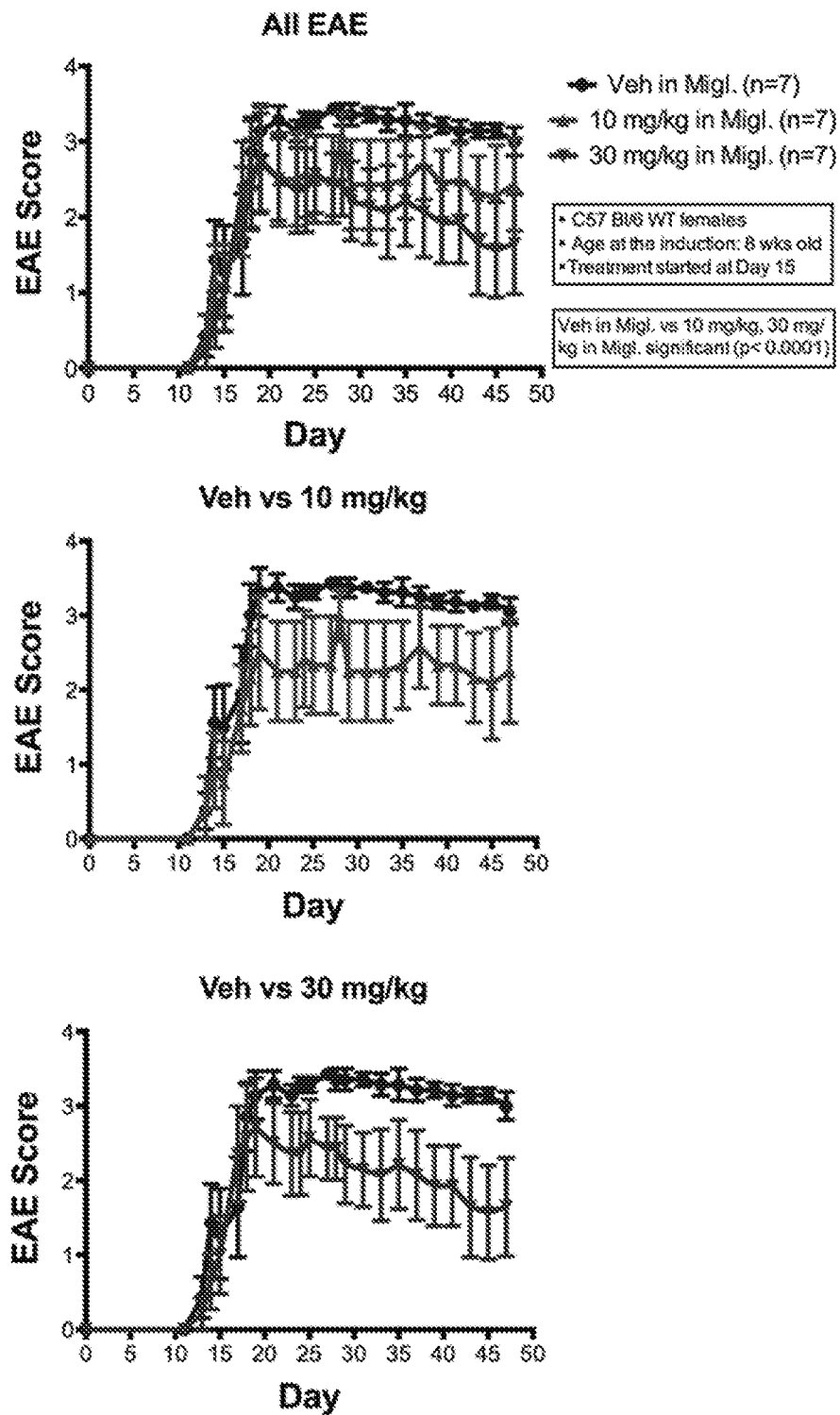


Figure 19

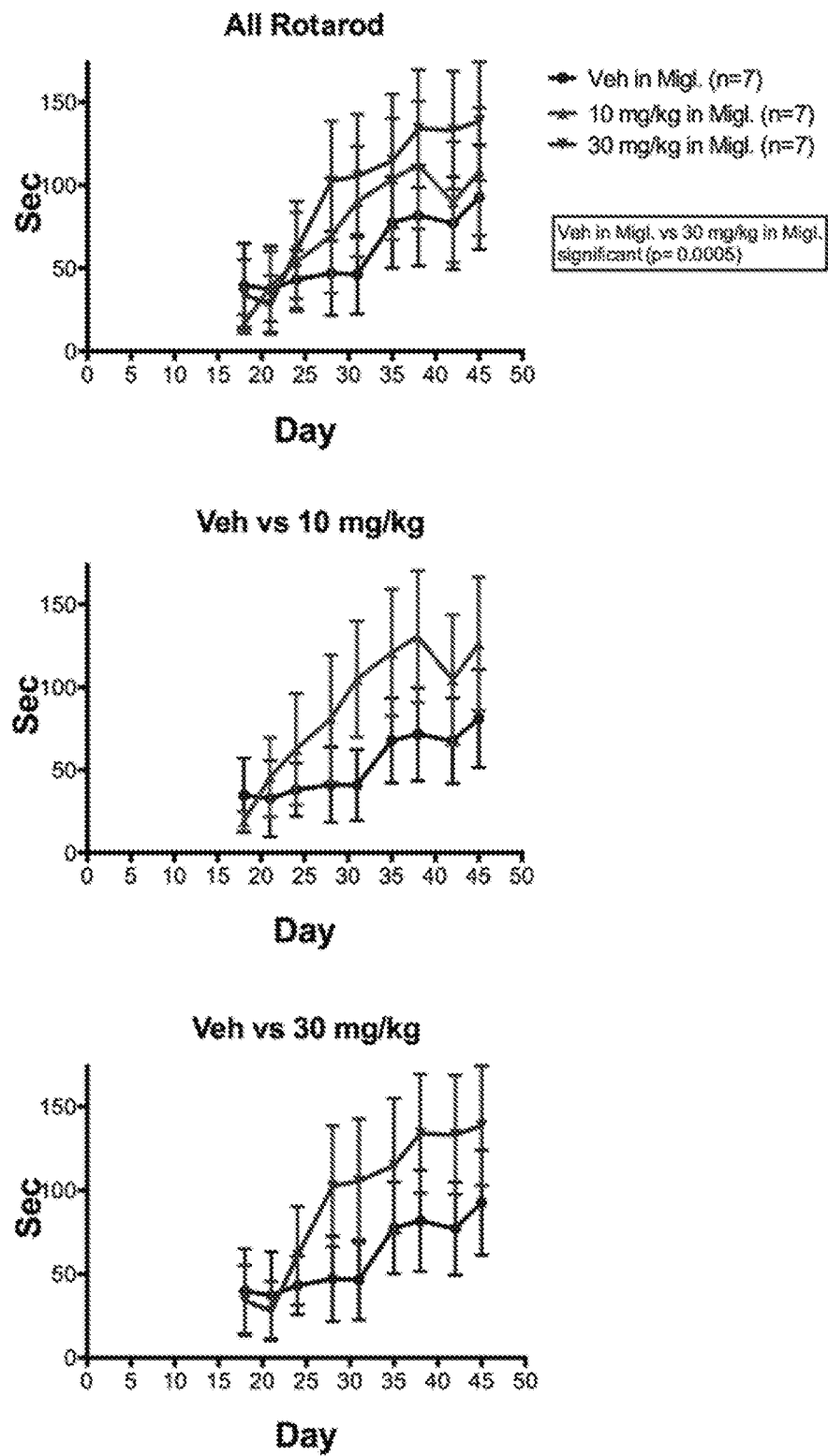


