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(54) **Title:** REDUCING SYSTEMIC REGULATORY T CELL LEVELS OR ACTIVITY FOR TREATMENT OF DISEASE AND INJURY OF THE CNS

(57) **Abstract:** A pharmaceutical composition comprising an active agent that causes reduction of the level of systemic immunosuppression in an individual by release of a restraint imposed on the immune system by one or more immune checkpoints, for use in treating a disease, disorder, condition or injury of the CNS that does not include the autoimmune neuroinflammatory disease, relapsing-remitting multiple sclerosis (RRMS), and said one or more immune checkpoints is selected from the group consisting of ICOS-B7RP1, V-domain Ig suppressor of T cell activation (VISTA), B7-CD28-like molecule, CD40L-CD40, CD28-CD80, CD28-CD86, B7H3, B7H4, B7H7, BTLA-HVEM, CD137-CD137L, OX40L, CD27-CD70, STimulator of INterferon Genes (STING), T cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT) and A2a R-Adenosine and indoleamine-2,3-dioxygenase (IDO)-L-tryptophan is provided. The pharmaceutical composition is for administration by a dosage regimen comprising at least two courses of therapy, each course of therapy comprising in sequence a treatment session followed by an interval session of non-treatment.



REDUCING SYSTEMIC REGULATORY T CELL LEVELS OR ACTIVITY FOR TREATMENT OF DISEASE AND INJURY OF THE CNS

FIELD OF THE INVENTION

5 The present invention relates in general to methods and compositions for treating disease, disorder, condition or injury of the Central Nervous System (CNS) by transiently reducing the level of systemic immunosuppression in the circulation.

BACKGROUND OF THE INVENTION

10 Most central nervous system (CNS) pathologies share a common neuroinflammatory component, which is part of disease progression, and contributes to disease escalation. Among these pathologies is Alzheimer's disease (AD), an age-related neurodegenerative disease characterized by progressive loss of memory and cognitive functions, in which accumulation of amyloid-beta ($A\beta$) peptide aggregates was suggested to play a key role in the inflammatory
15 cascade within the CNS, eventually leading to neuronal damage and tissue destruction (Akiyama et al, 2000; Hardy & Selkoe, 2002; Vom Berg et al, 2012). Despite the chronic neuroinflammatory response in neurodegenerative diseases, clinical and pre-clinical studies over the past decade, investigating immunosuppression-based therapies in neurodegenerative diseases, have raised the question as to why anti-inflammatory drugs fall short (Breitner et al,
20 2009; Group et al, 2007; Wyss-Coray & Rogers, 2012). We provide a novel answer that overcomes the drawbacks of existing therapies of AD and similar diseases and injuries of the CNS; this method is based on our unique understanding of the role of the different components of systemic and central immune system in CNS maintenance and repair.

SUMMARY OF INVENTION

25 In one aspect, the present invention provides a pharmaceutical composition comprising an active agent that causes reduction of the level of systemic immunosuppression in an individual by release of a restraint imposed on the immune system by one or more immune checkpoints for use in treating a disease, disorder, condition or injury of the CNS that does not
30 include the autoimmune neuroinflammatory disease, relapsing-remitting multiple sclerosis (RRMS), wherein said pharmaceutical composition is for administration by a dosage regimen comprising at least two courses of therapy, each course of therapy comprising in sequence a treatment session followed by an interval session of non-treatment; and said one or more

immune checkpoints is selected from the group consisting of ICOS-B7RP1, V-domain Ig suppressor of T cell activation (VISTA), B7-CD28-like molecule, CD40L-CD40, CD28-CD80, CD28-CD86, B7H3, B7H4, B7H7, BTLA-HVEM, CD137-CD137L, OX40L, CD27-CD70, STimulator of INterferon Genes (STING), T cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT) and A2aR-Adenosine and indoleamine-2,3-dioxygenase (IDO)-L-tryptophan.

In another aspect, the present invention provides a method for treating a disease, disorder, condition or injury of the Central Nervous System (CNS) that does not include the autoimmune neuroinflammatory disease relapsing-remitting multiple sclerosis (RRMS), said method comprising administering to an individual in need thereof a pharmaceutical composition comprising an active agent that causes reduction of the level of systemic immunosuppression by release of a restraint imposed on the immune system by one or more immune checkpoints according to the present invention, wherein said pharmaceutical composition is administered by a dosage regime comprising at least two courses of therapy, each course of therapy comprising in sequence a treatment session followed by an interval session of a non-treatment period, and said one or more immune checkpoints is selected from the group consisting of ICOS-B7RP1, V-domain Ig suppressor of T cell activation (VISTA), B7-CD28-like molecule, CD40L-CD40, CD28-CD80, CD28-CD86, B7H3, B7H4, B7H7, BTLA-HVEM, CD137-CD137L, OX40L, CD27-CD70, STimulator of INterferon Genes (STING), T cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT) and A2aR-Adenosine and indoleamine-2,3-dioxygenase (IDO)-L-tryptophan.

BRIEF DESCRIPTION OF DRAWINGS

Figs. 1A-B depict the choroid plexus (CP) activity along disease progression in the 5XFAD transgenic mouse model of AD (AD-Tg). **(A)** mRNA expression levels for the genes *icam1*, *vcam1*, *cxcl10* and *ccl2*, measured by RT-qPCR, in CPs isolated from 1, 2, 4 and 8-month old AD-Tg mice, shown as fold-change compared to age-matched WT controls (n=6-8 per group; Student's t test for each time point). **(B)** Representative microscopic images of CPs of 8-month old AD-Tg mice and age-matched WT controls, immunostained for the epithelial tight junction molecule Claudin-1, Hoechst nuclear staining, and the integrin ligand, ICAM-1 (scale bar, 50µm). In all panels, error bars represent mean $\pm$ s.e.m.; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Figs. 2A-C show **(A)** Quantification of ICAM-1 immunoreactivity in human postmortem CP of young and aged non-CNS diseased, and AD patients (n=5 per group; one-way ANOVA

followed by Newman–Keuls post hoc analysis); **(B)** flow cytometry analysis for IFN- γ -expressing immune cells (intracellularly stained, and pre-gated on CD45) in CPs of 8-month old AD-Tg mice and age-matched WT controls. Shaded histogram represents isotype control (n=4-6 per group; Student's t test); and **(C)** mRNA expression levels of *ifn- γ* , measured by RT-qPCR, in CP tissues isolated from 4- and 8-month old AD-Tg mice, compared to age-matched WT controls (n=5-8 per group; Student's t test for each time point). In all panels, error bars represent mean $\pm$ s.e.m.; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Figs. 3A-B depict **(A)** representative flow cytometry plots of CD4⁺Foxp3⁺ splenocyte frequencies (pre-gated on TCR β) in 8-month old AD-Tg and WT control mice; and **(B)** quantitative analysis of splenocytes from 1, 2, 4 and 8-month AD-Tg and WT control mice (n=6-8 per group; Student's t test for each time point). In all panels, error bars represent mean $\pm$ s.e.m.; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Fig. 4 shows gating strategy and representative flow cytometry plots of splenocytes from AD-Tg/Foxp3-DTR^{+/-} mice, 1 day after the last injection of DTx. DTx was injected i.p. for 4 constitutive days, achieving ~99% depletion of Foxp3⁺ cells.

Figs. 5A-G show the effects of transient depletion of Tregs in AD-Tg mice. **(A)** AD-Tg/Foxp3-DTR⁺ (which express the DTR transgene) and a non-DTR-expressing AD-Tg littermate (AD-Tg/Foxp3-DTR⁻) control group were treated with DTx for 4 constitutive days. CP mRNA expression levels for the genes *icam1*, *cxcl10* and *ccl2*, measured by RT-qPCR, in 6-month old DTx-treated AD-Tg mice, 1 day after the last DTx injection (n=6-8 per group; Student's t test). **(B-D)** Flow cytometry analysis of the brain parenchyma (excluding the choroid plexus, which was separately excised) of 6-month old DTx-treated AD-Tg mice and controls, 3 weeks following the last DTx injection. Quantitative flow cytometry analysis showing increased numbers of CD11b^{high}/CD45^{high} mo-M Φ and CD4⁺ T cells **(B)**, and representative flow cytometry plots **(C)** and quantitative analysis **(D)** of CD4⁺Foxp3⁺ Treg frequencies, in the brain parenchyma of AD-Tg/Foxp3-DTR⁺ mice and AD-Tg/Foxp3-DTR⁻ controls treated with DTx (n=3-7 per group; Student's t test). **(E)** mRNA expression levels of *foxp3* and *il10* in the brain parenchyma of 6-month old DTx-treated AD-Tg AD-Tg/Foxp3-DTR⁺ and AD-Tg/Foxp3-DTR⁻ controls, 3 weeks after the last DTx injection (n=6-8 per group; Student's t test). **(F)** quantitative analysis of GFAP immunostaining, showing reduced astrogliosis in hippocampal sections from 6-month old DTx-treated AD-Tg/Foxp3-DTR⁺ and AD-Tg/Foxp3-DTR⁻ control mice, 3 weeks following the last DTx injection (scale bar, 50 μ m; n=3-5 per group; Student's t test). **(G)** mRNA expression levels of *il-12p40* and *tnf-a* in the brain parenchyma, 3 weeks following the last DTx

injection (n=6-8 per group; Student's t test). In all panels, error bars represent mean $\pm$ s.e.m.; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Figs. 6A-E show the effect of transient depletion of Tregs on A β plaques learning/memory performance. **(A)** Representative microscopic images and **(B)** quantitative analysis of the brains of 5-month old DTx-treated AD-Tg/Foxp3-DTR⁺ and AD-Tg/Foxp3-DTR⁻ control mice, 3 weeks after the last DTx injection, immunostained for A β plaques and Hoechst nuclear staining (scale bar, 250 μ m). Mean A β plaque area and numbers in the hippocampal dentate gyrus (DG) and the 5th layer of the cerebral cortex were quantified (in 6 μ m brain slices; n=5-6 per group; Student's t test). **Figs. 6C-E** show Morris water maze (MWM) test performance of 6-month old DTx-treated AD-Tg/Foxp3-DTR⁺ and control mice, 3 weeks after the last DTx injection. Following transient Treg depletion, AD-Tg mice showed better spatial learning/memory performance in the **(C)** acquisition, **(D)** probe and **(E)** reversal phases of the MWM, relative to AD-Tg controls (n=7-9 per group; two-way repeated measures ANOVA followed by Bonferroni post-hoc analysis for individual pair comparisons; *, $P < 0.05$ for overall acquisition, probe, and reversal). In all panels, error bars represent mean $\pm$ s.e.m.; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Fig. 7 shows mRNA expression levels of *ifn- γ* , measured by RT-qPCR, in CPs isolated from 6- and 12-month old APP/PS1 AD-Tg mice (a mouse model for Alzheimer's disease (see Materials and Methods)), compared to age-matched WT controls (n=5-8 per group; Student's t test). Error bars represent mean $\pm$ s.e.m.; *, $P < 0.05$.

Figs. 8A-I show the therapeutic effect of administration of weekly Glatiramer acetate (GA) in AD-Tg mice. **(A)** Schematic representation of weekly-GA treatment regimen. Mice (5-month old) were s.c. injected with GA (100 μ g), twice during the first week (on day 1 and 4), and once every week thereafter, for an overall period of 4 weeks. The mice were examined for cognitive performance, 1 week (MWM), 1 month (RAWM) and 2 months (RAWM, using different experimental spatial settings) after the last injection, and for hippocampal inflammation. **Figs. 8B-D** show mRNA expression levels of genes in the hippocampus of untreated AD-Tg mice, and AD-Tg mice treated with weekly-GA, at the age of 6m, showing **(B)** reduced expression of pro-inflammatory cytokines such as TNF- α , IL-1 β and IL-12p40, **(C)** elevation of the anti-inflammatory cytokines IL-10 and TGF- β , and of **(D)** the neurotrophic factors, IGF-1 and BDNF, in weekly-GA treated mice (n=6-8 per group; Student's t test). In **Figs. 8E-G**, AD-Tg mice (5 months old) were treated with either weekly-GA or with vehicle (PBS), and compared to age-matched WT littermates in the MWM task at the age of 6m. Treated mice showed better spatial learning/memory performance in the acquisition **(E)**, probe **(F)** and

reversal (**G**) phases of the MWM, relative to controls (n=6-9 per group; two-way repeated measures ANOVA followed by Bonferroni post-hoc for individual pair comparisons; WT mice, black circles; AD-Tg controls, white circles; treated AD-Tg, grey circles). **Figs. 8H-I** show cognitive performance of the same mice in the RAWM task, 1 month (**H**) or 2 months (**I**) following the last GA injection (n=6-9 per group; two-way repeated measures ANOVA followed by Bonferroni post-hoc for individual pair comparisons). Data are representative of at least three independent experiments. In all panels, error bars represent mean $\pm$ s.e.m.; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Figs. 9A-H show further therapeutic effects of administration of weekly-GA in AD-Tg mice. **A-B** shows 5XFAD AD-Tg mice that were treated with either weekly-GA, or vehicle (PBS), and were examined at the end of the 1st week of the administration regimen (after a total of two GA injections). Flow cytometry analysis for CD4⁺Foxp3⁺ splenocyte frequencies (**A**), and CP IFN- γ -expressing immune cells (**B**; intracellularly stained and pre-gated on CD45), in treated 6-month old AD-Tg mice, compared to age-matched WT controls (n=4-6 per group; one-way ANOVA followed by Newman-Keuls post hoc analysis). (**C**) mRNA expression levels for the genes *icam1*, *cxcl10* and *ccl2*, measured by RT-qPCR, in CPs of 4-month old AD-Tg mice, treated with either weekly-GA or vehicle, and examined either at the end of the 1st or 4th week of the weekly-GA regimen (n=6-8 per group; one-way ANOVA followed by Newman-Keuls post hoc analysis). **Figs. 9D-E** show representative images of brain sections from 6-month old AD-Tg/CX₃CR1^{GFP/+} BM chimeras following weekly-GA. CX₃CR1^{GFP} cells were localized at the CP of the third ventricle (3V; **i**), the adjacent ventricular spaces (**ii**), and the CP of the lateral ventricles (LV; **iii**) in AD-Tg mice treated with weekly-GA (**D**; scale bar, 25 μ m). Representative orthogonal projections of confocal z-axis stacks, showing co-localization of GFP⁺ cells with the myeloid marker, CD68, in the CP of 7-month old AD-Tg/CX₃CR1^{GFP/+} mice treated with weekly-GA, but not in control PBS-treated AD-Tg/CX₃CR1^{GFP/+} mice (**E**; scale bar, 25 μ m). (**F**) CX₃CR1^{GFP} cells are co-localized with the myeloid marker IBA-1 in brains of GA-treated AD-Tg/CX₃CR1^{GFP/+} mice in the vicinity of A β plaques, and co-expressing the myeloid marker, IBA-1 (scale bar, 25 μ m). **Figs. 9G-H** show representative flow cytometry plots of cells isolated from the hippocampus of 4-month old WT, untreated AD-Tg, and AD-Tg mice, on the 2nd week of the weekly-GA regimen. CD11b^{high}/CD45^{high} mo-M Φ were gated (**G**) and quantified (**H**; n=4-5 per group; one-way ANOVA followed by Newman-Keuls post hoc analysis). In all panels, error bars represent mean $\pm$ s.e.m.; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Figs. 10A-H depict the therapeutic effect of administration of a p300 inhibitor (C646) in AD-Tg mice. In **Figs. 10A-B**, aged mice (18 months) were treated with either p300i or vehicle

(DMSO) for a period of 1 week, and examined a day after cessation of treatment. Representative flow cytometry plots showing elevation in the frequencies of CD4⁺ T cells expressing IFN- γ in the spleen (A), and IFN- γ -expressing immune cell numbers in the CP (B), following p300i treatment. **Figs. 10C-E** show representative microscopic images (C), and quantitative analysis, of A β plaque burden in the brains of 10-month old AD-Tg mice, which received either p300i or vehicle (DMSO) for a period of 1 week, and were subsequently examined after 3 additional weeks. Brains were immunostained for A β plaques and by Hoechst nuclear staining (n=5 per group; Scale bar, 250 μ m). Mean A β plaque area and plaque numbers were quantified in the hippocampal DG (D) and the 5th layer of the cerebral cortex (E) (in 6 μ m brain slices; n=5-6 per group; Student's t test). (F) Schematic representation of the p300i treatment (or DMSO as vehicle) administration regimen to the different groups of AD-Tg mice at the age of 7 months, in either 1 or 2 sessions. **Figs. 10G-H** show the change mean of A β plaque percentage coverage of the cerebral cortex (5th layer) (G), and the change in mean cerebral soluble A β ₁₋₄₀ and A β ₁₋₄₂ protein levels (H), relative to the untreated AD-Tg group (A β ₁₋₄₀ and A β ₁₋₄₂ mean level in untreated group, 90.5 $\pm$ 11.2 and 63.8 $\pm$ 6.8 pg/mg total portion, respectively; n=5-6 per group; one-way ANOVA followed by Newman-Keuls post hoc analysis). In all panels, error bars represent mean $\pm$ s.e.m.; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Figs. 11 A-D show that PD-1 blockade augments IFN- γ -dependent choroid plexus activity in AD-Tg mice. 10-month old AD-Tg mice were i.p. injected on day 1 and day 4 with 250ug of either anti-PD-1 or control IgG, and examined at days 7-10 for the effect on the systemic immune response and CP activity. (A-B) Representative flow cytometry plots (A), and quantitative analysis (B), of CD4⁺IFN- γ ⁺ splenocyte frequencies (intracellularly stained and pre-gated on CD45 and TCR- β), in α PD-1 or IgG treated AD-Tg mice, and untreated AD-Tg and WT controls (n=4-6 per group; one-way ANOVA followed by Newman-Keuls post hoc analysis; **, P < 0.01 between the indicated treated groups; error bars represent mean $\pm$ s.e.m.). (C) mRNA expression levels of *ifn-g*, measured by RT-qPCR in the CP of AD-Tg mice treated with anti-PD-1 when compared to IgG treated and untreated AD-Tg controls (D) GO annotation terms enriched in RNA-Seq in CPs of the same mice (n=3-5 per group; one-way ANOVA followed by Newman-Keuls post hoc analysis; *, P < 0.05) (gray scale corresponds to negative log-base 10 of P-value).

Figs. 12A-B show that PD-1 blockade mitigates cognitive decline in AD-Tg mice. 10-month old AD-Tg mice were i.p. injected on day 1 and day 4 with 250ug of either anti-PD-1 or control IgG, and examined 1 or 2 months later for the effect on pathology. (A-B) Scheme of the experimental design. Single arrows indicate time points of treatment, and double arrows indicate

time points of cognitive testing. Cognitive performance of anti-PD-1 and IgG treated mice, compared to age-matched WT and untreated AD-Tg mice, assessed by the average number of errors per day in the RAWM learning and memory task (n=6-8 per group; two-way repeated measures ANOVA followed by Bonferroni post-hoc for individual pair comparisons). (A) Performance of AD-Tg mice in the RAWM after 1 treatment session with anti-PD-1 or IgG control. (B) Effect of single anti-PD-1 treatment session, or 2 sessions with a 1 month interval on performance.