Figure 20

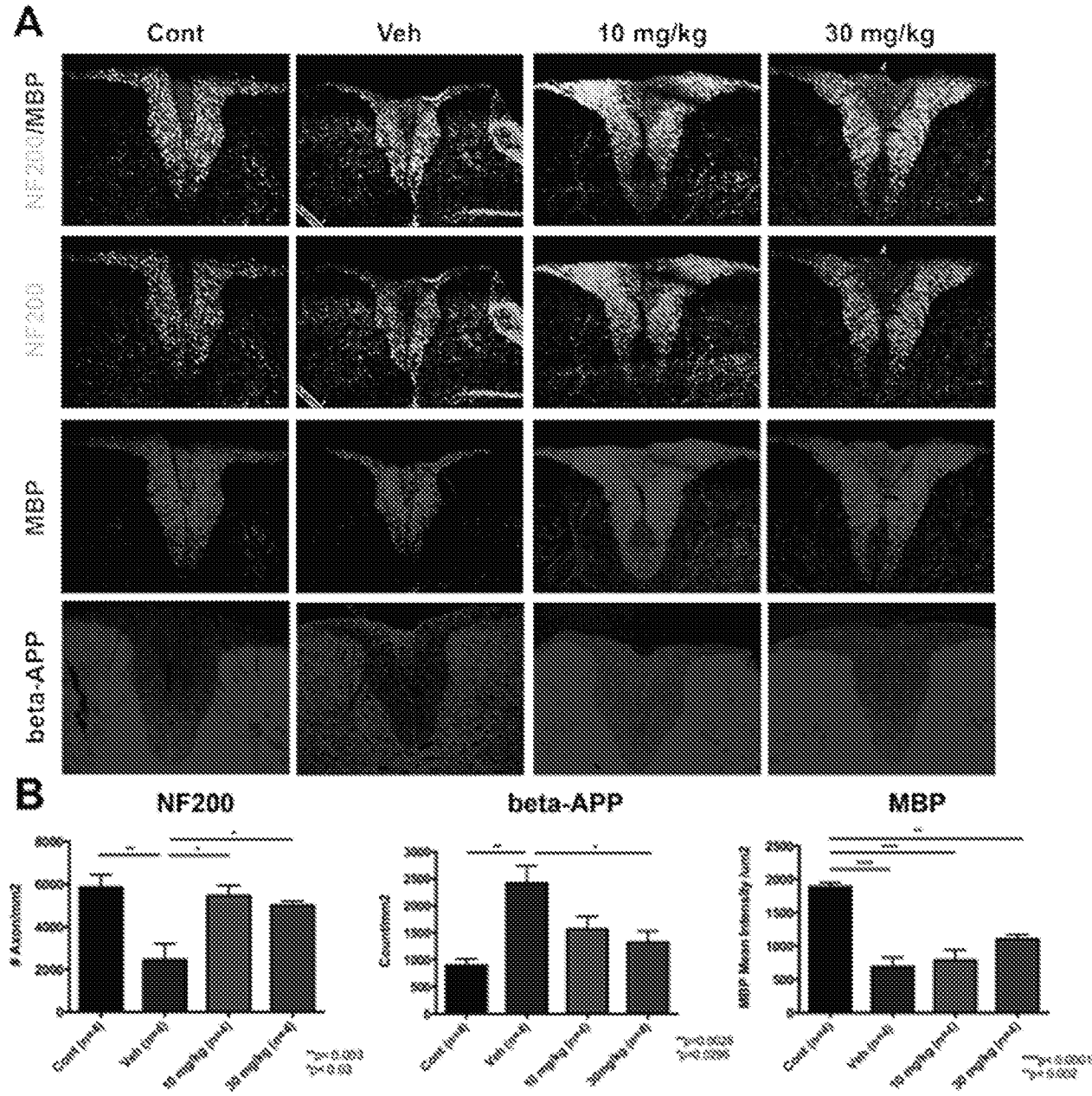


Figure 21

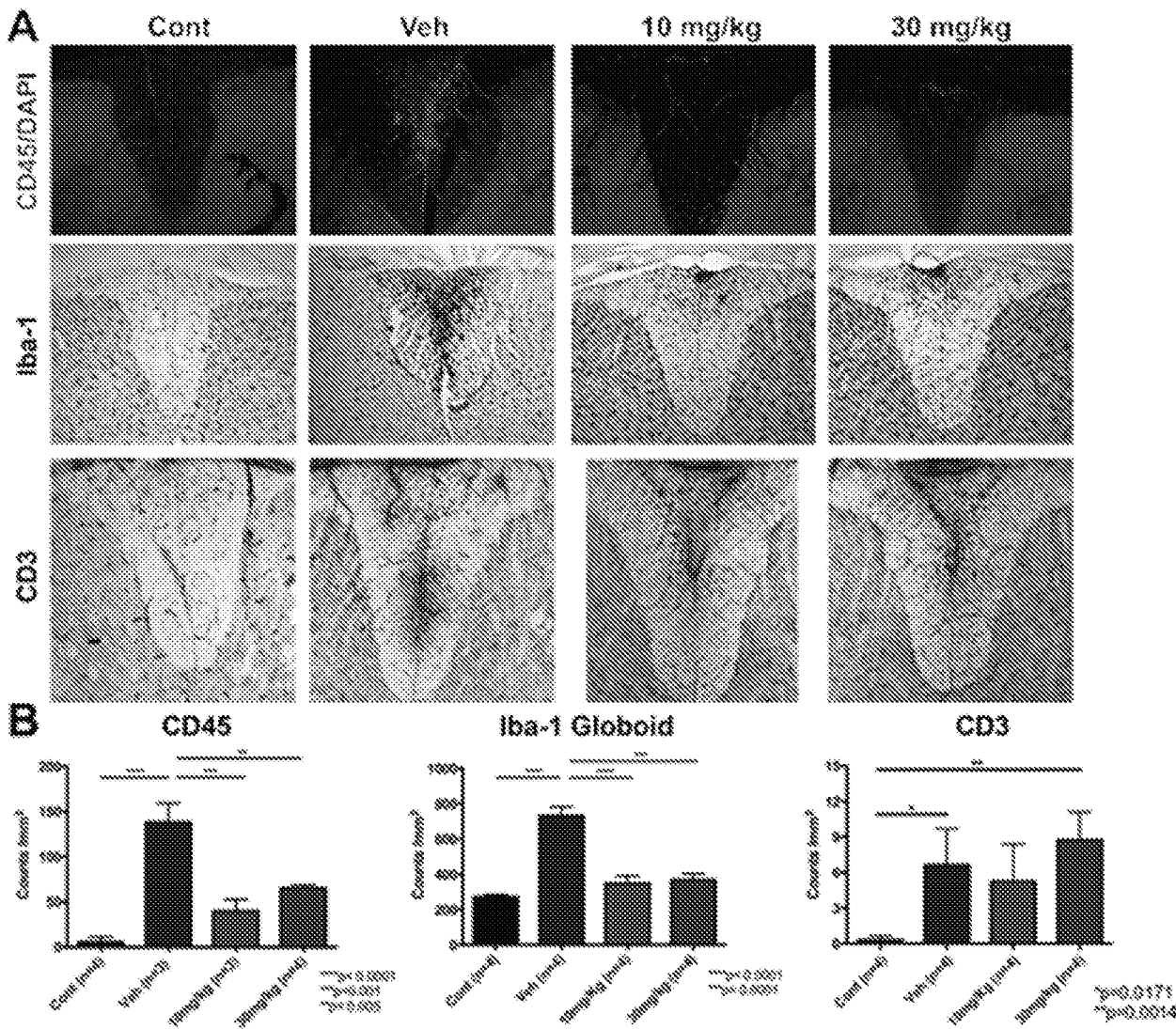


Figure 22

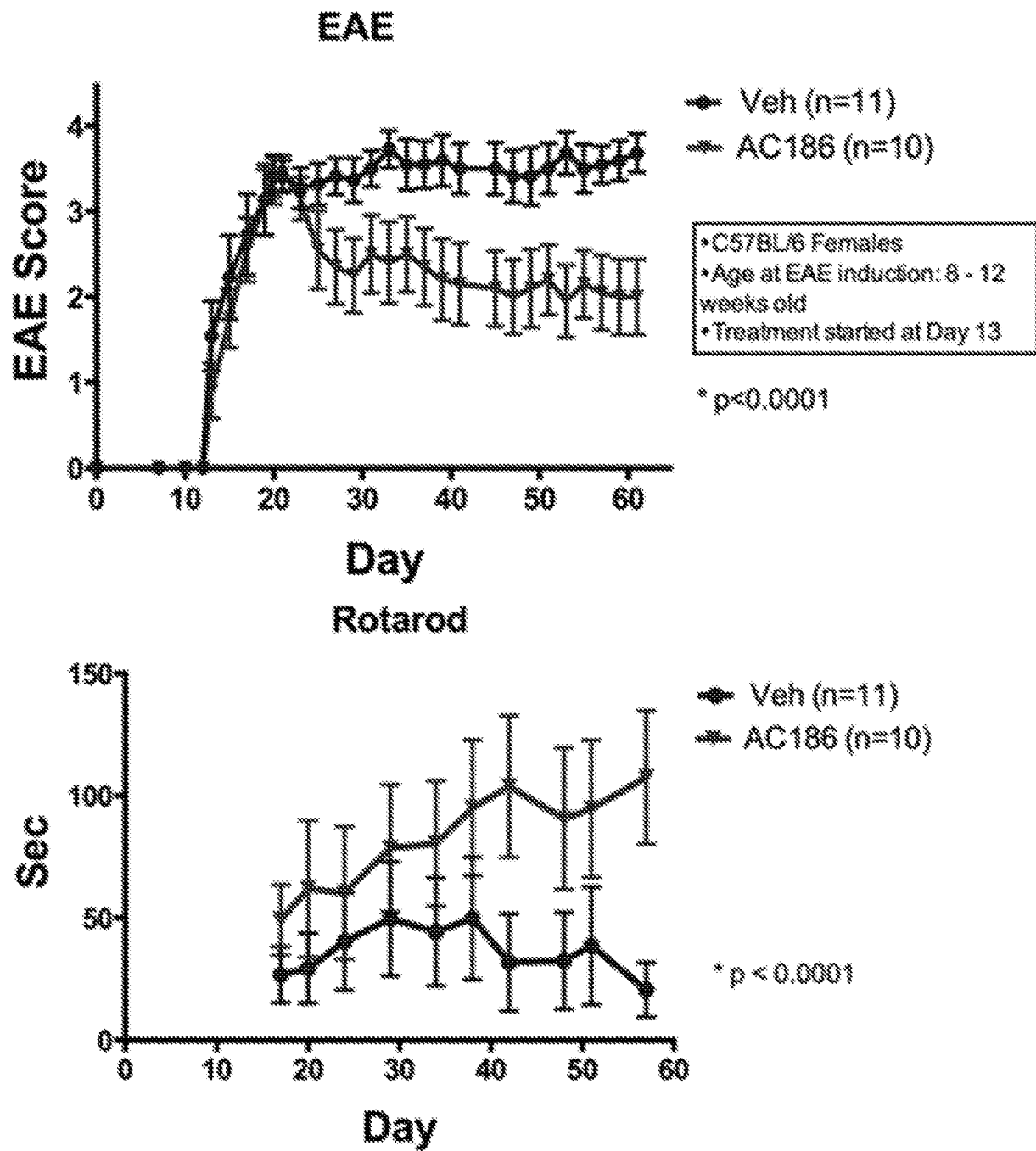


Figure 23