Figs. 13A-D depict representative microscopic images showing that PD-1 blockade mitigates AD pathology (A), and quantitative analyses (B, C, D), of A β plaque burden and astrogliosis in the brains of AD-Tg mice, which were treated at the age of 10-months with either anti-PD-1 (in 1 or 2 sessions, as depicted in Fig. 12a-b) or IgG control, and subsequently examined at the age of 12 months. Brains were immunostained for A β plaques (in red), GFAP (marking astrogliosis, in green), and by Hoechst nuclear staining (n=4-5 per group; Scale bar, 50 μ m). Mean A β plaque area and plaque numbers were quantified in the hippocampal dentate gyrus (DG) and the 5th layer of the cerebral cortex, and GFAP immunoreactivity was measured in the hippocampus (in 6 μ m brain slices; n=5-6 per group; Student's t test). In all panels, error bars represent mean $\pm$ s.e.m.; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Fig. 14 shows the effect of different dosing and frequency of administration of anti-PD-1 antibody on cognitive decline in AD-Tg mice. Female 5XFAD AD transgenic mice (average cohorts age of 6 months) were treated with either anti-PD-1-specific antibody (IgG2a anti-mouse PD-1 or IgG control (Rat IgG2a). anti-PD-1-treated mice received either 1 injection of 500ug of antibody on day 1 of the experiment, or two injections of 250ug with a 3-day interval between injections. Aged matched wild-type (WT) mice were used as additional control group. We evaluated the effect on spatial learning and memory performance, using the radial arm water maze (RAWM) task. Experimental design is presented. Black arrows indicate time points of treatment, and illustrations indicate time points of cognitive testing. RAWM performance of anti-PD-1-treated 5XFAD mice – one injection (n=7) or two injections (n=11), IgG2a-treated 5XFAD mice (n = 10), and WT (n = 14) controls; two-way repeated-measures ANOVA and Dunnett post-test). Error bars represent mean $\pm$ s.e.m.; *P < 0.05, **P < 0.01, ***P < 0.001, anti-PD-1-treated (1 injection) versus IgG-treated controls.

Figs. 15A-C show the effect of repeated administration of anti-PD-1 antibody on cognitive decline in AD-Tg mice. Male 5XFAD AD transgenic mice were treated in a repeated treatment session, once a month, with either anti-PD-1-specific antibody (IgG2a anti-mouse PD-1) or IgG control (Rat IgG2a). The first injection was at the age of 3 months, the second at the

age of 4 months, and the third at the age of 5 months. Dosage is indicated in the scheme of the experimental design (A). Aged matched wild-type (WT) mice were used as additional control group. We evaluated the effect on spatial learning and memory performance, using the radial arm water maze (RAWM) task, at two different time points – the age of 5 months (B), and the age of 6 months (C). Experimental design is presented. Black arrows indicate time points of treatment, and illustrations indicate time points of cognitive testing. RAWM performance of anti-PD-1-treated 5XFAD mice (n=7; empty circles), IgG2a-treated 5XFAD mice (n = 9; empty squares), and WT (n = 8) controls (filled circles); two-way repeated-measures ANOVA and Dunnett post-test). Error bars represent mean $\pm$ s.e.m.; *P < 0.05, **P < 0.01, ***P < 0.001, anti-PD-1-treated versus IgG-treated controls.

DETAILED DESCRIPTION

Immune checkpoint mechanisms, which include cell-intrinsic downregulation of activated T cell responsiveness and effector function by inhibitory receptors, maintain systemic immune homeostasis and autoimmune tolerance (Joller et al, 2012; Pardoll, 2012). In recent years, blockade of these immune checkpoints, such as the programmed death-1 (PD-1) pathway (Francisco et al, 2010), has demonstrated notable anti-tumor efficacy, highlighting the potential of unleashing the power of the immune system in fighting various malignancies. Recently, it was shown (WO 2015/136541; Baruch et al., 2016) that administration of anti-PD-1 antibodies to an animal model of Alzheimer's disease leads to clearance of A β , reversal of cognitive decline, and is associated with resolution of the neuroinflammatory response.

Thus, systemic immunosuppression interferes with the ability to fight off AD pathology, and by releasing restraints on the systemic immune system, AD pathology could be mitigated.

The present invention provides a method for treating a disease, disorder, condition or injury of the Central Nervous System (CNS) that does not include the autoimmune neuroinflammatory disease relapsing-remitting multiple sclerosis (RRMS), said method comprising administering to an individual in need thereof an active agent that causes reduction of the level of systemic immunosuppression by release of a restraint imposed on the immune system by one or more immune checkpoints, wherein said active agent is administered by a dosage regime comprising at least two courses of therapy, each course of therapy comprising in sequence a treatment session followed by an interval session of non-treatment and said one or more immune checkpoints is selected from the group consisting of ICOS-B7RP1, V-domain Ig suppressor of T cell activation (VISTA), B7-CD28-like molecule, CD40L-CD40, CD28-CD80, CD28-CD86, B7H3, B7H4, B7H7, BTLA-HVEM, CD137-CD137L, OX40L, CD27-CD70,

STimulator of INterferon Genes (STING), T cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT) and A2aR-Adenosine and indoleamine-2,3-dioxygenase (IDO)-L-tryptophan.

5 In another aspect, the present invention is directed to an active agent that causes reduction of the level of systemic immunosuppression in an individual by release of a restraint imposed on the immune system by one or more immune checkpoints, or a pharmaceutical composition comprising the active agent, for use in treating a disease, disorder, condition or injury of the CNS that does not include the autoimmune neuroinflammatory disease, relapsing-remitting multiple sclerosis (RRMS), wherein said pharmaceutical composition is for
10 administration by a dosage regimen comprising at least two courses of therapy, each course of therapy comprising in sequence a treatment session followed by an interval session of non-treatment; and said one or more immune checkpoints is selected from the group consisting of ICOS-B7RP1, VISTA, B7-CD28-like molecule, CD40L-CD40, CD28-CD80, CD28-CD86, B7H3, B7H4, B7H7, BTLA-HVEM, CD137-CD137L, OX40L, CD27-CD70, STING, TIGIT
15 and A2aR-Adenosine and indoleamine-2,3-dioxygenase (IDO)-L-tryptophan.

In certain embodiments, the dosage regimen is calibrated such that the level of systemic immunosuppression is transiently reduced.

The term "treating" as used herein refers to means of obtaining a desired physiological effect. The effect may be therapeutic in terms of partially or completely curing a disease and/or
20 symptoms attributed to the disease. The term refers to inhibiting the disease, i.e. arresting or slowing its development; or ameliorating the disease, i.e. causing regression of the disease.

The term "non-treatment session" is used interchangeably herein with the term "period of no treatment" and refers to a session during which no active agent is administered to the individual being treated.

25 The term "systemic presence" of regulatory or effector T cells as used herein refers to the presence of the regulatory or effector T cells (as measured by their level or activity) in the circulating immune system, i.e. the blood, spleen and lymph nodes. It is a well-known fact in the field of immunology that the cell population profile in the spleen is reflected in the cell population profile in the blood (Zhao et al, 2007).

30 The present treatment is applicable to both patients that show elevation of systemic immune suppression, as well as to patients that do not show such an elevation. Sometimes the individual in need for the treatment according to the present invention has a certain level of peripheral immunosuppression, which is reflected by elevated frequencies or numbers of Tregs in the circulation, and/or their enhanced functional activity and/or a decrease in IFN γ -producing

leukocytes and/or decreased proliferation of leukocytes in response to stimulation. The elevation of frequencies or numbers of Tregs can be in total numbers or as percentage of the total CD4 cells. For example, it has been found in accordance with the present invention that an animal model of Alzheimer's disease has higher frequencies of Foxp3 out of CD4 cells as compared with wild-type mice. However, even if the levels of systemic Treg cells is not elevated, their functional activity is not enhanced, the level of IFN γ -producing leukocytes is not reduced or the proliferation of leukocytes in response to stimulation is not decreased, in said individual, the method of the present invention that reduces the level or activity of systemic immunosuppression is effective in treating disease, disorder, condition or injury of the CNS that does not include the autoimmune neuroinflammatory disease RRMS. Importantly, said systemic immune suppression can also involve additional immune cell types except of Tregs, such as myeloid-derived suppressor cells (MDSCs) (Gabrilovich & Nagaraj, 2009).

The level of systemic immunosuppression may be detected by various methods that are well known to those of ordinary skill in the art. For example, the level of Tregs may be measured by flow cytometry analysis of peripheral blood mononuclear cells or T lymphocytes, immunostained either for cellular surface markers or nuclear intracellular markers of Treg (Chen & Oppenheim, 2011), CD45, TCR- β , or CD4 markers of lymphocytes, and measuring the amount of antibody specifically bound to the cells. The functional activity of Tregs may be measured by various assays; For example the thymidine incorporation assay is being commonly used, in which suppression of anti-CD3 mAb stimulated proliferation of CD4⁺CD25⁻ T cells (conventional T cells) is measured by [³H]thymidine incorporation or by using CFSE (5-(and 6)-carboxyfluorescein diacetate succinimidyl ester, which is capable of entering the cells; cell division is measured as successive halving of the fluorescence intensity of CFSE). The number of IFN γ -producing leukocytes or their activity or their proliferation capacity can easily be assessed by a skilled artisan using methods known in the art; For example, the level of IFN γ -producing leukocytes may be measured by flow cytometry analysis of peripheral blood mononuclear cells, following short *ex-vivo* stimulation and golgi-stop, and immunostaining by IFN γ intracellular staining (using e.g., BD Biosciences Cytofix/cytopermTM fixation/permeabilization kit), by collecting the condition media of these cells and quantifying the level of secreted cytokines using ELISA, or by comparing the ratio of different cytokines in the condition media, for example IL2/IL10, IL2/IL4, INF γ /TGF β , etc. The levels of MDSCs in the human peripheral blood easily can be assessed by a skilled artisan, for example by using flow cytometry analysis of frequency of DR⁻/LIN⁻/CD11b⁺, DR⁻/LIN⁻/CD15⁺, DR⁻/LIN⁻/CD33⁺ and DR(-/low)/CD14⁺ cells, as described (Kotsakis et al, 2012).

In humans, the peripheral/systemic immunosuppression may be considered elevated when the total number of Tregs in the circulation is higher than 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% or more than in a healthy control population, the percentage of Treg cells out of the total CD4+ cells is elevated by 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% or more than in a healthy control population, or the functional activity of Tregs is elevated by 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% or more than in a healthy control population. Alternatively, the peripheral/systemic immunosuppression may be considered elevated when the level of IFN γ -producing leukocytes or their activity is reduced relative to that of a healthy control population by 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100%; or the proliferation of leukocytes in response to stimulation is reduced relative to that of a healthy control population by 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100%.

An agent may be considered an agent that causes reduction of the level of systemic immunosuppression when, upon administration of the agent to an individual, the total number of Tregs in the circulation of this individual is reduced by 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100% as compared with the level before administration of the agent, the percentage of Treg cells out of the total CD4+ cells drops by 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100% relative to that of a healthy control population or the functional activity of Tregs is reduced by 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100% as compared with the level before administration of the agent. Alternatively, an agent may be considered an agent that causes reduction of the level of systemic immunosuppression when, upon administration of the agent to an individual, the total number of IFN γ -producing leukocytes or their activity is increased by 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% or more; or the proliferation of leukocytes in response to stimulation is increased relative to that of a healthy control population by 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100% or more.

In certain embodiments, the active agent causes reduction of the level of systemic immunosuppression by release of a restraint imposed on the immune system by one or more immune checkpoints, for example by blockade of the one or more immune checkpoints.

In certain embodiments, the reduction of the level of systemic immunosuppression is associated with an increase in systemic presence or activity of IFN γ -producing leukocytes.

In certain embodiments, the active agent causes reduction of the level of systemic immunosuppression and thereby an increase in the systemic presence or activity of effector T cells.

The checkpoints that may be manipulated to release the systemic immunosuppression are referred to herein as a pair of an immune checkpoint receptor and its native ligand or either one of the two partners. For example, PD1, which has two known ligands is referred to herein as

"PD1-PDL1" or "PD1-PDL2", while B7H3, the ligand of which has not yet been identified, is referred to simply by "B7H3".

In certain embodiments, the active agent that may be used according to the present invention may be selected from the group consisting of (i) an antibody, such as a humanized antibody; a human antibody; a functional fragment of an antibody; a single-domain antibody, such as a Nanobody; a recombinant antibody; and a single chain variable fragment (ScFv) (ii) an antibody mimetic, such as an affibody molecule; an affilin; an affimer; an affitin; an alphabody; an anticalin; an avimer; a DARPin; a fynomer; a Kunitz domain peptide; and a monobody; (iii) an aptamer; and (iv) a small molecule inhibitor.

Non-limiting examples of antibody mimetics are presented in Table 1:

Table 1. Antibody mimetics

Antibody mimetic*	Scaffold	Molar mass	Example drug
Affibody molecules ^[1]	Z domain of Protein A	6 kDa	ABY-025
Affilins ^[2]	Gamma-B crystallin	20 Da	
	Ubiquitin	10 Da	SPVF 2801
Affimers ^[3]	Cystatin	12–14 kDa	
Affitins ^[4]	Sac7d (from <i>Sulfolobus acidocaldarius</i>)	7 kDa	
Alphabodies ^[5]	Triple helix coiled coil	10 Da	CMPX-1023
Anticalins ^[6]	Lipocalins	20 Da	
Avimers ^[7]	A domains of various membrane receptors	9–18 kDa	
DARPin ^[8]	Ankyrin repeat motif	10–19 kDa	MP0112
Fynomers ^[9]	SH3 domain of Fyn	7 kDa	
Kunitz domain peptides ^[10]	Kunitz domains of various protease inhibitors	6 kDa	Ecaltantide (Kalbitor)
Monobodies ^[11]	10 th type III domain of fibronectin	10 kDa	Pegdinetanib (Angiocept)

*According to Wikipedia

[1]Nygren PA (June 2008); [2] Ebersbach et al. (2007); [3] Johnson et al. (2012); [4] Krehenbrink et al. (2008); [5] Desmet et al. (2014); [6] Skerra A (2008); [7] Silverman et al. (2005); [8] Stumpp et al. (2008)' [9] Grabulovski et al. (2007); [10] Nixon et al. (2006); [11] Koide et al. (2007).

Aptamers are oligonucleotide or peptide molecules that bind to a specific target molecule.

In certain embodiments, the active agent that may be used according to the present invention may be selected from the group consisting of:

(i) an antibody selected from the group consisting of: (a) anti-ICOS; (b) anti-B7RP1; (c) anti-VISTA; (d) anti-CD40; (e) anti-CD40L; (f) anti-CD80; (g) anti-CD86; (h) anti-B7-H3; (i) anti-B7-H4; (j) B7-H7; (k) anti-BTLA; (l) anti-HVEM; (m) anti-CD137; (n) anti-CD137L; (o) anti-OX40L; (p) anti-CD-27; (q) anti-CD70; (r) anti-STING; (s) anti-TIGIT; and (t) any combination of (a) to (s);

- (ii) any combination of (a) to (s) in combination with an adjuvant;
- (iii) a small molecule selected from the group consisting of: (a) an adenosine A1 receptor antagonist; (b) an adenosine A2a receptor; and (c) an A3 receptor antagonist;
- (iv) any combination of (iii) (a-c) and (i) (a-s); and
- (v) any combination of (i) to (iv).

In certain embodiments, the antibody described above may be administered to a human at a dosage of for example about 0.1 mg/kg - 20 mg/kg, 0.1 mg/kg - 15 mg/kg, 0.1 mg/kg - 10 mg/kg, 0.1 mg/kg - 5 mg/kg, 0.2 mg/kg - 20 mg/kg, 0.2 mg/kg - 15 mg/kg, 0.2 mg/kg - 10 mg/kg, 0.2 mg/kg - 6 mg/kg, 0.2 mg/kg - 5 mg/kg, 0.3 mg/kg - 20 mg/kg, 0.3 mg/kg - 15 mg/kg, 0.3 mg/kg - 10 mg/kg, 0.3 mg/kg - 5 mg/kg, , 1 mg/kg - 20 mg/kg, 1 mg/kg - 15 mg/kg, 1 mg/kg - 10 mg/kg, 1 mg/kg - 5 mg/kg, 1.5 mg/kg - 20 mg/kg, 1.5 mg/kg - 15 mg/kg, 1.5 mg/kg - 10 mg/kg, 1.5 mg/kg - 6 mg/kg or 1.5 mg/kg - 5 mg/kg.

In certain embodiments, the adenosine A1 receptor antagonist may be PSB 36 (1-Butyl-8-(hexahydro-2,5-methanopentalen-3a(1H)-yl)-3,7-dihydro-3-(3-hydroxypropyl)-1H-purine-2,6-dione); the adenosine A2a receptor antagonist may be SCH58261 (5-Amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo(4,3-e)-1,2,4-triazolo(1,5-c)pyrimidine), SYN115 (4-Hydroxy-N-[4-methoxy-7-(4-morpholinyl)-2-benzothiazolyl]-4-methyl-1-piperidinecarboxamide), FSPTP (also called SCH58261 (5-amino-7-[2-(4-fluorosulfonyl)phenylethyl]-2-(2-furyl)-pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine), SCH442416 (2-(2-Furanyl)-7-[3-(4-methoxyphenyl)propyl]-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-5-amine), or ZM241385 (also called tozadenant (4-Hydroxy-N-(4-methoxy-7-morpholinobenzo[d]thiazol-2-yl)-4-methylpiperidine-1-carboxamide); and the A3 receptor antagonist may be MRS3777 (2-Phenoxy-6-(cyclohexylamino)purine hemioxalate).

As stated above, the active agent is administered by a dosage regime comprising at least two courses of therapy, each course of therapy comprising in sequence a treatment session followed by an interval session of non-treatment. The dosage regime may be determined in a number of ways. For example, the level of immunosuppression may be calibrated to a desired level for each patient who is being treated (personalized medicine), by monitoring the level or activity of IFN- γ -producing leukocytes or proliferation rate of leukocytes in response to stimulation individually, and adjusting the treatment session, the frequency of administration and the interval session empirically and personally as determined from the results of the monitoring.

Thus, the treatment session may comprise administering the active agent or pharmaceutical composition to the individual and the treatment session is maintained at least until the systemic presence or level of IFN- γ -producing leukocytes, or the rate of proliferation of

leukocytes in response to stimulation rises above a reference, the administering is paused during the interval session, and the interval session is maintained as long as the level is above the reference, wherein the reference is selected from (a) the level of systemic presence or activity of IFN- γ -producing leukocytes, or the rate of proliferation of leukocytes in response to stimulation, measured in the most recent blood sample obtained from said individual before said administering; or (b) the level of systemic presence or activity of IFN- γ -producing leukocytes, or the rate of proliferation of leukocytes in response to stimulation, characteristic of a population of individuals afflicted with a disease, disorder, condition or injury of the CNS.

The length of the treatment and interval sessions may be determined by physicians in clinical trials directed to a certain patient population and then applied consistently to this patient population, without the need for monitoring the level of immunosuppression on a personal basis.

In certain embodiments, the treatment session comprises administering the active agent to the individual and the treatment session is maintained at least until the systemic presence of the active agent reaches therapeutic levels, the administering is paused during the interval session, and the interval session is maintained as long as the level is above about 95%, 90%, 80%, 70%, 60% or 50% of said therapeutic level. The term "therapeutic level" as used herein refers to generally accepted systemic levels of drugs used to block immune checkpoints in known therapies, such as cancer therapy (see above).