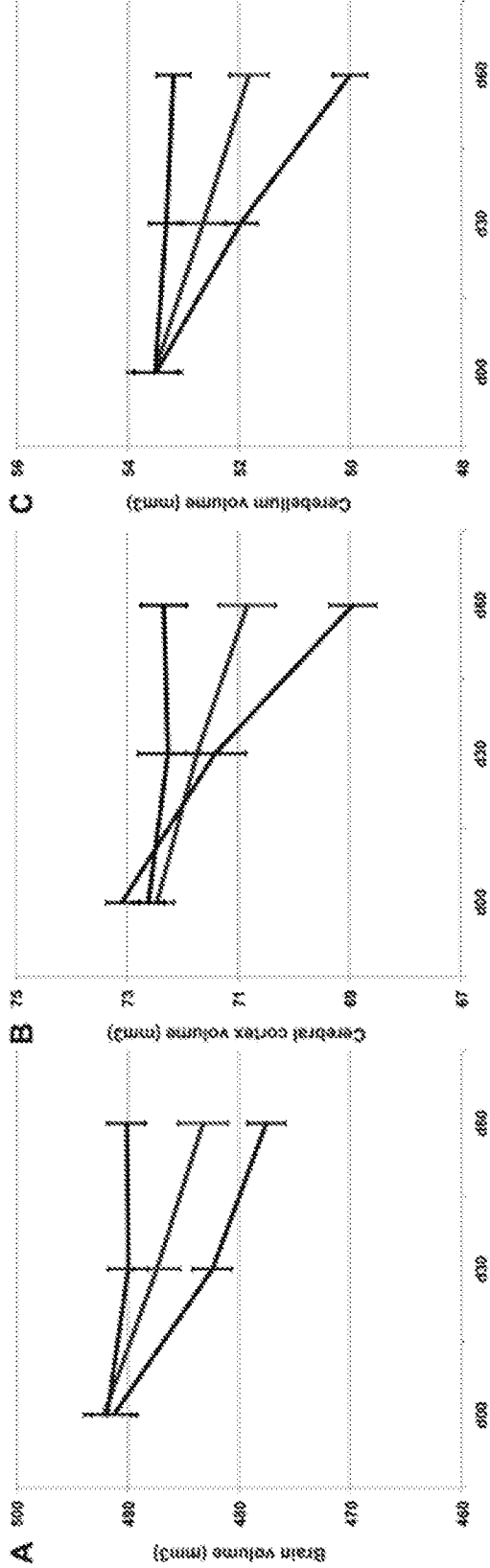
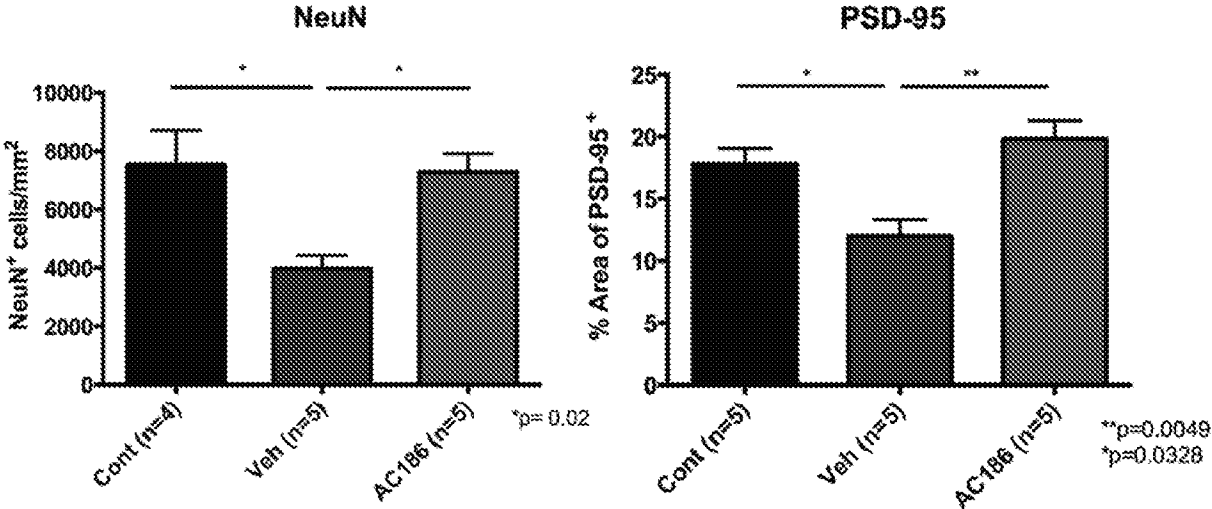