In certain embodiments, the treatment session may be a single administration or it may comprise multiple administrations given in the course of between 1 day and four weeks, for example 1 day, 2 days or 3 days or between one and four weeks. For example, the treatment session may comprise two administrations both given within one week, such as, *e.g.*, the second administration given 1, 2, 3, 4, 5 or 6 days after the first administration. As another example, the treatment session may comprise three administrations all given within one week such as, *e.g.*, given 1, 2 or 3 days after the preceding administration. As another example, the treatment session may comprise three administrations all given within two week such as, *e.g.*, given 1, 2, 3, 4 or 5 days after the preceding administration. As another example, the treatment session may comprise four administrations all given within two week such as, *e.g.*, given 1, 2, 3 or 4 days after the preceding administration. As another example, the treatment session may comprise four administrations all given within three week such as, *e.g.*, given 1, 2, 3, 4, 5 or 6 days after the preceding administration. As another example, the treatment session may comprise five administrations all given within three week such as, *e.g.*, given 1, 2, 3, 4 or 5 days after the preceding administration.

In certain embodiments, the interval session of non-treatment may be between one week and six months, for example between two to four weeks, between three to four weeks, between two weeks and six months long, between 3 weeks and six months long and in particular 2, 3 or 4 weeks long. In certain embodiments, the interval session of non-treatment may be 1 to 2 months in length, 1 to 3 months in length or 2 to 3 months in length.

In the treatments session, the administration of the active agent or pharmaceutical composition may be a single administration or repeated administration, for example the active agent or pharmaceutical composition may be administered only once and then immediately followed by an interval, or it may be administered daily, or once every two, three, four, five or six days, or once weekly, once every two weeks, once every three weeks or once every four weeks. These frequencies are applicable to any active agent, may be based on commonly used practices in the art, and may finally be determined by physicians in clinical trials. Alternatively, the frequency of the repeated administration in the treatment session could be adapted according to the nature of the active agent, wherein for example, a small molecule may be administered daily and an antibody may be administered once every 3 days. It should be understood that when an agent is administered during a treatment session at a relatively low frequency, for example once per week during a treatment session of one month, or once per month during a treatment session of six months, this treatment session is followed by a non-treatment interval session, the length of which is longer than the period between the repeated administrations during the treatment session (i.e. longer than one week or one month, respectively, in this example). The pause of one week or one month between the administrations during the treatment session in this example is not considered an interval session.

The lengths of the treatment session and the interval session may be adjusted to the frequency of the administration such that, for example, a frequency of administering the active agent once every 3 days may result in a treatment session of 6 or 9 days and an interval session that is commenced accordingly.

If the treatment session consists of a single administration, the dosage regimen is determined by the length of the interval, so that a single administration is followed by an interval of 7, 8, 9, 10, 14, 18, 21, 24 or 28 days or longer before the next single-administration treatment session. In particular, the dosage regimen consists of single administrations interspersed with intervals of non-treatment of 2, 3 or 4 weeks. In addition, the dosage regimen may consist of single administrations interspersed with intervals of non-treatment of 2 to 4 weeks, 2 to 3 weeks or 3 to 4 weeks.

If the treatment session consists of a multiple administrations, the dosage regimen is determined by the length of the interval, so that multiple administrations given within one week is followed by an interval of 7, 10, 14, 18, 21, 24 or 28 days or longer before the next multiple-administration treatment session. In particular, the dosage regimen may consist of multiple administrations given within one week interspersed with intervals of non-treatment of 2 or 3 or 4 weeks. In addition, the dosage regimen may consist of multiple administrations given within one week interspersed with intervals of non-treatment of 2 to 4 weeks, 2 to 3 weeks or 3 to 4 weeks.

As another example, the dosage regimen may comprise multiple administrations given within two weeks followed by an interval of 2 weeks, 3 weeks or 1, 2, 3 or 4 months or longer before the next multiple-administration treatment session. In particular, the dosage regimen may consist of multiple administrations given within two weeks interspersed with intervals of non-treatment of 1, 2, 3 or 4 months. In addition, the dosage regimen may consists of multiple administrations given within two week interspersed with intervals of non-treatment of 1 to 2 months, 1 to 3 months, 1 to 4 months, 2 to 3 months, 2 to 4 months or 3 to 4 months.

As another example, the dosage regimen may comprise multiple administrations given within three week followed by 1, 2, 3, 4, 5 or 6 months or longer before the next multiple-administration treatment session. In particular, the dosage regimen may consist of multiple administrations given within three weeks interspersed with intervals of non-treatment of 1, 2, 3, 4, 5 or 6 months. In addition, the dosage regimen may consists of multiple administrations given within three weeks interspersed with intervals of non-treatment of 1 to 2 months, 1 to 3 months, 1 to 4 months, 1 to 5 months, 1 to 6 months, 2 to 3 months, 2 to 4 months, 2 to 5 months, 2 to 6 months, 3 to 4 months, 3 to 5 months, 3 to 6 months, 4 to 5 months, 4 to 6 months or 5 to 6 months.

Of course, a flexible dosage regimen is envisioned that starts with a certain regimen and is replaced with another. For example, treatment sessions, each one including 2 single administrations 3 days apart, with an interval of for example 1 week between the treatment sessions, could be replaced when considered appropriate by a dosage regimen including treatment sessions of single administrations separated by for example 2, 3 or 4 weeks intervals. As another example, treatment sessions, each one including 2 single administrations 7 days apart, with an interval of for example 2 weeks between the treatment sessions, could be replaced when considered appropriate by a dosage regimen including treatment sessions of single administrations separated by for example 2, 3, 4, 5 or 6 weeks intervals. As another example, treatment sessions, each one including 3 single administrations 3 days apart, with an interval of for example 2 weeks between the treatment sessions, could be replaced when considered

appropriate by a dosage regimen including treatment sessions of single administrations separated by for example 2, 3, 4, 5 or 6 weeks intervals.

In any case, the dosage regimen, i.e. the length of the treatment session and the interval session, is calibrated such that the reduction in the level of immunosuppression, for example as measured by a reduction in the level of systemic presence or activity of regulatory T cells or the increase in the level of systemic presence or activity of IFN- γ producing leukocytes in the individual, is transient.

The method, active agent or pharmaceutical composition according to the present invention may be for treating a disease, disorder or condition of the CNS that is a neurodegenerative disease, disorder or condition selected from Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease Huntington's disease, primary progressive multiple sclerosis; secondary progressive multiple sclerosis, corticobasal degeneration, Rett syndrome, a retinal degeneration disorder selected from the group consisting of age-related macular degeneration and retinitis pigmentosa; anterior ischemic optic neuropathy; glaucoma; uveitis; depression; trauma-associated stress or post-traumatic stress disorder, frontotemporal dementia, Lewy body dementias, mild cognitive impairments, posterior cortical atrophy, primary progressive aphasia or progressive supranuclear palsy. In certain embodiments, the condition of the CNS is aged-related dementia.

In certain embodiments, the condition of the CNS is Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease Huntington's disease.

In certain embodiments, each one of the active agents described above that blocks one of the immune checkpoints selected from ICOS-B7RP1, V-domain Ig suppressor of T cell activation (VISTA), B7-CD28-like molecule, CD40L-CD40, CD28-CD80, CD28-CD86, B7H3, B7H4, B7H7, BTLA-HVEM, CD137-CD137L, OX40L, CD27-CD70, STING, TIGIT and A2aR-Adenosine and indoleamine-2,3-dioxygenase (IDO)-L-tryptophan, such as an antibody against one of the two partners of the immune checkpoint, is for use in treating either one of a neurodegenerative disease, disorder or condition selected from Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease Huntington's disease, primary progressive multiple sclerosis; secondary progressive multiple sclerosis, corticobasal degeneration, Rett syndrome, a retinal degeneration disorder selected from the group consisting of age-related macular degeneration and retinitis pigmentosa; anterior ischemic optic neuropathy; glaucoma; uveitis; depression; trauma-associated stress or post-traumatic stress disorder, frontotemporal dementia, Lewy body dementias, mild cognitive impairments, posterior cortical atrophy, primary progressive aphasia or progressive supranuclear palsy. The treatment of any one of these

diseases comprising the use of any one of these active agents can be done according to the regiment described above.

The method, active agent and pharmaceutical composition according to the present invention may further be for treating an injury of the CNS selected from spinal cord injury, closed head injury, blunt trauma, penetrating trauma, hemorrhagic stroke, ischemic stroke, cerebral ischemia, optic nerve injury, myocardial infarction, organophosphate poisoning and injury caused by tumor excision.

In certain embodiments, each one of the active agents described above that blocks one of the immune checkpoints selected from ICOS-B7RP1, V-domain Ig suppressor of T cell activation (VISTA), B7-CD28-like molecule, CD40L-CD40, CD28-CD80, CD28-CD86, B7H3, B7H4, B7H7, BTLA-HVEM, CD137-CD137L, OX40L, CD27-CD70, STING, TIGIT and A2aR-Adenosine and indoleamine-2,3-dioxygenase (IDO)-L-tryptophan, such as an antibody against one of the two partners of the immune checkpoint, is for use in treating an injury of the CNS selected from spinal cord injury, closed head injury, blunt trauma, penetrating trauma, hemorrhagic stroke, ischemic stroke, cerebral ischemia, optic nerve injury, myocardial infarction, organophosphate poisoning and injury caused by tumor excision. The treatment of any one of these injuries comprising the use of any one of these active agents can be done according to the regiment described above.

As stated above, the inventors have found that the present invention improves the cognitive function in mice that emulates Alzheimer's disease. Thus, the method, active agent and pharmaceutical composition may be for use in improving CNS motor and/or cognitive function, for example for use in alleviating age-associated loss of cognitive function, which may occur in individuals free of a diagnosed disease, as well as in people suffering from neurodegenerative disease. Furthermore, the method, active agent and pharmaceutical composition may be for use in alleviating loss of cognitive function resulting from acute stress or traumatic episode. The cognitive function mentioned herein above may comprise learning, memory or both.

It should be emphasized, that the improvement of cognitive function in mice that emulates Alzheimer's disease (5XFAD AD-Tg mice) were observed and characterized by the inventors in various stages of disease manifestation; both early and late progressive stages of disease pathology could be mitigated by the treatment. 5XFAD AD-Tg mice begin to display cerebral plaque pathology at the ages of 2.5 months and cognitive deficits at the ages of 5 months (Oakley et al, 2006). Of note, while in Example 2 below the inventors describe the therapeutic effect in 5XFAD mice at 6 months of age, in Example 5 they characterize the therapeutic effect in 5XFAD mice at 11 and 12 months of age – an extremely progressive stage

of amyloid beta plaque deposition and cognitive deficits in this model. It is therefore expected that the proposed invention would be of relevance to patients of different stages of disease progression, such as Stage 1 – Mild/Early (lasts 2-4 years); Stage 2 – Moderate/Middle (lasts 2-10 years); and Stage 3 – Severe/Late (lasts 1-3+ years).

5 The term "CNS function" as used herein refers, *inter alia*, to receiving and processing sensory information, thinking, learning, memorizing, perceiving, producing and understanding language, controlling motor function and auditory and visual responses, maintaining balance and equilibrium, movement coordination, the conduction of sensory information and controlling such autonomic functions as breathing, heart rate, and digestion.

10 The terms "cognition", "cognitive function" and "cognitive performance" are used herein interchangeably and are related to any mental process or state that involves but is not limited to learning, memory, creation of imagery, thinking, awareness, reasoning, spatial ability, speech and language skills, language acquisition and capacity for judgment attention. Cognition is formed in multiple areas of the brain such as hippocampus, cortex and other brain structures.
15 However, it is assumed that long term memories are stored at least in part in the cortex and it is known that sensory information is acquired, consolidated and retrieved by a specific cortical structure, the gustatory cortex, which resides within the insular cortex.

 In humans, cognitive function may be measured by any know method, for example and without limitation, by the clinical global impression of change scale (CIBIC-plus scale); the Mini
20 Mental State Exam (MMSE); the Neuropsychiatric Inventory (NPI); the Clinical Dementia Rating Scale (CDR); the Cambridge Neuropsychological Test Automated Battery (CANTAB) or the Sandoz Clinical Assessment-Geriatric (SCAG). Cognitive function may also be measured indirectly using imaging techniques such as Positron Emission Tomography (PET), functional magnetic resonance imaging (fMRI), Single Photon Emission Computed Tomography (SPECT),
25 or any other imaging technique that allows one to measure brain function.

 An improvement of one or more of the processes affecting the cognition in a patient will signify an improvement of the cognitive function in said patient, thus in certain embodiments improving cognition comprises improving learning, plasticity, and/or long term memory. The terms "improving" and "enhancing" may be used interchangeably.

30 The term "learning" relates to acquiring or gaining new, or modifying and reinforcing, existing knowledge, behaviors, skills, values, or preferences.

 The term "plasticity" relates to synaptic plasticity, brain plasticity or neuroplasticity associated with the ability of the brain to change with learning, and to change the already acquired memory. One measurable parameter reflecting plasticity is memory extinction.

The term "memory" relates to the process in which information is encoded, stored, and retrieved. Memory has three distinguishable categories: sensory memory, short-term memory, and long-term memory.

5 The term "long term memory" is the ability to keep information for a long or unlimited period of time. Long term memory comprises two major divisions: explicit memory (declarative memory) and implicit memory (non-declarative memory). Long term memory is achieved by memory consolidation which is a category of processes that stabilize a memory trace after its initial acquisition. Consolidation is distinguished into two specific processes, synaptic consolidation, which occurs within the first few hours after learning, and system consolidation,
10 where hippocampus-dependent memories become independent of the hippocampus over a period of weeks to years.

The embodiments above that describe different features of the pharmaceutical composition of the present invention are relevant also for the method of the invention, because the method employs the same pharmaceutical composition.

15 In yet another aspect, the present invention provides methods for reducing A β -plaque burden in a patient diagnosed with Alzheimer's disease, comprising administering to said patient an active agent or pharmaceutical composition as defined herein above that causes reduction of the level of systemic immunosuppression by release of a restraint imposed on the immune system by one or more immune checkpoints.

20 In still another aspect, the present invention provides a method for reducing hippocampal gliosis in a patient diagnosed with Alzheimer's disease, comprising administering to said patient an active agent or pharmaceutical composition as defined herein above that causes reduction of the level of systemic immunosuppression by release of a restraint imposed on the immune system by one or more immune checkpoints.

25 Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof.

The following exemplification of carriers, modes of administration, dosage forms, etc.,
30 are listed as known possibilities from which the carriers, modes of administration, dosage forms, etc., may be selected for use with the present invention. Those of ordinary skill in the art will understand, however, that any given formulation and mode of administration selected should first be tested to determine that it achieves the desired results.

Methods of administration include, but are not limited to, parenteral, e.g., intravenous, intraperitoneal, intramuscular, subcutaneous, mucosal (e.g., oral, intranasal, buccal, vaginal, rectal, intraocular), intrathecal, topical and intradermal routes. Administration can be systemic or local.

5 The term “carrier” refers to a diluent, adjuvant, excipient, or vehicle with which the active agent is administered. The carriers in the pharmaceutical composition may comprise a binder, such as microcrystalline cellulose, polyvinylpyrrolidone (polyvidone or povidone), gum tragacanth, gelatin, starch, lactose or lactose monohydrate; a disintegrating agent, such as alginic acid, maize starch and the like; a lubricant or surfactant, such as magnesium stearate, or sodium
10 lauryl sulphate; and a glidant, such as colloidal silicon dioxide.

For oral administration, the pharmaceutical preparation may be in liquid form, for example, solutions, syrups or suspensions, or may be presented as a drug product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending
15 agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding
20 agents (e.g., pregelatinized maize starch, polyvinyl pyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well-known in the art.

25 Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

The compositions may be formulated for parenteral administration by injection, e.g., by
30 bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing

agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen free water, before use.

The compositions may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

For administration by inhalation, the compositions for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin, for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The determination of the doses of the active ingredient to be used for human use is based on commonly used practices in the art, and will be finally determined by physicians in clinical trials. An expected approximate equivalent dose for administration to a human can be calculated based on the *in vivo* experimental evidence disclosed herein below, using known formulas (e.g. Reagan-Show et al. (2007) Dose translation from animal to human studies revisited. The FASEB Journal 22:659-661). According to this paradigm, the adult human equivalent dose (mg/kg body weight) equals a dose given to a mouse (mg/kg body weight) multiplied with 0.081.

The invention will now be illustrated by the following non-limiting examples.

EXAMPLES

Materials and Methods

Animals. 5XFAD transgenic mice (Tg6799) that co-overexpress familial AD mutant forms of human APP (the Swedish mutation, K670N/M671L; the Florida mutation, I716V; and the London mutation, V717I) and PS1 (M146L/L286V) transgenes under transcriptional control of the neuron-specific mouse Thy-1 promoter (Oakley et al, 2006), and AD double transgenic B6.Cg-Tg (APP^{swe}, PSEN1^{dE9}) 85Dbo/J mice (Borchelt et al, 1997) were purchased from The Jackson Laboratory. Genotyping was performed by PCR analysis of tail DNA, as previously described (Oakley et al, 2006). Heterozygous mutant *Cx3cr1*^{GFP/+} mice (Jung et al, 2000) (B6.129P-*Cx3cr1*^{tm1Litt}/J, in which one of the CX₃CR1 chemokine receptor alleles was replaced with a gene encoding GFP) were used as donors for BM chimeras. Foxp3.LuciDTR mice (Suffner et al, 2010) were bred with 5XFAD mice to enable conditional depletion of Foxp3⁺

Tregs. Animals were bred and maintained by the Animal Breeding Center of the Weizmann Institute of Science. All experiments detailed herein complied with the regulations formulated by the Institutional Animal Care and Use Committee (IACUC) of the Weizmann Institute of Science.

5 **RNA purification, cDNA synthesis, and quantitative real-time PCR analysis.** Total RNA of the hippocampal dentate gyrus (DG) was extracted with TRI Reagent (Molecular Research Center) and purified from the lysates using an RNeasy Kit (Qiagen). Total RNA of the choroid plexus was extracted using an RNA MicroPrep Kit (Zymo Research). mRNA (1 µg) was converted into cDNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The expression of specific mRNAs was assayed using fluorescence-based quantitative real-time PCR (RT-qPCR). RT-qPCR reactions were performed using Fast-SYBR PCR Master Mix (Applied Biosystems). Quantification reactions were performed in triplicate for each sample using the standard curve method. Peptidylprolyl isomerase A (*ppia*) was chosen as a reference (housekeeping) gene. The amplification cycles were 95°C for 5 s, 60°C for 20 s, and 15 72°C for 15 s. At the end of the assay, a melting curve was constructed to evaluate the specificity of the reaction. For *ifn-γ* and *ppia* gene analysis, the cDNA was pre-amplified in 14 PCR cycles with non-random PCR primers, thereby increasing the sensitivity of the subsequent real-time PCR analysis, according to the manufacturer's protocol (PreAmp Master Mix Kit; Applied Biosystems). mRNA expression was determined using TaqMan RT-qPCR, according to the 20 manufacturer's instructions (Applied Biosystems). All RT-qPCR reactions were performed and analyzed using StepOne software V2.2.2 (Applied Biosystems). The following TaqMan Assays-on-Demand™ probes were used: Mm02342430_g1 (*ppia*) and Mm01168134_m1 (*ifn-γ*).