Figure 24

Top panel: cerebral gray matter neuropathology



Bottom panel: cerebellar gray matter neuropathology

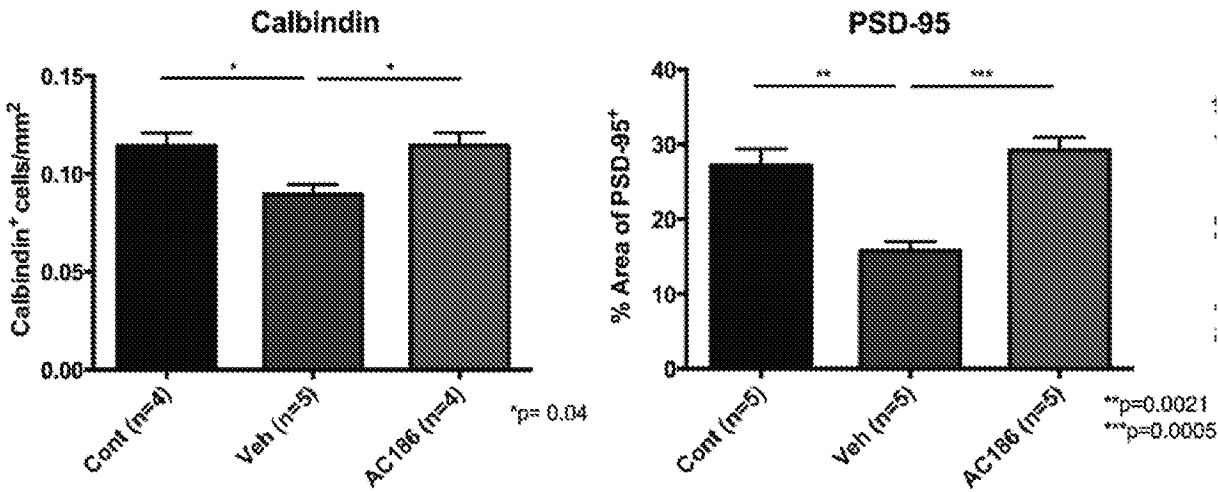