For all other genes examined, the following primers were used:

25 *ppia* forward 5'-AGCATAACAGGTCCTGGCATCTTGT-3' (SEQ ID NO: 33) and reverse 5'-CAAAGACCACATGCTTGCCATCCA-3' (SEQ ID NO: 34);
icam1 forward 5'-AGATCACATTCACGGTGCTGGCTA-3' (SEQ ID NO: 35) and reverse 5'-AGCTTTGGGATGGTAGCTGGAAGA-3' (SEQ ID NO: 36);
vcam1 forward 5'-TGTGAAGGGATTAACGAGGCTGGA-3' (SEQ ID NO: 37) and reverse 5'-CCATGTTTCGGGCACATTTCCACA-3' (SEQ ID NO: 38);
30 *cxcl10* forward 5'-AACTGCATCCATATCGATGAC-3' (SEQ ID NO: 39) and reverse 5'-GTGGCAATGATCTCAACAC-3' (SEQ ID NO: 40);
ccl2 forward 5'-CATCCACGTGTTGGCTCA-3' (SEQ ID NO: 41) and reverse 5'-GATCATCTTGCTGGTGAATGAGT-3' (SEQ ID NO: 42);

tnf-γ forward 5'-GCCTCTTCTCATTCTGCTT-3' (SEQ ID NO: 43) reverse CTCCTCCACTTGGTGGTTTG-3' (SEQ ID NO: 44);

il-1β forward 5'-CCAAAAGATGAAGGGCTGCTT-3' (SEQ ID NO: 45) and reverse 5'-TGCTGCTGCGAGATTTGAAG-3' (SEQ ID NO: 46);

5 *il-12p40* forward 5'-GAAGTTCAACATCAAGAGCA-3' (SEQ ID NO: 47) and reverse 5'-CATAGTCCCTTTGGTCCAG-3' (SEQ ID NO: 48);

il-10 forward 5'-TGAATTCCCTGGGTGAGAAGCTGA-3' (SEQ ID NO: 49) and reverse 5'-TGGCCTTG TAGACACCTTGGTCTT-3' (SEQ ID NO: 50);

10 *tgfb2* forward 5'-AATTGCTGCCTTCGCCCTCTTTAC-3' (SEQ ID NO: 51) and reverse 5'-TGTACAGGCTGAGGACTTTGGTGT-3' (SEQ ID NO: 52);

igf-1 forward 5'-CCGGACCAGAGACCCTTTG (SEQ ID NO: 53) and reverse 5'-CCTGTGGGCTTGTTGAAGTAAAA-3' (SEQ ID NO: 54);

bdnf forward 5'-GATGCTCAGCAGTCAAGTGCCTTT-3' (SEQ ID NO: 55) and reverse 5'-GACATGTTTGCGGCATCCAGGTAA-3' (SEQ ID NO: 56);

15 **Immunohistochemistry.** Tissue processing and immunohistochemistry were performed on paraffin embedded sectioned mouse (6 μm thick) and human (10 μm thick) brains. For human ICAM-1 staining, primary mouse anti-ICAM (1:20 Abcam; ab2213) antibody was used. Slides were incubated for 10 min with 3% H₂O₂, and a secondary biotin-conjugated anti-mouse antibody was used, followed by biotin/avidin amplification with Vectastain ABC kit (Vector
20 Laboratories). Subsequently, 3,3'-diaminobenzidine (DAB substrate) (Zytomed kit) was applied; slides were dehydrated and mounted with xylene-based mounting solution. For tissue stainings, mice were transcardially perfused with PBS prior to tissue excision and fixation. CP tissues were isolated under a dissecting microscope (Stemi DV4; Zeiss) from the lateral, third, and fourth ventricles of the brain. For whole mount CP staining, tissues were fixated with 2.5%
25 paraformaldehyde (PFA) for 1 hour at 4°C, and subsequently transferred to PBS containing 0.05% sodium azide. Prior to staining, the dissected tissues were washed with PBS and blocked (20% horse serum, 0.3% Triton X-100, and PBS) for 1h at room temperature. Whole mount staining with primary antibodies (in PBS containing 2% horse serum and 0.3% Triton X-100), or secondary antibodies, was performed for 1h at room temperature. Each step was followed by
30 three washes in PBS. The tissues were applied to slides, mounted with Immu-mount (9990402, from Thermo Scientific), and sealed with cover-slips. For staining of sectioned brains, two different tissue preparation protocols (paraffin embedded or microtomed free-floating sections) were applied, as previously described (Baruch et al, 2013; Kunis et al, 2013). The following primary antibodies were used: mouse anti-Aβ (1:300, Covance, #SIG-39320); rabbit anti-GFP

(1:100, MBL, #598); rat anti-CD68 (1:300, eBioscience, #14-0681); rat anti-ICAM-1 (1:200, Abcam, #AB2213); goat anti-GFP (1:100, Abcam, #ab6658); rabbit anti-IBA-1 (1:300, Wako, #019-19741); goat anti-IL-10 (1:20, R&D systems, #AF519); rat anti-Foxp3 (1:20, eBioscience, #13-5773-80); rabbit anti-CD3 (1:500, Dako, #IS503); mouse anti-ZO-1, mouse anti-E-Cadherin, and rabbit anti-Claudin-1 (all 1:100, Invitrogen, #33-9100, #33-4000, #51-9000); rabbit anti-GFAP (1:200, Dako, #Z0334). Secondary antibodies included: Cy2/Cy3/Cy5-conjugated donkey anti-mouse/goat/rabbit/rat antibodies (1:200; all from Jackson ImmunoResearch). The slides were exposed to Hoechst nuclear staining (1:4000; Invitrogen Probes) for 1 min. Two negative controls were routinely used in immunostaining procedures, staining with isotype control antibody followed by secondary antibody, or staining with secondary antibody alone. For Foxp3 intracellular staining, antigen retrieval from paraffin-embedded slides was performed using Retrieval Kit (#550524, #550527; BD Pharmingen™). Microscopic analysis, was performed using a fluorescence microscope (E800; Nikon) or laser-scanning confocal microscope (Carl Zeiss, Inc.). The fluorescence microscope was equipped with a digital camera (DXM 1200F; Nikon), and with either a 20× NA 0.50 or 40× NA 0.75 objective lens (Plan Fluor; Nikon). The confocal microscope was equipped with LSM 510 laser scanning capacity (three lasers: Ar 488, HeNe 543, and HeNe 633). Recordings were made on postfixed tissues using acquisition software (NIS-Elements, F3 [Nikon] or LSM [Carl Zeiss, Inc.]). For quantification of staining intensity, total cell and background staining was measured using ImageJ software (NIH), and intensity of specific staining was calculated, as previously described (Burgess et al, 2010). Images were cropped, merged, and optimized using Photoshop CS6 13.0 (Adobe), and were arranged using Illustrator CS5 15.1 (Adobe).

Paraffin embedded sections of human CP. Human brain sections of young and aged postmortem non-CNS-disease individuals, as well as AD patients, were obtained from the Oxford Brain Bank (formerly known as the Thomas Willis Oxford Brain Collection (TWBC)) with appropriate consent and Ethics Committee approval (TW220). The experiments involving these sections were approved by the Weizmann Institute of Science Bioethics Committee.

Flow cytometry, sample preparation and analysis. Mice were transcardially perfused with PBS, and tissues were treated as previously described (Baruch et al, 2013). Brains were dissected and the different brain regions were removed under a dissecting microscope (Stemi DV4; Zeiss) in PBS, and tissues were dissociated using the gentleMACS™ dissociator (Miltenyi Biotec). Choroid plexus tissues were isolated from the lateral, third and fourth ventricles of the brain, incubated at 37°C for 45 min in PBS (with $\text{Ca}^{2+}/\text{Mg}^{2+}$) containing 400 U/ml collagenase type IV (Worthington Biochemical Corporation), and then manually homogenized by pipetting.

Spleens were mashed with the plunger of a syringe and treated with ACK (ammonium chloride potassium) lysing buffer to remove erythrocytes. In all cases, samples were stained according to the manufacturers' protocols. All samples were filtered through a 70µm nylon mesh, and blocked with anti-Fc CD16/32 (1:100; BD Biosciences). For intracellular staining of IFN-γ, the cells were incubated with para-methoxyamphetamine (10 ng/ml; Sigma-Aldrich) and ionomycin (250 ng/ml; Sigma-Aldrich) for 6h, and Brefeldin-A (10 µg/ml; Sigma-Aldrich) was added for the last 4h. Intracellular labeling of cytokines was done with BD Cytofix/Cytoperm™ Plus fixation/permeabilization kit (cat. no. 555028). For Treg staining, an eBioscience FoxP3 staining buffer set (cat. no. 00-5523-00) was used. The following fluorochrome-labeled monoclonal antibodies were purchased from BD Pharmingen, BioLegend, R&D Systems, or eBiosciences, and used according to the manufacturers' protocols: PE or Alexa Fluor 450-conjugated anti-CD4; PE-conjugated anti-CD25; PerCP-Cy5.5-conjugated anti-CD45; FITC-conjugated anti-TCRβ; APC-conjugated anti-IFN-γ; APC-conjugated anti-FoxP3; Brilliant-violet-conjugated anti-CD45. Cells were analyzed on an LSRII cytometer (BD Biosciences) using FlowJo software. In each experiment, relevant negative control groups, positive controls, and single stained samples for each tissue were used to identify the populations of interest and to exclude other populations.

Preparation of BM chimeras. BM chimeras were prepared as previously described (Shechter et al, 2009; Shechter et al, 2013). In brief, gender-matched recipient mice were subjected to lethal whole-body irradiation (950 rad) while shielding the head (Shechter et al, 2009). The mice were then injected intravenously with 5×10^6 BM cells from CX₃CR1^{GFP/+} donors. Mice were left for 8-10 weeks after BM transplantation to enable reconstitution of the hematopoietic lineage, prior to their use in experiments. The percentage of chimerism was determined by FACS analysis of blood samples according to percentages of GFP expressing cells out of circulating monocytes (CD11b⁺). In this head-shielded model, an average of 60% chimerism was achieved, and CNS-infiltrating GFP⁺ myeloid cells were verified to be CD45^{high}/CD11b^{high}, representing monocyte-derived macrophages and not microglia (Shechter et al, 2013).

Morris Water Maze. Mice were given three trials per day, for 4 consecutive days, to learn to find a hidden platform located 1.5 cm below the water surface in a pool (1.1 m in diameter). The water temperature was kept between 21-22°C. Water was made opaque with milk powder. Within the testing room, only distal visual shape and object cues were available to the mice to aid in location of the submerged platform. The escape latency, i.e., the time required to find and climb onto the platform, was recorded for up to 60 s. Each mouse was allowed to remain on the platform for 15 s and was then removed from the maze to its home cage. If the

mouse did not find the platform within 60 s, it was manually placed on the platform and returned to its home cage after 15 s. The inter-trial interval for each mouse was 10 min. On day 5, the platform was removed, and mice were given a single trial lasting 60s without available escape. On days 6 and 7, the platform was placed in the quadrant opposite the original training quadrant, and the mouse was retrained for three sessions each day. Data were recorded using the EthoVision V7.1 automated tracking system (Noldus Information Technology). Statistical analysis was performed using analysis of variance (ANOVA) and the Bonferroni post-hoc test. All MWM testing was performed between 10 a.m. and 5 p.m. during the lights-off phase.

Radial Arm Water Maze. The radial-arm water maze (RAWM) was used to test spatial learning and memory, as was previously described in detail (Alamed et al, 2006). Briefly, six stainless steel inserts were placed in the tank, forming six swim arms radiating from an open central area. The escape platform was located at the end of one arm (the goal arm), 1.5 cm below the water surface, in a pool 1.1 m in diameter. The water temperature was kept between 21-22°C. Water was made opaque with milk powder. Within the testing room, only distal visual shape and object cues were available to the mice to aid in location of the submerged platform. The goal arm location remained constant for a given mouse. On day 1, mice were trained for 15 trials (spaced over 3 h), with trials alternating between a visible and hidden platform, and the last 4 trails with hidden platform only. On day 2, mice were trained for 15 trials with the hidden platform. Entry into an incorrect arm, or failure to select an arm within 15 sec, was scored as an error. Spatial learning and memory were measured by counting the number of arm entry errors or the escape latency of the mice on each trial. Training data were analyzed as the mean errors or escape latency, for training blocks of three consecutive trials.

GA administration. Each mouse was subcutaneously (s.c.) injected with a total dose of 100µg of GA (batch no. P53640; Teva Pharmaceutical Industries, Petah Tiqva, Israel) dissolved in 200µl of PBS. Mice were either injected according to a weekly-GA regimen (Butovsky et al, 2006), or daily-GA administration (**Fig. 8 and Fig. 16**). Mice were euthanized either 1 week after the last GA injection, or 1 month after treatment, as indicated for each experiment.

Conditional ablation of Treg. Diphtheria toxin (DTx; 8 ng/g body weight; Sigma) was injected intraperitoneally (i.p.) daily for 4 consecutive days to Foxp3.LuciDTR mice (Suffner et al, 2010). The efficiency of DTx was confirmed by flow cytometry analysis of immune cells in the blood and spleen, achieving almost complete (>99%) depletion of the GFP-expressing FoxP3⁺ CD4⁺ Treg cells (**Fig. 4**).

P300 inhibition. Inhibition of p300 in mice was performed similarly to previously described (Liu et al, 2013). p300i (C646; Tocris Bioscience) was dissolved in DMSO and

injected i.p. daily ($8.9 \text{ mg kg}^{-1} \text{ d}^{-1}$, i.p.) for 1 week. Vehicle-treated mice were similarly injected with DMSO.

ATRA treatment. All-trans retinoic acid (ATRA) administration to mice was performed similarly to previously described (Walsh et al, 2014). ATRA (Sigma) was dissolved in DMSO and injected i.p. ($8 \text{ mg kg}^{-1} \text{ d}^{-1}$) every other day over the course of 1 week. Vehicle-treated mice were similarly injected with DMSO.

Soluble A β (sA β) protein isolation and quantification. Tissue homogenization and sA β protein extraction was performed as previously described (Schmidt et al, 2005). Briefly, cerebral brain parenchyma was dissected and snap-frozen and kept at -80°C until homogenization. Proteins were sequentially extracted from samples to obtain separate fractions containing proteins of differing solubility. Samples were homogenized in 10 volumes of ice-cold tissue homogenization buffer, containing 250mM of sucrose, 20mM of Tris base, 1mM of ethylenediaminetetraacetic acid (EDTA), and 1mM of ethylene glycol tetraacetic acid (pH 7.4), using a ground glass pestle in a Dounce homogenizer. After six strokes, the homogenate was mixed 1:1 with 0.4% diethylamine (DEA) in a 100-mM NaCl solution before an additional six strokes, and then centrifuged at $135,000g$ at 4°C for 45 min. The supernatant (DEA-soluble fraction containing extracellular and cytosolic proteins) was collected and neutralized with 10% of 0.5M of Tris-HCl (pH 6.8). A β_{1-40} and A β_{1-42} were individually measured by enzyme-linked immunosorbent assay (ELISA) from the soluble fraction using commercially available kits (Biolegend; #SIG-38954 and #SIG-38956, respectively) according to the manufacturer instructions.

A β plaque quantitation. From each brain, $6 \mu\text{m}$ coronal slices were collected, and eight sections per mouse, from four different pre-determined depths throughout the region of interest (dentate gyrus or cerebral cortex) were immunostained. Histogram-based segmentation of positively stained pixels was performed using the Image-Pro Plus software (Media Cybernetics, Bethesda, MD, USA). The segmentation algorithm was manually applied to each image, in the dentate gyrus area or in the cortical layer V, and the percentage of the area occupied by total A β immunostaining was determined. Plaque numbers were quantified from the same $6 \mu\text{m}$ coronal brain slices, and are presented as average number of plaques per brain region. Prior to quantification, slices were coded to mask the identity of the experimental groups, and plaque burden was quantified by an observer blinded to the identity of the groups.

Statistical analysis. The specific tests used to analyze each set of experiments are indicated in the figure legends. Data were analyzed using a two-tailed Student's t test to compare between two groups, one-way ANOVA was used to compare several groups, followed by the

Newman–Keuls post-hoc procedure for pairwise comparison of groups after the null hypothesis was rejected ($P < 0.05$). Data from behavioral tests were analyzed using two-way repeated-measures ANOVA, and Bonferroni post-hoc procedure was used for follow-up pairwise comparison. Sample sizes were chosen with adequate statistical power based on the literature and past experience, and mice were allocated to experimental groups according to age, gender, and genotype. Investigators were blinded to the identity of the groups during experiments and outcome assessment. All inclusion and exclusion criteria were pre-established according to the IACUC guidelines. Results are presented as means $\pm$ s.e.m. In the graphs, y-axis error bars represent s.e.m. Statistical calculations were performed using the GraphPad Prism software (GraphPad Software, San Diego, CA).

Example 1. Choroid plexus (CP) gateway activity along disease progression in the mouse model of AD.

We first examined CP activity along disease progression in the 5XFAD transgenic mouse model of AD (AD-Tg); these mice co-express five mutations associated with familial AD and develop cerebral A β pathology and gliosis as early as 2 months of age (Oakley et al, 2006). We found that along the progressive stages of disease pathology, the CP of AD-Tg mice, compared to age-matched wild-type (WT) controls, expressed significantly lower levels of leukocyte homing and trafficking determinants, including *icam1*, *vcam1*, *cxcl10*, and *ccl2* (**Fig. 1A**), shown to be upregulated by the CP in response to acute CNS damage, and needed for transepithelial migration of leukocytes (Kunis et al, 2013; Shechter et al, 2013). Immunohistochemical staining for the integrin ligand, ICAM-1, confirmed its reduced expression by the CP epithelium of AD-Tg mice (Fig. 1b). In addition, staining for ICAM-1 in human postmortem brains, showed its age-associated reduction in the CP epithelium, in line with our previous observations (Baruch et al, 2014), and quantitative assessment of this effect revealed further decline in AD patients compared to aged individuals without CNS disease (**Fig. 2A**). Since the induction of leukocyte trafficking determinants by the CP is dependent on epithelial interferon (IFN)- γ signaling (Kunis et al, 2013), we next tested whether the observed effects could reflect loss of IFN- γ availability at the CP. Examining the CP of 5XFAD AD-Tg mice using flow cytometry intracellular staining, revealed significantly lower numbers of IFN- γ -producing cells in this compartment (**Fig. 2B**), and quantitative real-time PCR (RT-qPCR) analysis confirmed lower mRNA expression levels of *ifn- γ* at the CP of AD-Tg mice compared to age-matched WT controls (**Fig. 2C**).

Example 2. The functional relationships between Treg-mediated systemic immune suppression, CP gateway activity, and AD pathology.

Regulatory T cells (Tregs) play a pivotal role in suppressing systemic effector immune responses (Sakaguchi et al, 2008). We envisioned that Treg-mediated systemic immune suppression affects IFN- γ availability at the CP, and therefore focused on the involvement of Tregs in AD pathology. In line with previous reports of elevated Treg levels and suppressive activities in AD patients (Rosenkranz et al, 2007; Saresella et al, 2010; Torres et al, 2013), evaluating Foxp3⁺ Treg frequencies in splenocytes of 5XFAD AD-Tg mice, relative to their age-matched WT littermates, revealed their elevated levels along disease progression (**Fig. 3A, B**). To study the functional relationships between Treg-mediated systemic immune suppression, CP gateway activity, and AD pathology, we crossbred 5XFAD AD-Tg mice with Foxp3-diphtheria toxin receptor (DTR⁺) mice, enabling transient conditional *in vivo* depletion of Foxp3⁺ Tregs in AD-Tg/DTR⁺ mice by administration of diphtheria toxin (DTx) (**Fig. 4A**). Transient depletion of Tregs resulted in elevated mRNA expression of leukocyte trafficking molecules by the CP of AD-Tg/DTR⁺ mice relative to DTx-treated AD-Tg/DTR⁻ littermates (**Fig. 5A**). Analysis of the long-term effect of the transient Treg depletion on the brain parenchyma (3 weeks later), revealed immune cell accumulation in the brain, including elevated numbers of CD45^{high}/CD11b^{high} myeloid cells, representing infiltrating mo-M Φ (Shechter et al, 2013), and CD4⁺ T cells (**Fig. 5B**). In addition, the short and transient depletion of Tregs resulted in a marked enrichment of Foxp3⁺ Tregs among the CD4⁺ T cells that accumulated within the brain, as assessed by flow cytometry (**Fig. 5C, D**). RT-qPCR analysis of the hippocampus showed increased expression of *foxp3* and *il10* mRNA (**Fig. 5E**).

We next examined whether the short-term depletion of Tregs, which was followed by accumulation of immunoregulatory cells in sites of brain pathology, led to a long-term effect on brain function. We observed reduction in hippocampal gliosis (**Fig. 5F**), and reduced mRNA expression levels of pro-inflammatory cytokines, such as *il-12p40* and *tnf- α* (**Fig. 5G**). Moreover, cerebral A β plaque burden in the hippocampal dentate gyrus, and the cerebral cortex (5th layer), two brain regions exhibiting robust A β plaque pathology in 5XFAD AD-Tg mice (Oakley et al, 2006), was reduced (**Fig. 6A, B**). Evaluating the effect on cognitive function, using the Morris water maze (MWM) test, revealed a significant improvement in spatial learning and memory in AD-Tg/DTR⁺ mice following the Treg depletion, relative to DTx-treated AD-Tg/DTR⁻ aged matched mice, reaching performance similar to that of WT mice (**Fig. 6C-E**). Taken together, these data demonstrated that transiently breaking Treg-mediated systemic immune suppression in AD-Tg mice resulted in accumulation of inflammation-resolving cells,

including mo-MΦ and Tregs, in the brain, and was followed by resolution of the neuroinflammatory response, clearance of Aβ, and reversal of cognitive decline.

Example 3. Weekly administration of Copolymer-1 reduces Treg-mediated systemic immune suppression, improves CP gateway activity, and mitigates AD pathology.

To further substantiate the causal nature of the inverse relationship between systemic immune suppression, CP function and AD pathology, we next made use of the immunomodulatory compound, Glatiramer acetate (GA; also known as Copolymer-1, or Copaxone®), which in a weekly administration regimen was found to have a therapeutic effect in the APP/PS1 mouse model of AD (Butovsky et al, 2006); this effect was functionally associated with mo-MΦ recruitment to cerebral sites of disease pathology (Butovsky et al, 2007). Here, we first examined whether the CP in APP/PS1 AD-Tg mice, similarly to our observation in 5XFAD AD-Tg mice, is also deficient with respect to IFN-γ expression levels. We found that in APP/PS1 AD-Tg mice, IFN-γ levels at the CP were reduced relative to age-matched WT controls (**Fig. 7A**). These results encouraged us to test whether the therapeutic effect of weekly-GA in APP/PS1 mice (Butovsky et al, 2006), could be reproduced in 5XFAD AD-Tg mice, and if so, whether it would affect systemic Tregs, and activation of the CP for mo-MΦ trafficking. We therefore treated 5XFAD AD-Tg mice with a weekly administration regimen of GA over a period of 4 weeks (henceforth, “weekly-GA”; schematically depicted in **Fig. 8A**). We found that 5XFAD AD-Tg mice treated with weekly-GA, showed reduced neuroinflammation (**Fig. 8B-D**), and improved cognitive performance, which lasted up to 2 months after the treatment (**Fig. 8E-I**). Examining by flow cytometry the effect of weekly-GA on systemic immunity and on the CP, we found reduced splenocyte Foxp3⁺ Treg levels (**Fig. 9A**), and an increase in IFN-γ-producing cells at the CP of the treated 5XFAD AD-Tg mice, reaching similar levels as those observed in WT controls (**Fig. 9B**). The elevated level of IFN-γ-expressing cells at the CP in the weekly-GA treated mice, was accompanied by upregulated epithelial expression of leukocyte trafficking molecules (**Fig. 9C**).

To detect infiltrating mo-MΦ entry to the CNS, we used 5XFAD AD-Tg/CX₃CR1^{GFP/+} bone marrow (BM) chimeric mice (prepared using head protection), allowing the visualization of circulating (green fluorescent protein (GFP)⁺ labeled) myeloid cells (Shechter et al, 2009; Shechter et al, 2013). We found increased homing of GFP⁺ mo-MΦ to the CP and to the adjacent ventricular spaces following weekly-GA treatment, as compared to vehicle-treated AD-Tg/CX₃CR1^{GFP/+} controls (**Fig. 9D-E**). Immunohistochemistry of the brain parenchyma revealed the presence of GFP⁺ mo-MΦ accumulation at sites of cerebral plaque formation (**Fig. 9F**), and

quantification of infiltrating myeloid cells, by flow cytometry analysis of the hippocampus in AD-Tg non-chimeric mice, showed increased numbers of CD11b^{high}CD45^{high}-expressing cells (**Fig. 9G, H**). Together, these results substantiated the functional linkage between mo-MΦ recruitment to sites of AD pathology, reduction of systemic Treg levels and IFN-γ-dependent activation of the CP.

Example 4. Interference with Treg activity using a small molecule histone acetyltransferase inhibitor.

The findings above, which suggested that Treg-mediated systemic immune suppression interferes with the ability to fight AD pathology, are reminiscent of the function attributed to Tregs in cancer immunotherapy, in which these cells hinder the ability of the immune system to mount an effective anti-tumor response (Bos & Rudensky, 2012; Nishikawa & Sakaguchi, 2010). Therefore, we considered that a treatment that directly interferes with Foxp3⁺ Treg cell activity might be advantageous in AD. We tested p300i (C646 (Bowers et al, 2010)), a nonpeptidic inhibitor of p300, a histone acetyltransferase that regulates Treg function (Liu et al, 2013); this inhibitor was shown to affect Treg suppressive activities while leaving protective T effector cell responses intact (Liu et al, 2013). We found that mice treated with p300i, compared to vehicle (DMSO) treated controls, showed elevated levels of systemic IFN-γ-expressing cells in the spleen (**Fig. 10A**), as well as in the CP (**Fig. 10B**). We next treated AD-Tg mice with either p300i or vehicle over the course of 1 week, and examined them 3 weeks later for cerebral Aβ plaque burden. Immunohistochemical analysis revealed a significant reduction in cerebral Aβ plaque load in the p300i treated AD-Tg mice (**Fig. 10C-E**). We also tested whether the effect on plaque pathology following one course of treatment would last beyond the 3 weeks, and if so, whether additional courses of treatment would contribute to a long-lasting effect. We therefore compared AD-Tg mice that received a single course of p300i treatment and were examined 2 month later, to an age-matched group that received two courses of treatments during this period, with a 1-month interval in between (schematically depicted in **Fig. 10F**). We found that the reduction of cerebral plaque load was evident even two months after a single course of treatment, but was stronger in mice that received two courses of treatments with a 1-month interval in between (**Fig. 10G**). Since impaired synaptic plasticity and memory in AD is associated with elevated cerebral levels of soluble Aβ₁₋₄₀/Aβ₁₋₄₂ (sAβ) levels (Shankar et al, 2008), we also measured sAβ levels following a single or repeated cycles of p300i treatment. Again, we found that both one and two courses (with an interval of 1 month in between) were effective in reducing cerebral sAβ, yet this effect was stronger following repeated courses with respect to the

effect on sA β ₁₋₄₂ (**Fig. 10H**). These results indicated that while a single short-term course of treatment is effective, repeated courses of treatments would be advantageous to maintain a long-lasting therapeutic effect, similar to our observations following weekly-GA treatment.

5 Example 5. Therapeutic potential of PD-1 immune checkpoint blockade in Alzheimer's disease.

We first tested whether targeting the PD-1 inhibitory pathway could affect IFN- γ - associated systemic immunity in 5XFAD AD transgenic (AD-Tg) mice, which co-expresses five mutations associated with familial AD (Oakley et al, 2006). AD-Tg mice at the age of 10 months, a time point at which cerebral pathology is advanced, were administrated with two intraperitoneal (i.p.) injections of either blocking antibodies directed at PD-1 (anti-PD-1) or IgG control antibodies, on days 1 and 4, and then examined on day 7. Flow cytometry analysis revealed that blockade of the PD-1 pathway resulted in elevated frequencies of IFN- γ -producing CD4⁺ T splenocytes (**Fig. 11A, B**).

Table 2. GO annotation, related to Figure 11.

GO term	Description	P-value	FDR q-value
GO:0034341	response to interferon-gamma	2.13E-14	2.30E-10
GO:0048002	antigen processing and presentation of peptide antigen	3.05E-10	1.65E-06
GO:0019886	antigen processing and presentation of exogenous peptide antigen via MHC class II	4.11E-10	1.48E-06
GO:0002478	antigen processing and presentation of exogenous peptide antigen	5.26E-10	1.42E-06
GO:0034097	response to cytokine	5.67E-10	1.22E-06
GO:0002504	antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	1.04E-09	1.87E-06
GO:0002495	antigen processing and presentation of peptide antigen via MHC class II	1.04E-09	1.60E-06
GO:0019884	antigen processing and presentation of exogenous antigen	5.82E-09	7.86E-06
GO:0019882	antigen processing and presentation	1.43E-07	1.71E-04
GO:0035456	response to interferon-beta	6.67E-07	7.20E-04
GO:0006955	immune response	1.07E-06	1.05E-03
GO:0002819	regulation of adaptive immune response	1.92E-06	1.73E-03
GO:0071345	cellular response to cytokine stimulus	2.21E-06	1.84E-03
GO:0071346	cellular response to interferon-gamma	2.21E-06	1.71E-03
Gene ontology terms enriched in the CP of AD-Tg mice treated with anti-PD-1, when compared to IgG treated and untreated AD-Tg controls. Log 10 values of all RNA sequences of the CP were ranked according to their differential expression levels and analyzed.			

We next examined whether this systemic immune response affected the CP activity. Genome wide RNA-sequencing of the CP (Not shown; the full analysis will be disclosed in a

report by the present inventors having the title of Example 5 and it can be obtained from the inventors upon request) showed an expression profile associated with response to IFN- γ (**Fig. 11D** and **Table 2**), and real-time quantitative PCR (RT-qPCR) verified elevated IFN- γ mRNA levels at the CP, when compared to IgG-treated or untreated AD-Tg controls (**Fig. 11C**). These findings confirmed a systemic, and CP tissue-specific, IFN- γ immune response following PD-1 blockade, and encouraged us to next test the effect on disease pathology.

To examine the functional impact of PD-1 blockade on AD pathology, we treated 10-month old AD-Tg mice with either anti-PD-1 or IgG control antibodies, and evaluated the effect on spatial learning and memory performance, using the radial arm water maze (RAWM) task.

One month following treatment (two i.p. injections with 3-day interval), anti-PD1 treated AD-Tg mice exhibited a significant improvement in cognitive function relative to IgG-treated or untreated age-matched controls, reaching cognitive levels similar to that of age-matched WT mice (**Fig. 12A**). We next tested whether the benefit of PD-1 blockade on cognitive performance in AD-Tg mice would last beyond 1 month, and whether additional therapeutic sessions would be advantageous. We treated AD-Tg mice with anti-PD-1 at the age of 10 months ("1 session") or at both 10 and 11 months of age ("2 sessions"), and examined the outcome on cognitive performance at the age of 12 months (schematically depicted in **Fig. 12B**). Control groups included WT mice, untreated AD-Tg mice, and AD-Tg mice that received two sessions of IgG treatment. We found that while a single session of anti-PD-1 administration had a beneficial effect on spatial learning and memory 1 month following the treatment (**Fig. 12A**), no significant effect could be detected in mice that received a single session of treatment and were tested 2 months later (**Fig. 12B**). In contrast, AD-Tg mice that received two sessions of anti-PD-1, at a 1-month interval, displayed cognitive performance similar to that of WT mice, at the end of the 2-month timeframe (**Fig. 12B**).

We examined whether PD-1 blockade affected AD pathology as manifested by cerebral A β plaque load and gliosis. Brains of AD-Tg mice that received anti-PD-1 or IgG in either one or two sessions were examined by immunohistochemistry for A β and glial fibrillary acid protein (GFAP). We found that cerebral A β plaque burden was reduced in the hippocampal dentate gyrus (**Fig. 13A, B**), and the cerebral cortex (5th layer) (**Fig. 13A, C**), two brain regions exhibiting robust A β plaque pathology in 5XFAD mice (Oakley et al, 2006). The effect on A β clearance was evident following a single session of anti-PD-1 administration, and was more robust following two sessions. Quantitative analysis of GFAP immunostaining showed reduced hippocampal astrogliosis in both AD-Tg mice treated with 1 session, and those treated with 2 sessions of PD-1 blockade, relative to IgG-treated controls (**Fig. 13A, D**).

To investigate the effect of dosage and frequency of administration, female 5XFAD AD transgenic mice (average cohorts age of 6 months) were treated with either anti-PD-1-specific antibody (IgG2a anti-mouse PD-1 or IgG control (Rat IgG2a). anti-PD-1-treated mice received either 1 injection of 500ug of antibody on day 1 of the experiment, or two injections of 250ug with a 3-day interval between injections. Aged matched wild-type (WT) mice were used as additional control group. We evaluated the effect on spatial learning and memory performance, using the radial arm water maze (RAWM) task.

One month following treatment (two i.p. injections with 3-day interval), anti-PD1 treated AD-Tg mice exhibited a significant improvement in cognitive function relative to IgG-treated or untreated age-matched controls, reaching cognitive levels similar to that of age-matched WT mice (**Fig. 14**).

Finally, male 5XFAD AD transgenic mice were treated in a repeated treatment session, once a month, with either anti-PD-1-specific antibody (IgG2a anti-mouse PD-1) or IgG control (Rat IgG2a). The first injection was at the age of 3 months, the second at the age of 4 months, and the third at the age of 5 months. Dosage is indicated in the scheme of the experimental design (**Fig. 15A**). Aged matched wild-type (WT) mice were used as additional control group. We evaluated the effect on spatial learning and memory performance, using the radial arm water maze (RAWM) task, at two different time points – the age of 5 months (**Fig. 15B**), and the age of 6 months (**Fig. 15C**). Experimental design is presented. Black arrows indicate time points of treatment, and illustrations indicate time points of cognitive testing. RAWM performance of anti-PD-1-treated 5XFAD mice (n=7), IgG2a-treated 5XFAD mice (n = 9), and WT (n = 8) controls; two-way repeated-measures ANOVA and Dunnett post-test). Error bars represent mean $\pm$ s.e.m.; *P < 0.05, **P < 0.01, ***P < 0.001, anti-PD-1-treated versus IgG-treated controls. These findings demonstrate that repeated sessions of treatment with PD-1 blockade, could not only reverse disease progression when given to 5XFAD mice at advanced stages of disease, but also delay disease onset when the treatment commences at an early age, prior to cognitive decline (**Figs. 15B-C**).

Example 6. Therapeutic potential of immune checkpoint blockade in Alzheimer's disease.

To test whether blockade of immune checkpoints could attenuate AD pathology, AD-Tg mice are treated at ages between 6 to 10-month old with one of the following anti-checkpoint antibodies: anti-ICOS, anti-B7RP1, anti-VISTA, anti-CD40, anti-CD40L, anti-CD80, anti-CD86, anti-B7-H3, anti-B7-H4, B7-H7, anti-BTLA, anti-HVEM, anti-CD137, anti-CD137L, anti-OX40L, anti-CD-27, anti-CD70, anti-STING, or anti-TIGIT antibody. Some mice are

treated with anti-PD-1 antibody as positive control, IgG control as negative control or combinations of anti-PD1 and one of the other anti-checkpoint antibodies mentioned above. Treatment effect on spatial learning and memory performance, using the radial arm water maze (RAWM) task, A β plaque burden by immunohistochemistry for A β and hippocampal astrogliosis by immunohistochemistry for glial fibrillary acid protein (GFAP) will be measured one month following treatment.

It is expected that the mice treated with the antibodies display significant cognitive improvement in comparison to IgG-treated and untreated AD-Tg mice as well as a significant reduction of cerebral plaque load.

Example 7. Therapeutic potential of immune checkpoint blockade approach in PTSD pathology.

Severely stressful conditions or chronic stress can lead to posttraumatic stress disorder (PTSD) and depression. We adopted a physiological PTSD-like animal model in which the mice exhibit hypervigilant behavior, impaired attention, increased risk assessment, and poor sleep (Lebow et al, 2012). In this experimental model of PTSD induction, mice are habituated for 10 days to a reverse day/night cycle, inflicted with two episodes of electrical shocks (the trauma and the trigger), referred to as a “PTSD induction”, and evaluated at different time points subsequent to trauma. Following the traumatic event mice are injected with said compound which blocks immune checkpoints. The mice are treated according to one of the following regimens:

The mice are treated with one of the following anti-checkpoint antibodies: anti-ICOS, anti-B7RP1, anti-VISTA, anti-CD40, anti-CD40L, anti-CD80, anti-CD86, anti-B7-H3, anti-B7-H4, B7-H7, anti-BTLA, anti-HVEM, anti-CD137, anti-CD137L, anti-OX40L, anti-CD-27, anti-CD70, anti-STING, or anti-TIGIT antibody alone or in combination with an anti-CTLA-4 antibody. Some mice are treated with anti-PD-1 antibody as positive control, IgG control as negative control or combinations of anti-PD1 and one of the other anti-checkpoint antibodies mentioned above.

Some mice receive an additional treatment session with an appropriate interval session.

It is expected that mice that receive the treatment do not display anxiety behavior associated with PTSD in this experimental model, as assessed by time spent exploring and risk assessing in dark/light maze or the other behavioral tasks described in (Lebow et al, 2012).

Example 8. Therapeutic potential of immune checkpoint blockade approach in Parkinson's disease pathology.

Parkinson disease (PD) transgenic (Tg) mice or the MPTP-induced mouse models of PD are used in these experiment. The mice are treated at the progressive stages of disease according to one of the following regimens:

PD-Tg mice are treated with one of the following anti-checkpoint antibodies: anti-ICOS, anti-B7RP1, anti-VISTA, anti-CD40, anti-CD40L, anti-CD80, anti-CD86, anti-B7-H3, anti-B7-H4, B7-H7, anti-BTLA, anti-HVEM, anti-CD137, anti-CD137L, anti-OX40L, anti-CD-27, anti-CD70, anti-STING, or anti-TIGIT antibody alone or in combination with an anti-CTLA-4 antibody. Some mice are treated with anti-PD-1 antibody as positive control, IgG control as negative control or combinations of anti-PD1 and one of the other anti-checkpoint antibodies mentioned above.

Motor neurological functions are evaluated using for example the rotarod performance test, which assesses the capacity of the mice to stay on a rotating rod.

It is expected that PD-Tg mice treated with one treatment session show significant improved motor performance, compared to IgG-treated or vehicle treated control group, or untreated group. PD-Tg mice which receive two courses of therapy, and examined after an appropriate interval session are expected to show a long-lasting therapeutic effect. To maintain this therapeutic effect mice are subjected to an active session of treatment with an appropriate interval session of non-treatment between each treatment session.

Example 9. Therapeutic potential of immune checkpoint blockade in Huntington's disease pathology.