Figure 25

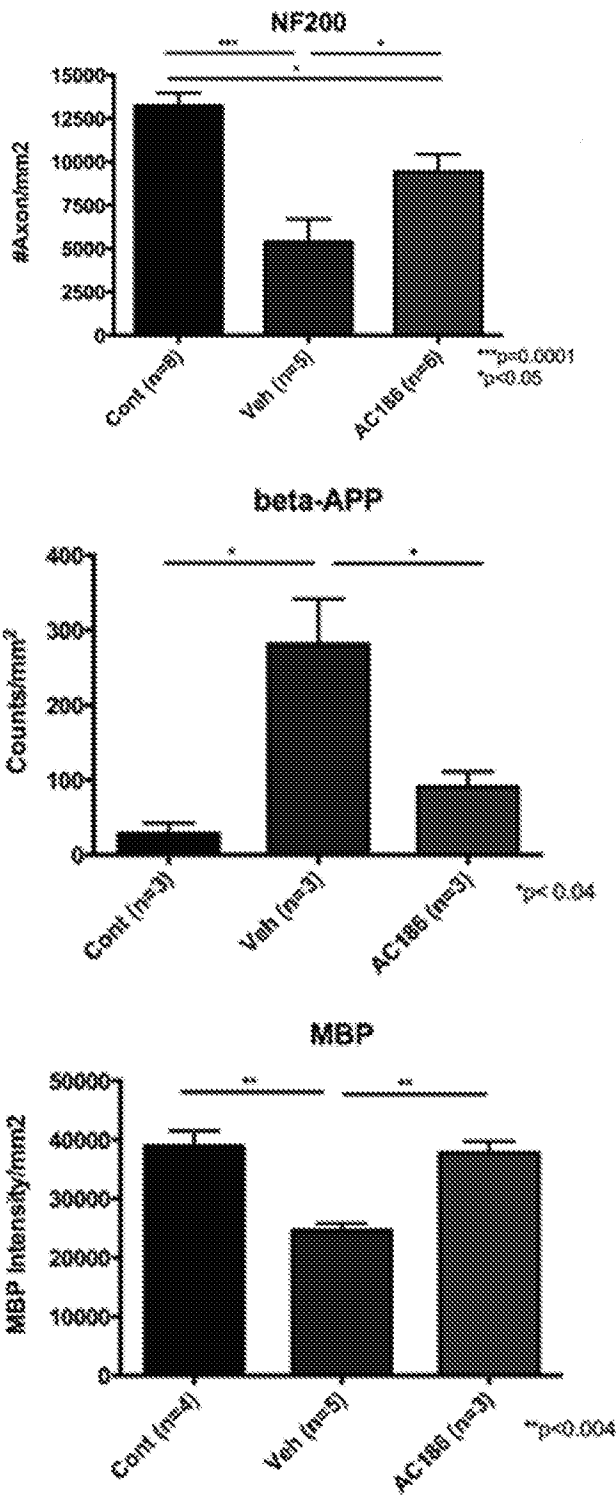


Figure 26

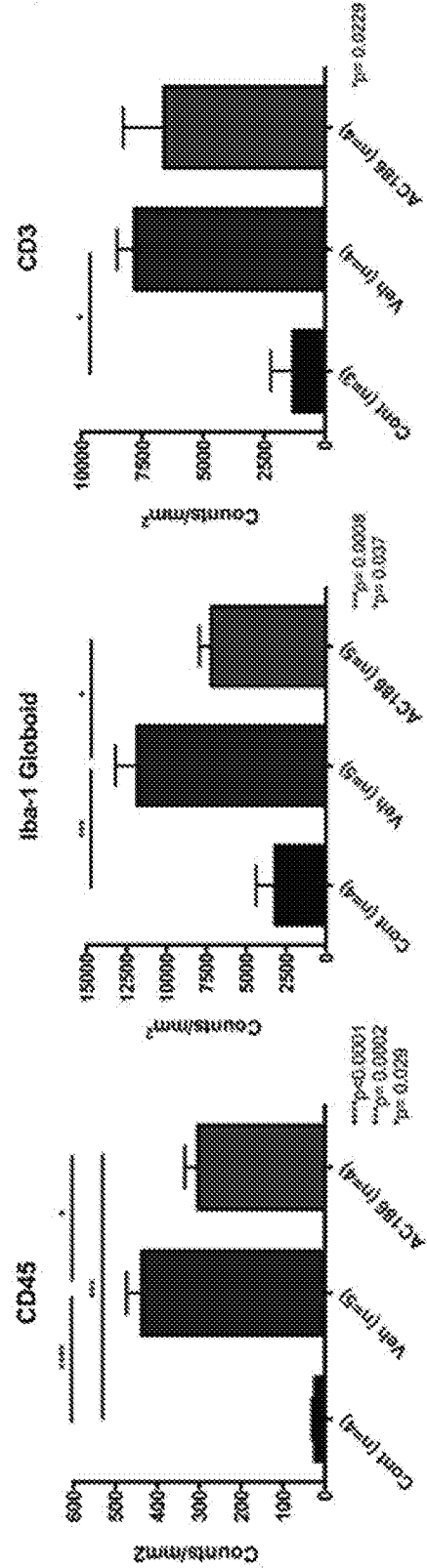


Figure 27