The model used in these experiments may be the Huntington's disease (HD) R6/2 transgenic mice (Tg) test system. R6/2 transgenic mice over express the mutated human huntingtin gene that includes the insertion of multiple CAG repeats mice at the progressive stages of disease. These mice show progressive behavioral-motor deficits starting as early as 5-6 weeks of age, and leading to premature death at 10-13 weeks. The symptoms include low body weight, clasping, tremor and convulsions.

The mice are treated according to one of the following regimens when they are 45 days old:

The mice are treated with one of the following anti-checkpoint antibodies: anti-ICOS, anti-B7RP1, anti-VISTA, anti-CD40, anti-CD40L, anti-CD80, anti-CD86, anti-B7-H3, anti-B7-H4, B7-H7, anti-BTLA, anti-HVEM, anti-CD137, anti-CD137L, anti-OX40L, anti-CD-27, anti-

CD70, anti-STING, or anti-TIGIT antibody alone or in combination with an anti-CTLA-4 antibody. Some mice are treated with anti-PD-1 antibody as positive control, IgG control as negative control or combinations of anti-PD1 and one of the other anti-checkpoint antibodies mentioned above.

5 Motor neurological functions are evaluated using for example the rotarod performance test, which assesses the capacity of the mice to stay on a rotating rod.

 It is expected that HD-Tg mice treated with one treatment session show significant improved motor performance, compared to IgG-treated or vehicle treated control group, or untreated group. HD-Tg mice which receive which receive two courses of therapy, and examined
10 after an appropriate interval session are expected to show a long-lasting therapeutic effect. To maintain this therapeutic effect mice are subjected to an active session of treatment with an appropriate interval session of non-treatment between each treatment session.

**Example 10. Therapeutic potential of immune checkpoint blockade approach in
15 amyotrophic lateral sclerosis pathology.**

 The model used in this experiment may be the transgenic mice overexpressing the defective human mutant SOD1 allele containing the Gly93→Ala (G93A) gene (B6SJL-TgN (SOD1-G93A)1Gur (herein “ALS mice”). This model develop motor neuron disease and thus constitute an accepted animal model for testing ALS.

20 The mice are treated according to one of the following regimens when they are 75 days old:

 The mice are treated with one of the following anti-checkpoint antibodies: anti-ICOS, anti-B7RP1, anti-VISTA, anti-CD40, anti-CD40L, anti-CD80, anti-CD86, anti-B7-H3, anti-B7-H4, B7-H7, anti-BTLA, anti-HVEM, anti-CD137, anti-CD137L, anti-OX40L, anti-CD-27, anti-
25 CD70, anti-STING, or anti-TIGIT antibody alone or in combination with an anti-CTLA-4 antibody. Some mice are treated with anti-PD-1 antibody as positive control, IgG control as negative control or combinations of anti-PD1 and one of the other anti-checkpoint antibodies mentioned above.

 Motor neurological functions are evaluated using for example the rotarod performance
30 test, which assesses the capacity of the mice to stay on a rotating rod, or mice are allowed to grasp and hold onto a vertical wire (2 mm in diameter) with a small loop at the lower end. A vertical wire allows mice to use both fore- and hindlimbs to grab onto the wire. The wire is maintained in a vertically oriented circular motion (the circle radius was 10 cm) at 24 rpm. The time that the mouse is able to hang onto the wire is recorded with a timer.

It is expected that ALS mice treated with one treatment session show significant improved motor performance, compared to IgG-treated or vehicle treated control group, or untreated group. ALS mice which receive which receive two courses of therapy, and examined after an appropriate interval session are expected to show a long-lasting therapeutic effect. To
5 maintain this therapeutic effect mice are subjected to an active session of treatment with an appropriate interval session of non-treatment between each treatment session.

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CLAIMS

1. An active agent that causes reduction of the level of systemic immunosuppression by release of a restraint imposed on the immune system by one or more immune checkpoints, for use in treating a disease, disorder, condition or injury of the Central Nervous System (CNS) that does not include the autoimmune neuroinflammatory disease relapsing-remitting multiple sclerosis (RRMS), wherein said active agent is administered by a dosage regime comprising at least two courses of therapy, each course of therapy comprising in sequence a treatment session followed by an interval session of non-treatment,

and said one or more immune checkpoints is selected from the group consisting of ICOS-B7RP1, V-domain Ig suppressor of T cell activation (VISTA), B7-CD28-like molecule, CD40L-CD40, CD28-CD80, CD28-CD86, B7H3, B7H4, B7H7, BTLA-HVEM, CD137-CD137L, OX40L, CD27-CD70, STimulator of INterferon Genes (STING), T cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT) and A2aR-Adenosine and indoleamine-2,3-dioxygenase (IDO)-L-tryptophan.

2. The active agent according to claim 1, wherein said reduction of the level of systemic immunosuppression is associated with an increase in systemic presence or activity of IFN γ -producing leukocytes.

3. The active agent according to claim 1, wherein said active agent causes reduction of the level of systemic immunosuppression and thereby an increase in the systemic presence or activity of effector T cells.

4. The active agent according to claim 1, wherein said active agent causes release from immunosuppression by blockade of said one or more immune checkpoints.

5. The active agent according to any one of claims 1 to 4, wherein said active agent is selected from the group consisting of:

(i) an antibody, such as a humanized antibody; a human antibody; a functional fragment of an antibody; a single-domain antibody, such as a Nanobody; a recombinant antibody; and a single chain variable fragment (ScFv);

(ii) an antibody mimetic, such as an affibody molecule; an affilin; an affimer; an affitin; an alphabody; an anticalin; an avimer; a DARPin; a fynomer; a Kunitz domain peptide; and a monobody;

(iii) an aptamer; and

(iv) a small molecule inhibitor.

6. The active agent according to any one of claims 1 to 5, wherein said active agent is selected from the group consisting of:

- (i) an antibody selected from the group consisting of:
 - (a) anti-ICOS;
 - 5 (b) anti-B7RP1;
 - (c) anti-VISTA;
 - (d) anti-CD40;
 - (e) anti-CD40L;
 - (f) anti-CD80;
 - 10 (g) anti-CD86;
 - (h) anti-B7-H3;
 - (i) anti-B7-H4;
 - (j) anti-B7-H7;
 - (k) anti-BTLA;
 - 15 (l) anti-HVEM;
 - (m) anti-CD137;
 - (n) anti-CD137L;
 - (o) anti-OX40L;
 - (p) anti-CD-27;
 - 20 (q) anti-CD70;
 - (r) anti-STING;
 - (s) anti-TIGIT; and
 - (t) any combination of (a) to (s);
- (ii) any combination of (a) to (s) in combination with an adjuvant;
- 25 (iii) a small molecule selected from the group consisting of:
 - (a) an adenosine A1 receptor antagonist;
 - (b) an adenosine A2a receptor antagonist;
 - (c) an A3 receptor antagonist;
- (iv) any combination of (iii) (a-c) and (i) (a-s);
- 30 and
- (v) any combination of (i) to (iv).

7. The active agent according to any one of claims 1 to 6, wherein said treatment session comprises administering said active agent to said individual and said treatment session is maintained at least until the systemic presence or level of IFN γ -producing leukocytes rises above

a reference, said administering is paused during the interval session, and said interval session is maintained as long as said level is above said reference,

wherein said reference is selected from:

- (a) the level of systemic presence or activity of IFN γ -producing leukocytes measured in the most recent blood sample obtained from said individual before said administering; or
- (b) the level of systemic presence or activity of IFN γ -producing leukocytes characteristic of a population of individuals afflicted with a disease, disorder, condition or injury of the CNS.

- 8. The active agent according to any one of claims 1 to 6, wherein said treatment session comprises administering said active agent to said individual and said treatment session is maintained at least until the systemic presence of said active agent reaches therapeutic levels, said administering is paused during the interval session, and said interval session is maintained as long as said level is above about 95%, 90%, 80%, 70%, 60% or 50% of said therapeutic level.
- 9. The active agent according to any one of claims 1 to 8, wherein the administration of the composition during the treatment session is a single administration.
- 10. The active agent according to any one of claims 1 to 8, wherein the administration of the composition during the treatment session is a repeated administration.
- 11. The active agent according to claim 10, wherein the repeated administration occurs once every two, three, four, five or six days.
- 12. The active agent according to claim 10, wherein the repeated administration occurs once weekly.
- 13. The active agent according to claim 10, wherein the repeated administration occurs once every four weeks.
- 14. The active agent according to any one of claims 1 to 13, wherein the treatment session is from 3 days to four weeks.
- 15. The active agent according to any one of claims 1 to 14, wherein the non-treatment period is from one week to six months.

16. The active agent according to any one of claims 1 to 15, for use in treating a disease, disorder or condition selected from the group consisting of a neurodegenerative disease selected from the group consisting of Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease and Huntington's disease; primary progressive multiple sclerosis; secondary progressive multiple sclerosis; corticobasal degeneration; Rett syndrome; a retinal degeneration disorder selected from the group consisting of age-related macular degeneration and retinitis pigmentosa; anterior ischemic optic neuropathy; glaucoma; uveitis; depression; trauma-associated stress or post-traumatic stress disorder; frontotemporal dementia; Lewy body dementias; mild cognitive impairments; posterior cortical atrophy; primary progressive aphasia; progressive supranuclear palsy and aged-related dementia.
17. The active agent according to claim 16, wherein said neurodegenerative disease, disorder or condition is selected from the group consisting of Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease and Huntington's disease.
18. The active agent according to claim 17, for use in treating Alzheimer's disease.
19. The active agent according to any one of claims 1 to 15, for use in treating an injury of the CNS selected from the group consisting of spinal cord injury, closed head injury, blunt trauma, penetrating trauma, hemorrhagic stroke, ischemic stroke, cerebral ischemia, optic nerve injury, myocardial infarction, organophosphate poisoning and injury caused by tumor excision.
20. The active agent according to any one of claims 1 to 15, wherein said treating improves CNS motor and/or cognitive function.
21. The active agent according to claim 20, for use in alleviating age-associated loss of cognitive function.
22. The active agent according to claim 21, wherein said age-associated loss of cognitive function occurs in individuals free of a diagnosed disease.
23. The active agent according to claim 21, for use in alleviating loss of cognitive function resulting from acute stress or traumatic episode.
24. The active agent according to claim 19, wherein said cognitive function is learning, memory or both.

Fig. 1A

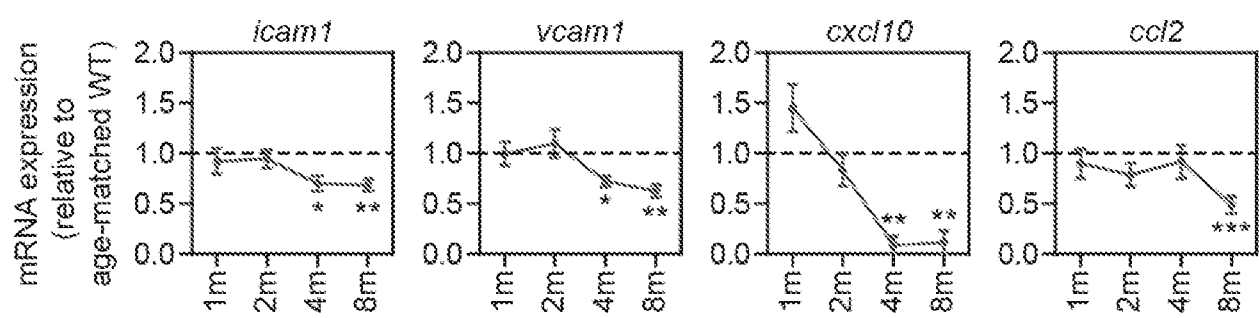


Fig. 1B

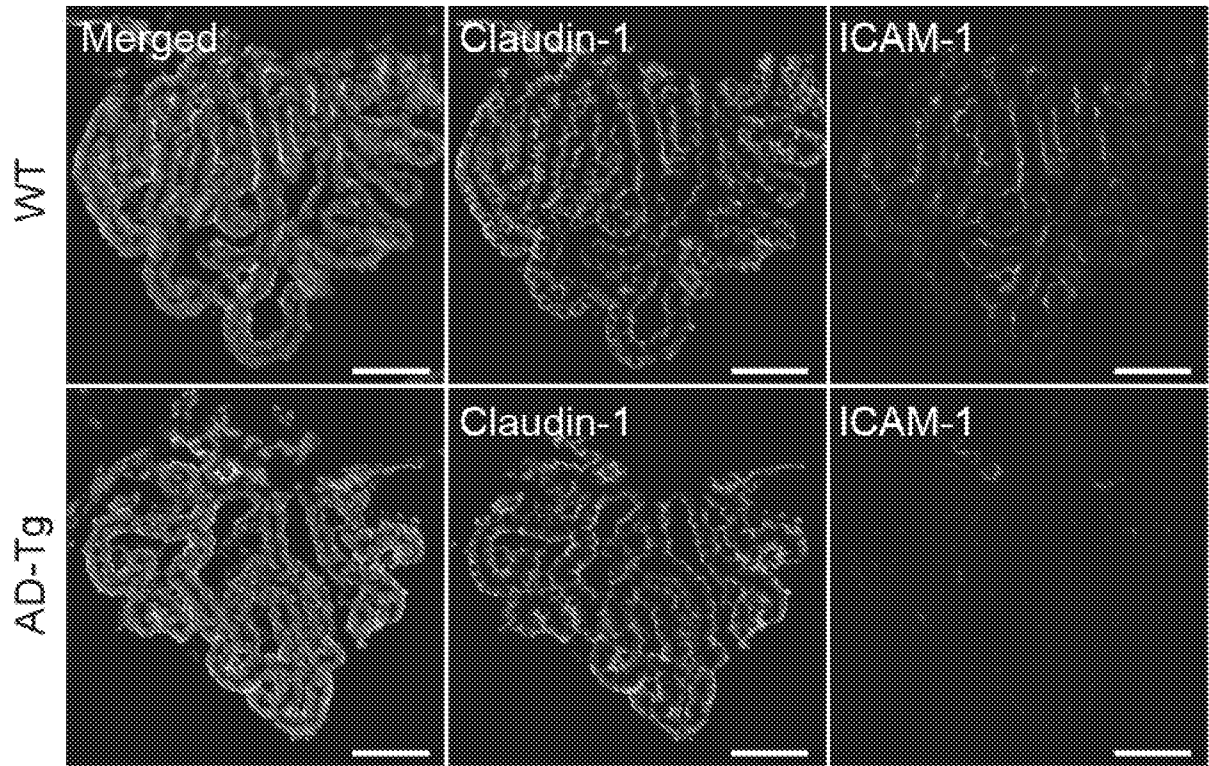


Fig. 2A

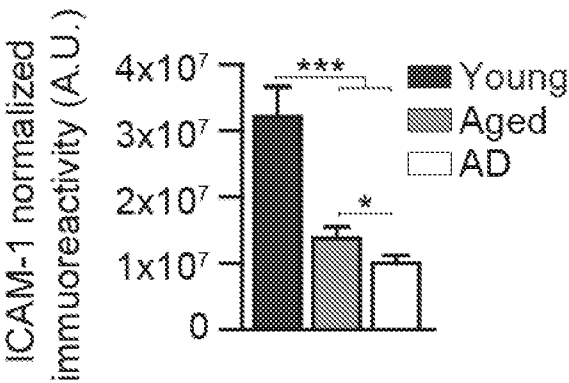


Fig. 2B

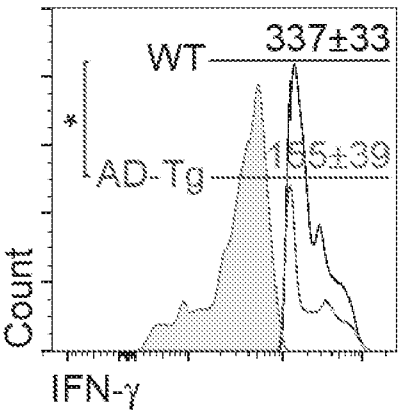


Fig. 2C

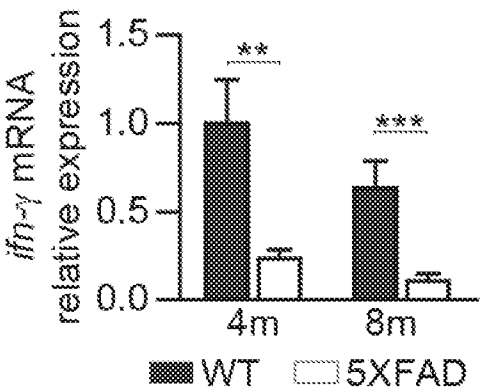


Fig. 3A

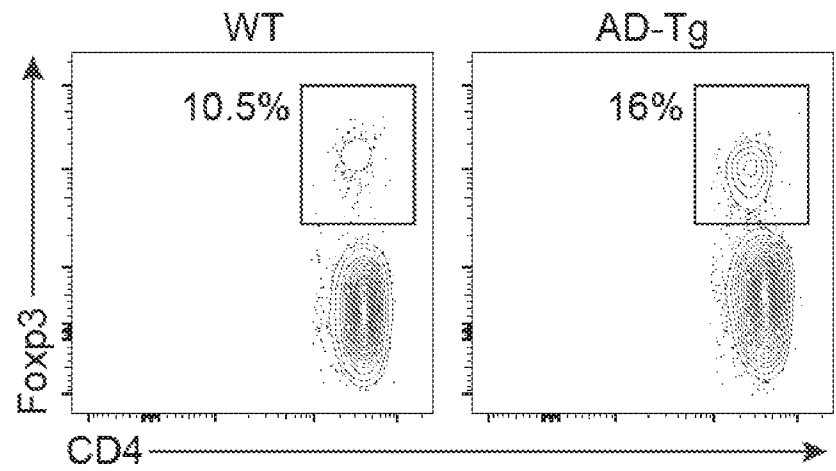


Fig. 3B

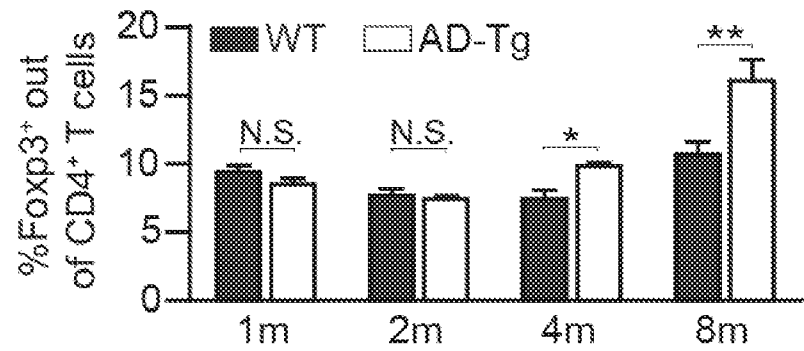
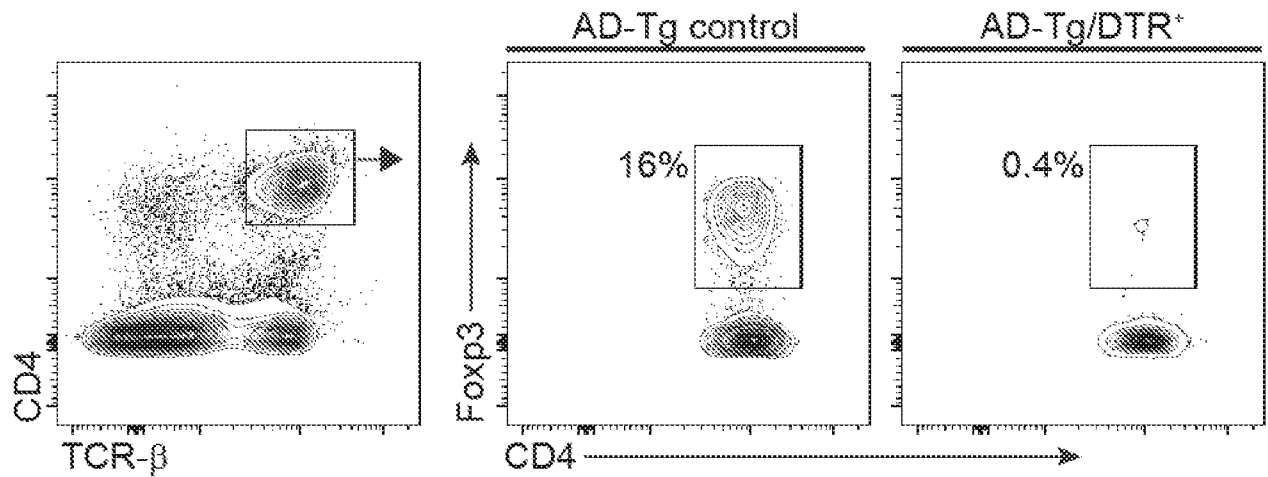


Fig. 4



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Fig. 5A

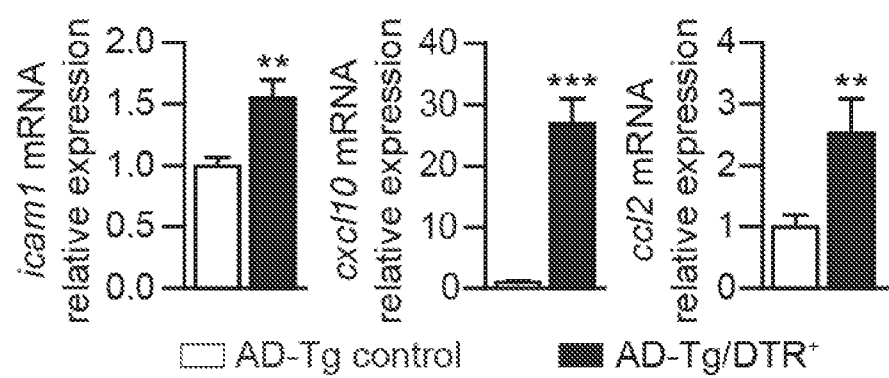


Fig. 5B

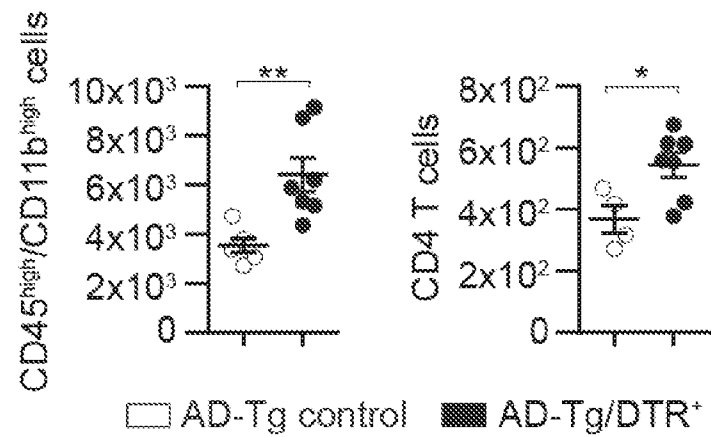
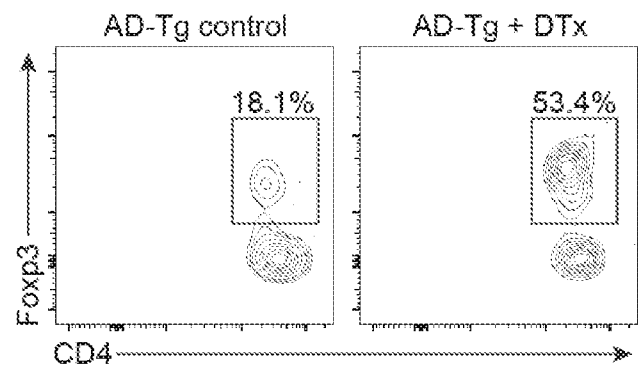


Fig. 5C



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Fig. 5D

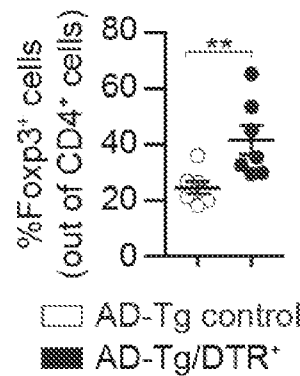


Fig. 5E

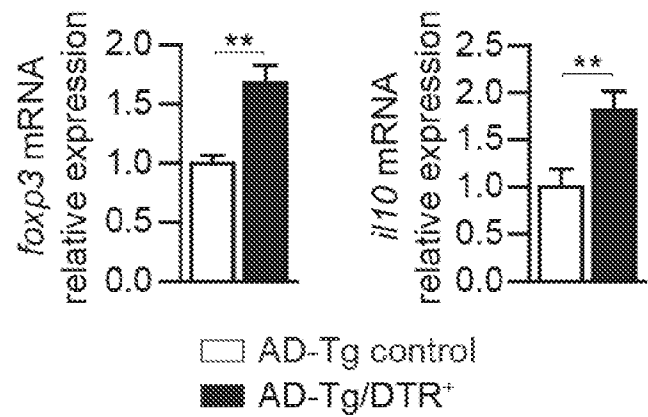


Fig. 5F

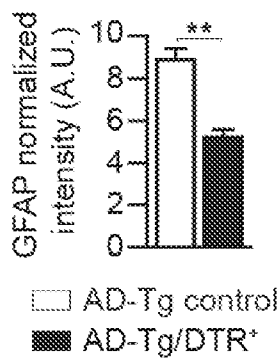


Fig. 5G

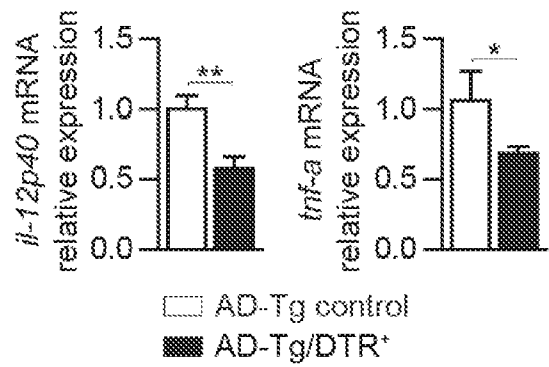


Fig. 6A

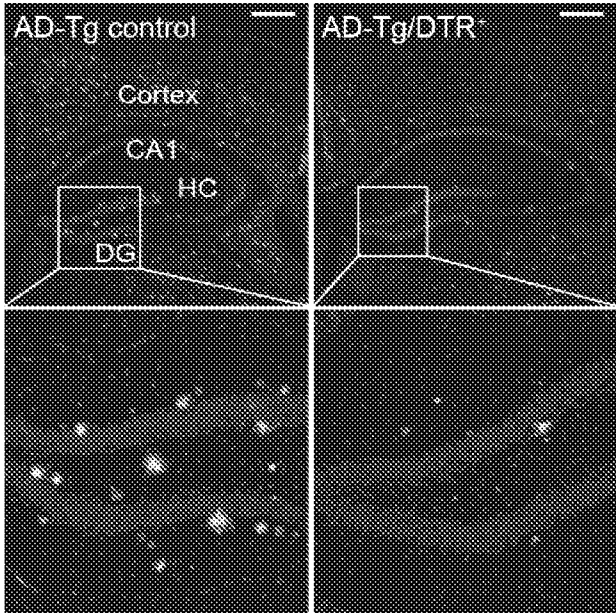


Fig. 6B

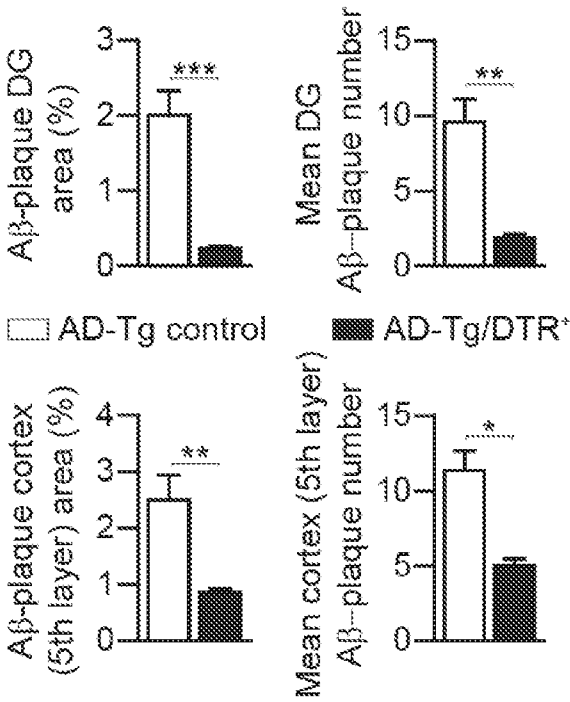


Fig. 6C

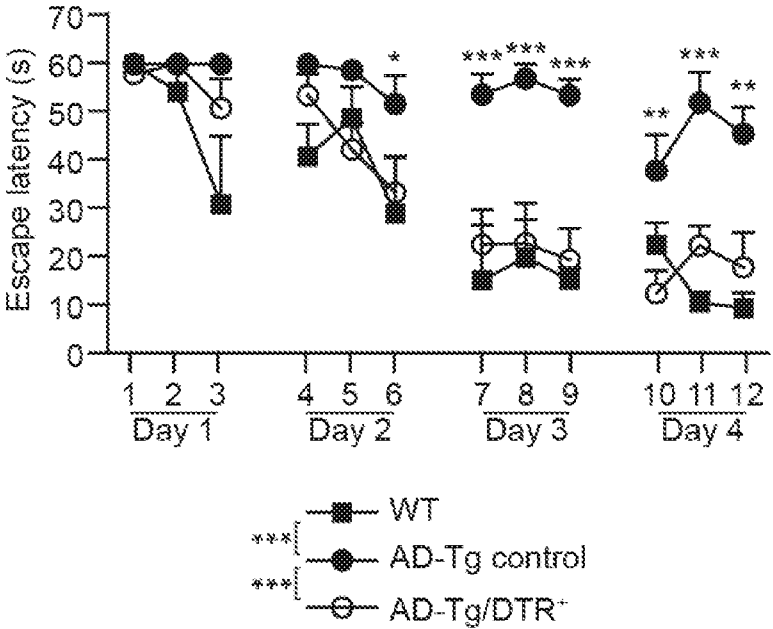
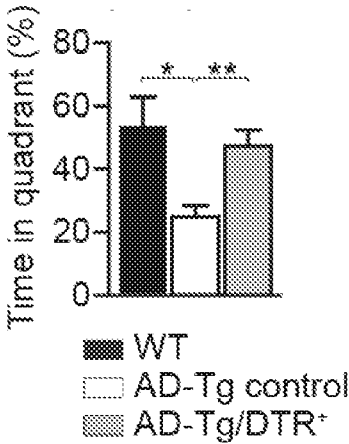


Fig. 6D



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Fig. 6E

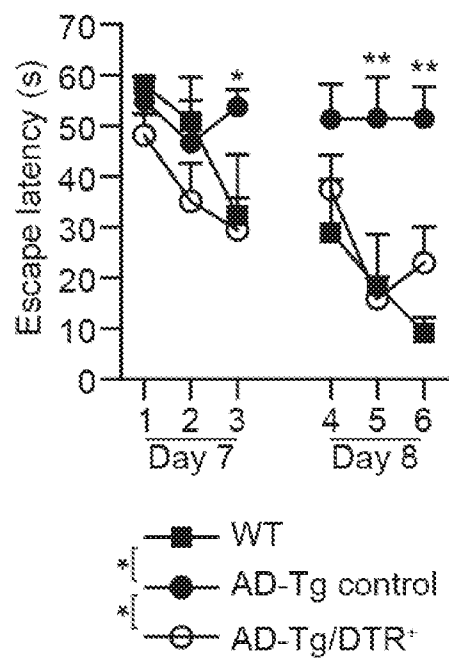


Fig. 7

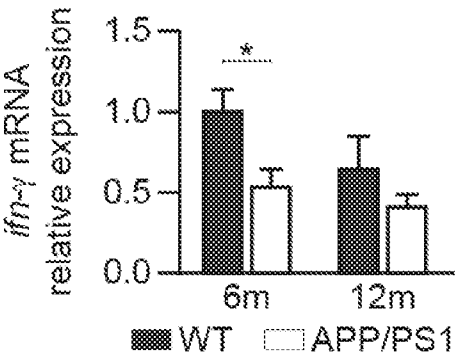
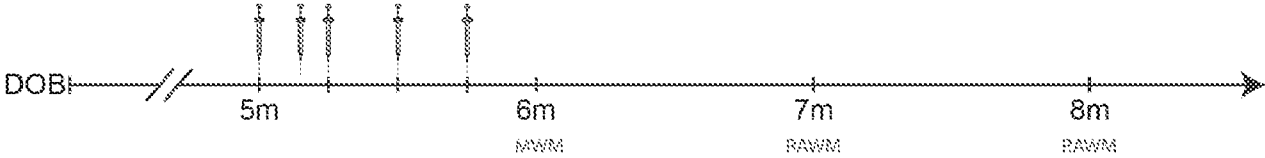


Fig. 8A



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Fig. 8B

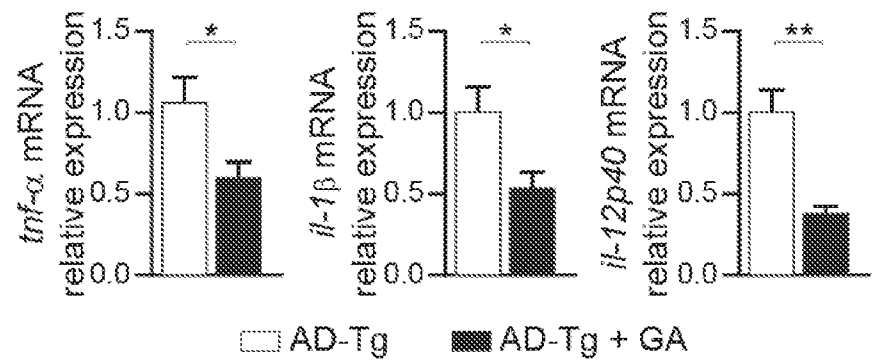


Fig. 8C

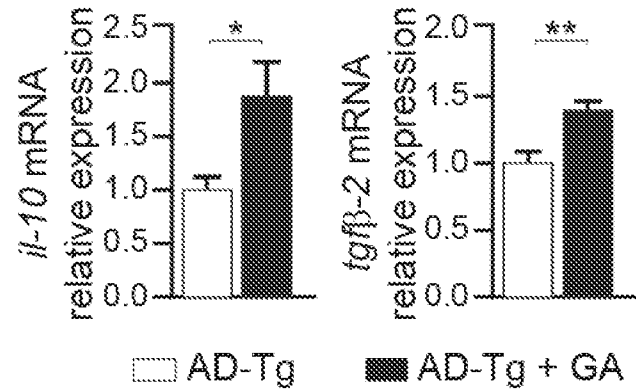
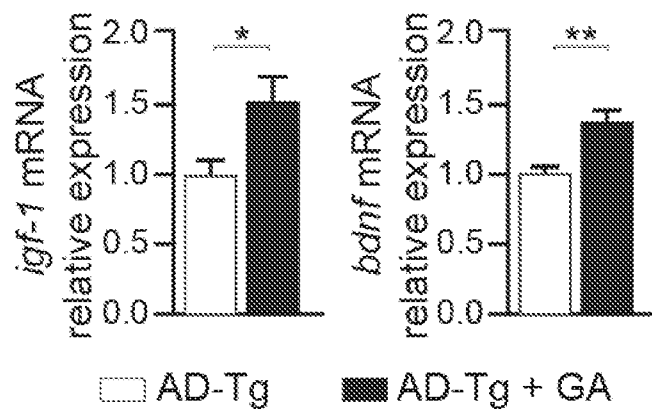


Fig. 8D



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Fig. 8E

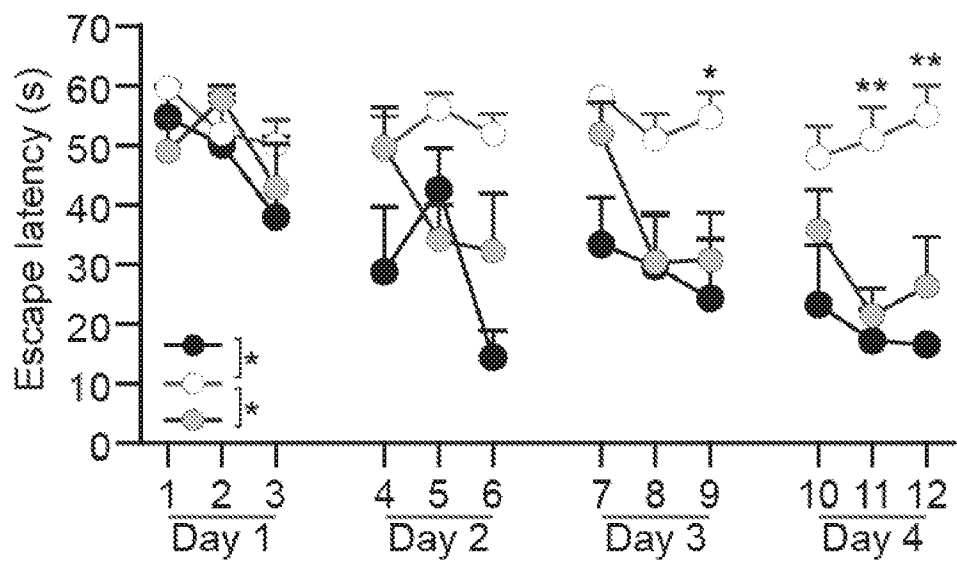


Fig. 8F

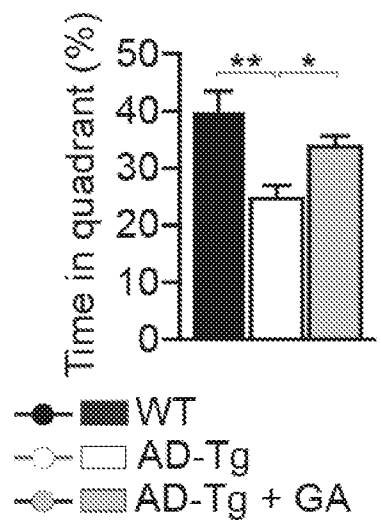
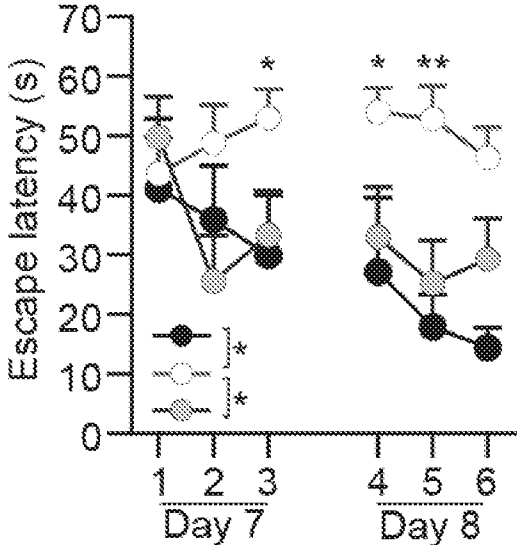