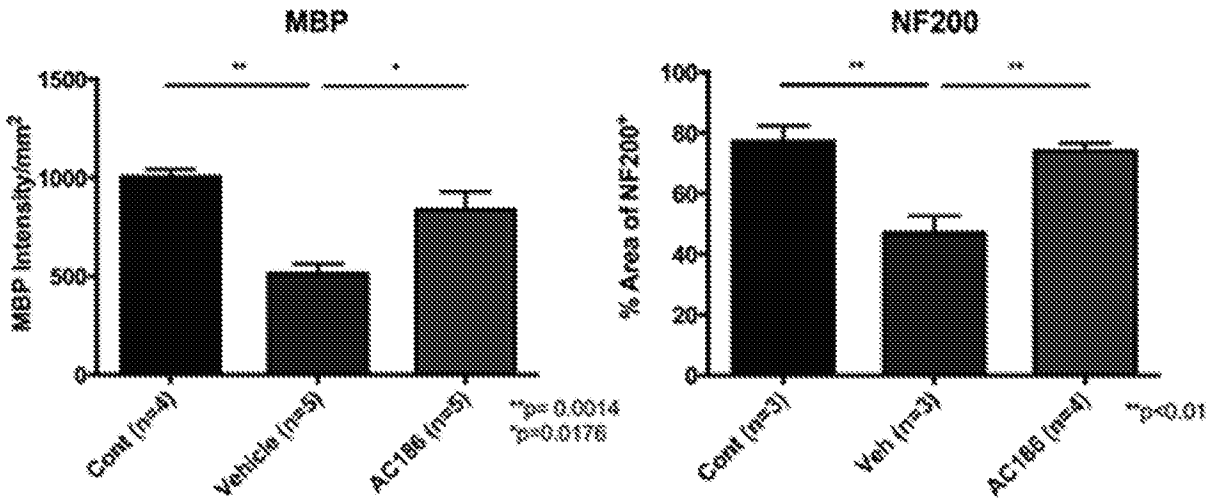


Figure 28

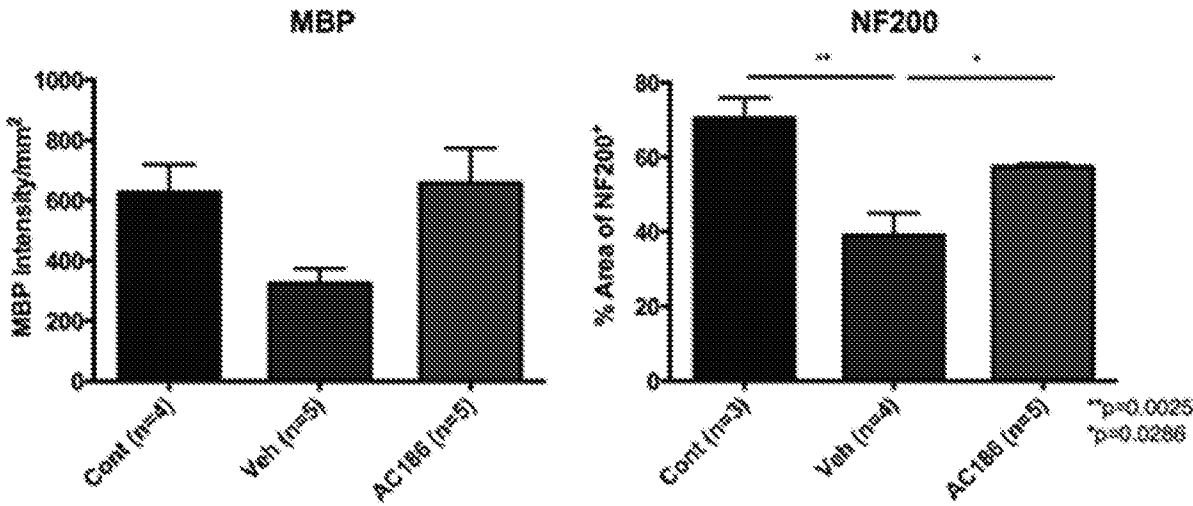
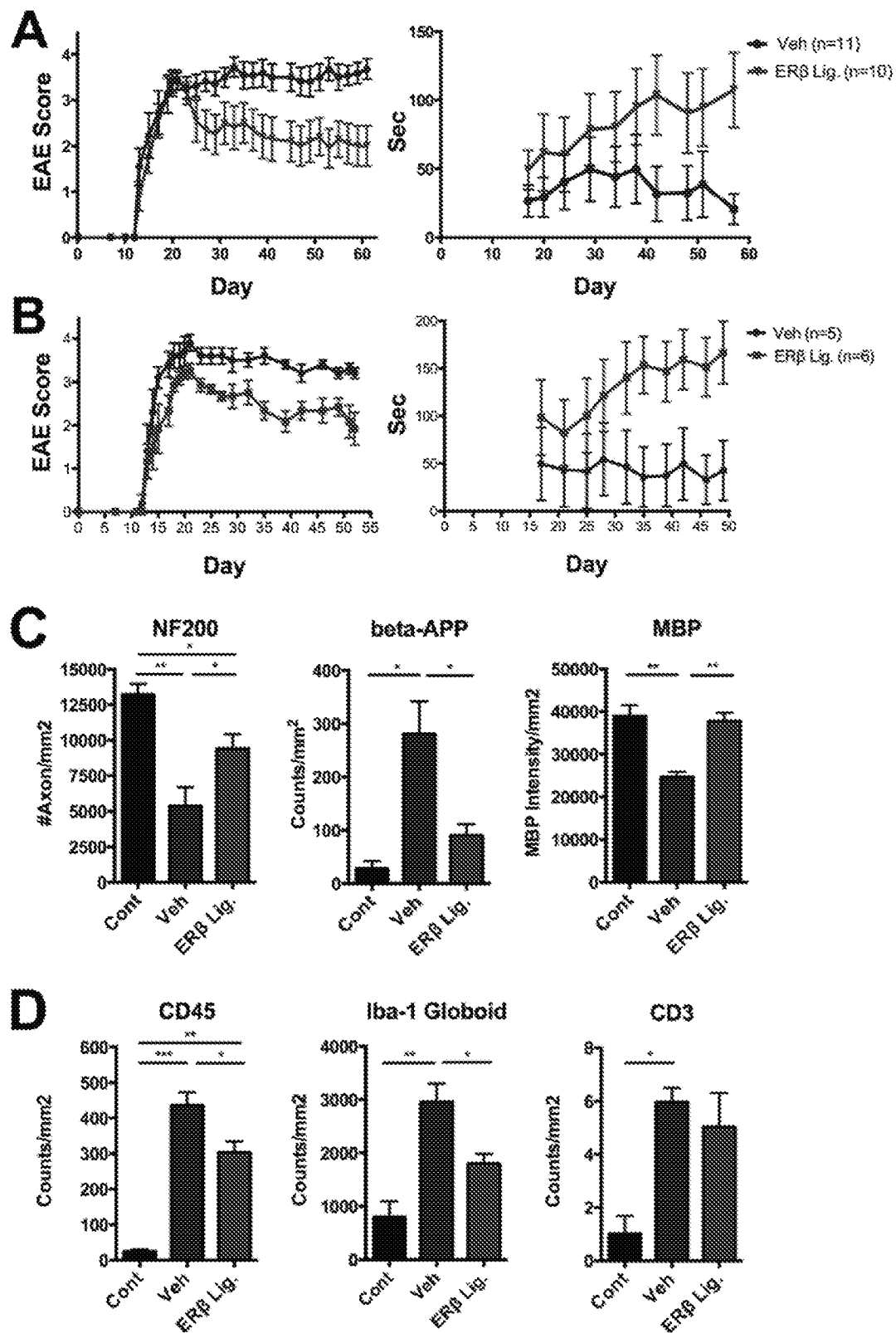


Figure 29



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/047485

A. CLASSIFICATION OF SUBJECT MATTER IPC (2016.01) A61K 31/05, A61P 25/28, C07C 39/17, C07C 39/42 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) IPC (2016.01) A61K 31/05, A61P 25/28, C07C 39/17, C07C 39/42 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 practicable, search terms used) See extra sheet.		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Seema Tiwari-Woodruff et al, Differential neuroprotective and antiinflammatory effects of estrogen receptor (ER) and ER ligand treatment, Article in Proceedings of the National Academy of Sciences, Vol.104(37), pages 14813-14818 11 Sep 2007 (2007/09/11) last paragraph of the results section, on page 14816, and page 14818, last paragraph of the discussion, left column	1-20,25-51
A	last paragraph of the results section, page 14816	21-24
A	WO 2013017619 A1 ACADIA PHARMACEUTICALS Inc. [US] 07 Feb 2013 (2013/02/07) abstract, claims 22 and 32	1-51
A	Wisdom, Amy J. et al, Estrogen receptor-beta ligand treatment after disease onset is neuroprotective in the multiple sclerosis model, J Neurosci Res., Vol. 91(7), pages 901-908 30 Apr 2013 (2013/04/30) Whole document	1-51
☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 29 Nov 2016		Date of mailing of the international search report 30 Nov 2016
Name and mailing address of the ISA: Israel Patent Office Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel Facsimile No. 972-2-5651616		Authorized officer Pinchover Miriam Telephone No. 972-2-5651642

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2016/047485

Patent document cited search report	Publication date	Patent family member(s)	Publication Date
WO 2013017619 A1	07 Feb 2013	WO 2013017619 A1	07 Feb 2013
		AU 2012292088 A1	30 Jan 2014
		CA 2842394 A1	07 Feb 2013
		CN 103781751 A	07 May 2014
		EP 2554532 A1	06 Feb 2013
		EP 2739600 A1	11 Jun 2014
		JP 2014527046 A	09 Oct 2014
		US 2014275284 A1	18 Sep 2014
		US 9061981 B2	23 Jun 2015

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/047485

B. FIELDS SEARCHED:

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

Databases consulted: Esp@cenet, Google Patents, CAPLUS, REGISTRY, Google Scholar

Search terms used: Multiple sclerosis, estrogen receptor beta, active lesion, neurodegenerative, AC-186, KBRV. General structure according to compound defined in claim 21, as well as compounds mentioned in the specification (pages 15-17).