Fig. 8G



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Fig. 8H

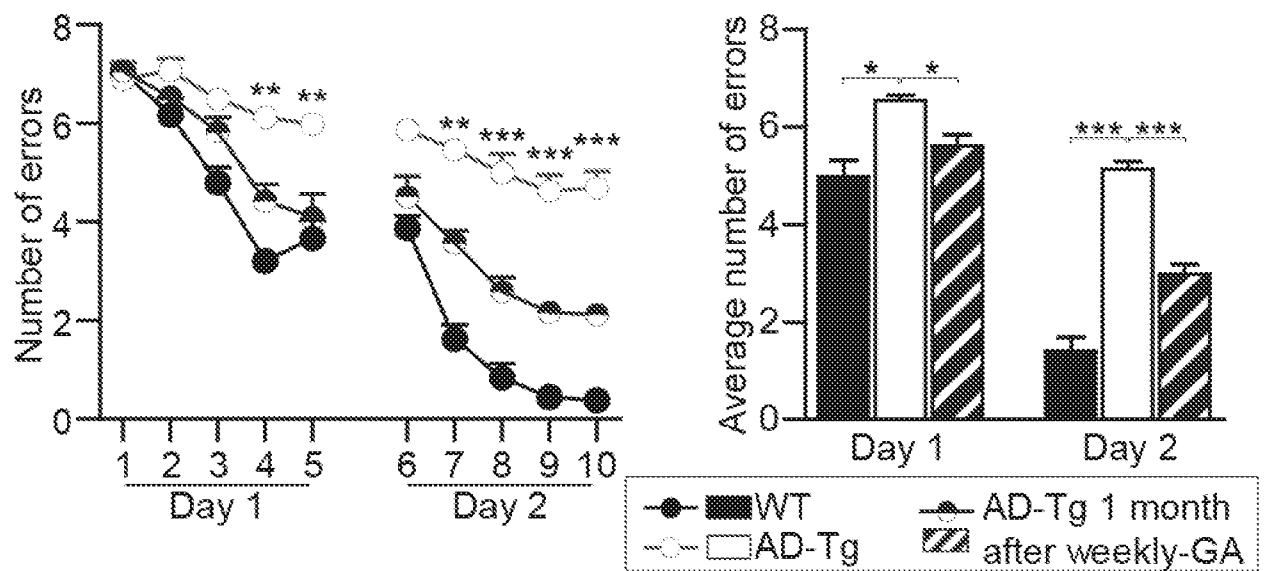
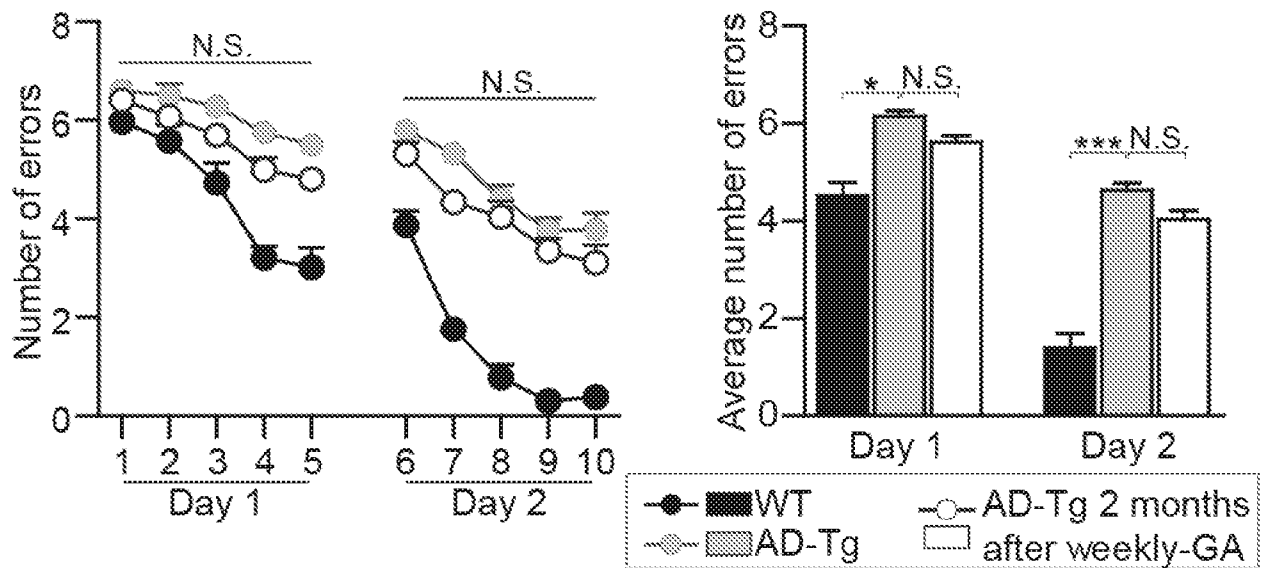


Fig. 8I



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Fig. 9A

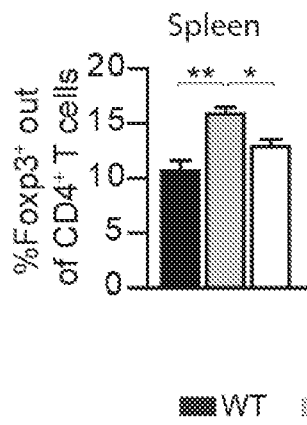


Fig. 9B

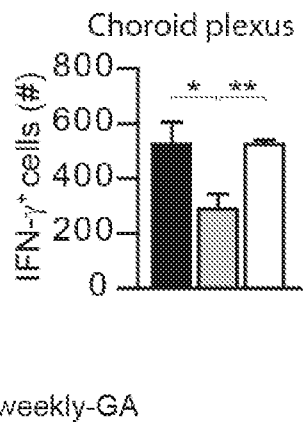
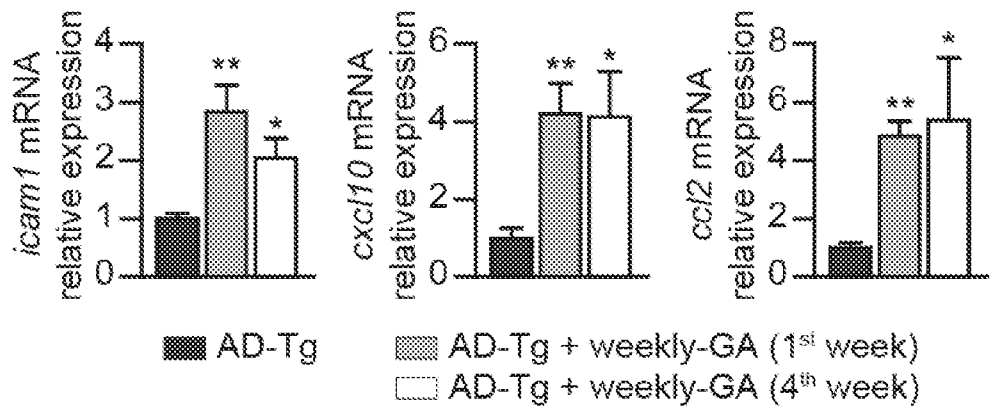


Fig. 9C



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Fig. 9D

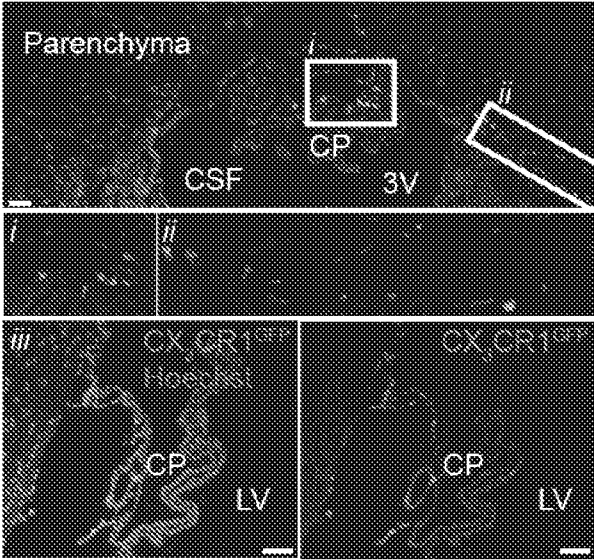


Fig. 9E

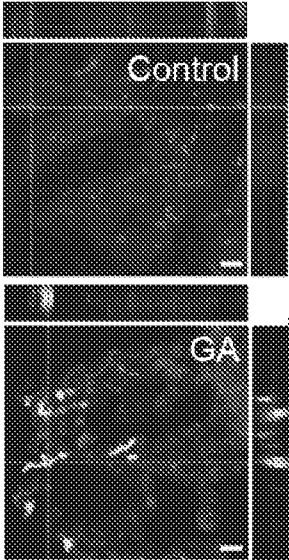
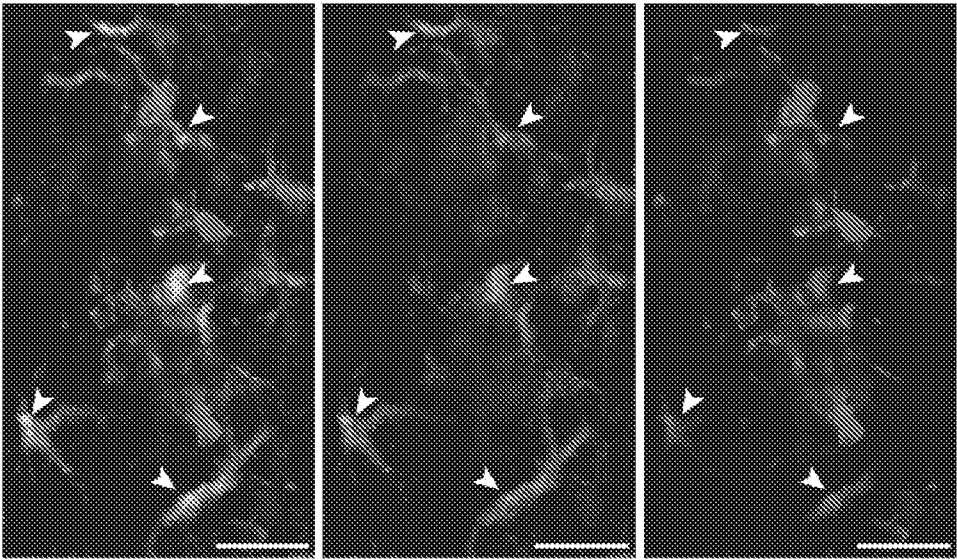


Fig. 9F



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Fig. 9G

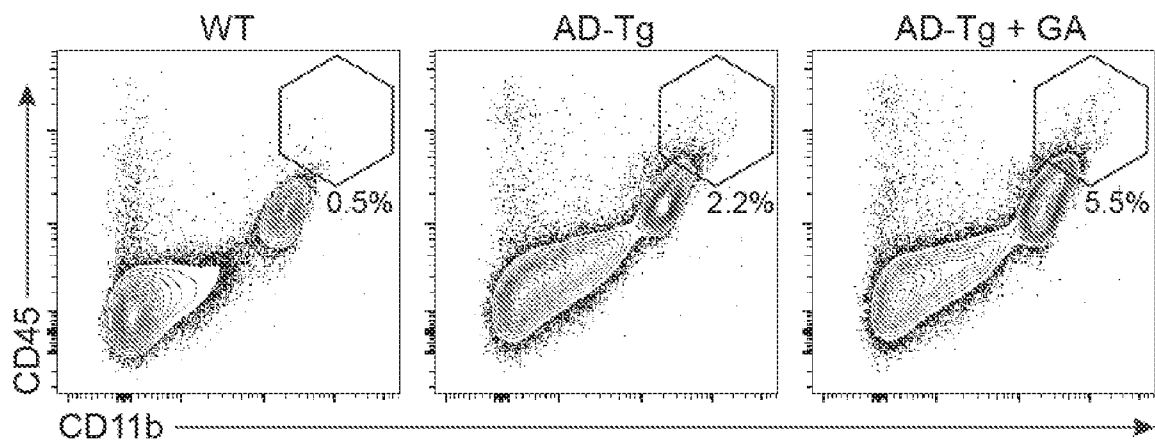


Fig. 9H

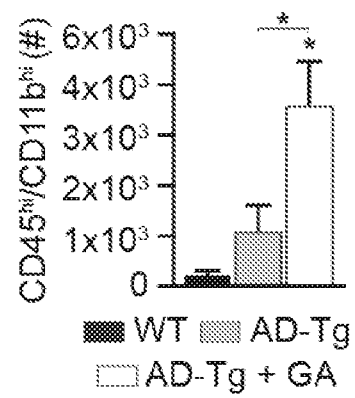


Fig. 10A

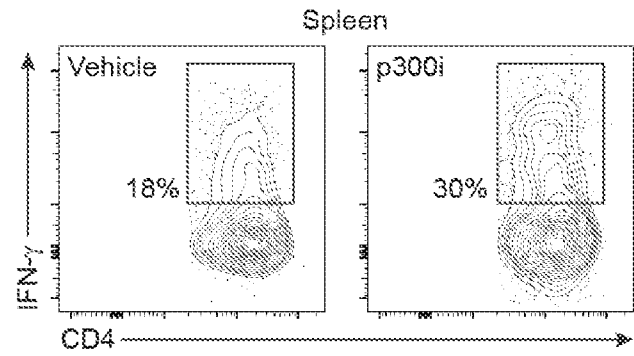


Fig. 10B

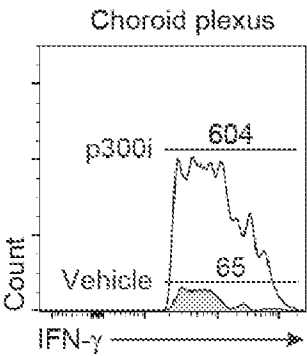
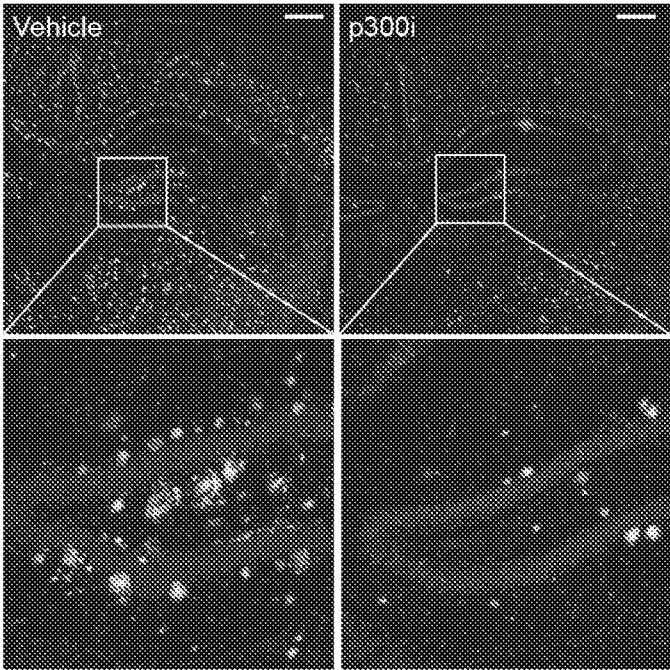
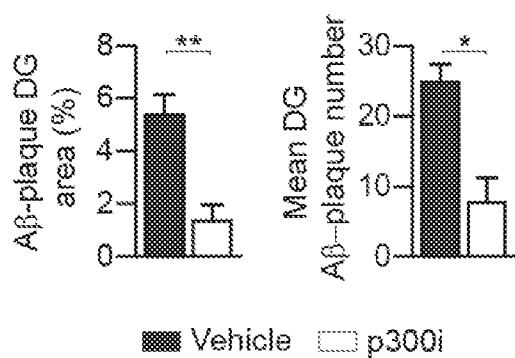
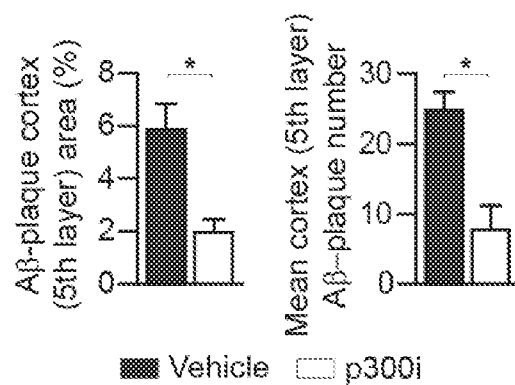
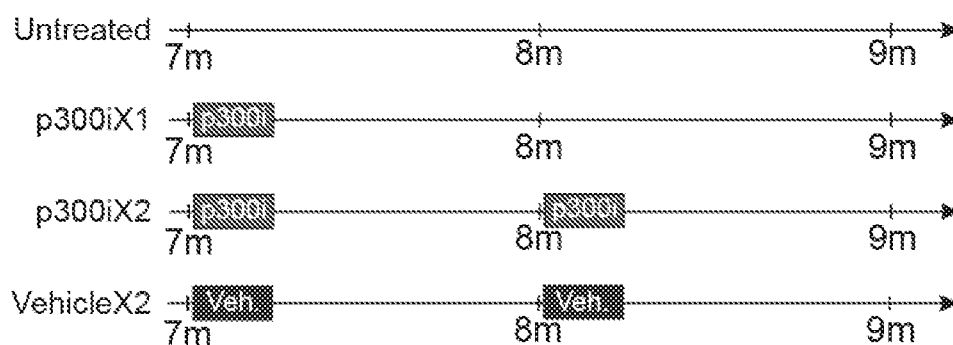
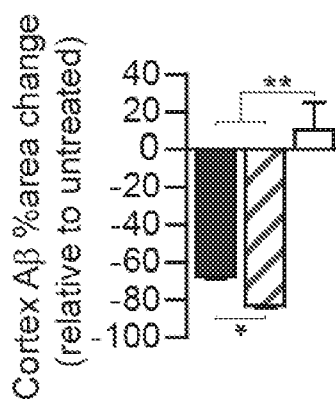
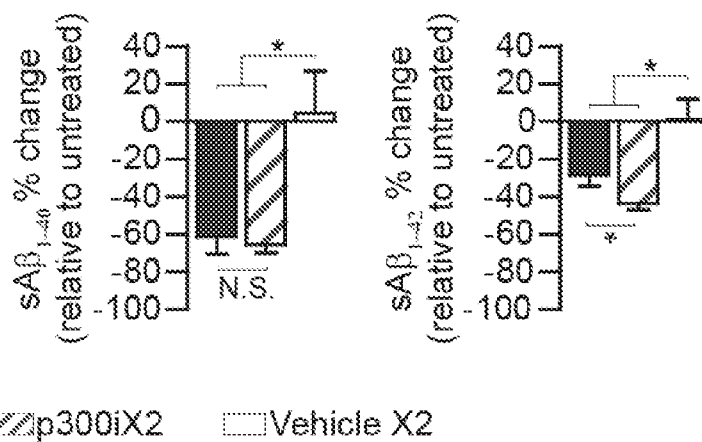


Fig. 10C



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Fig. 10D**Fig. 10E****Fig. 10F****Fig. 10G****Fig. 10H**

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Figure 11A

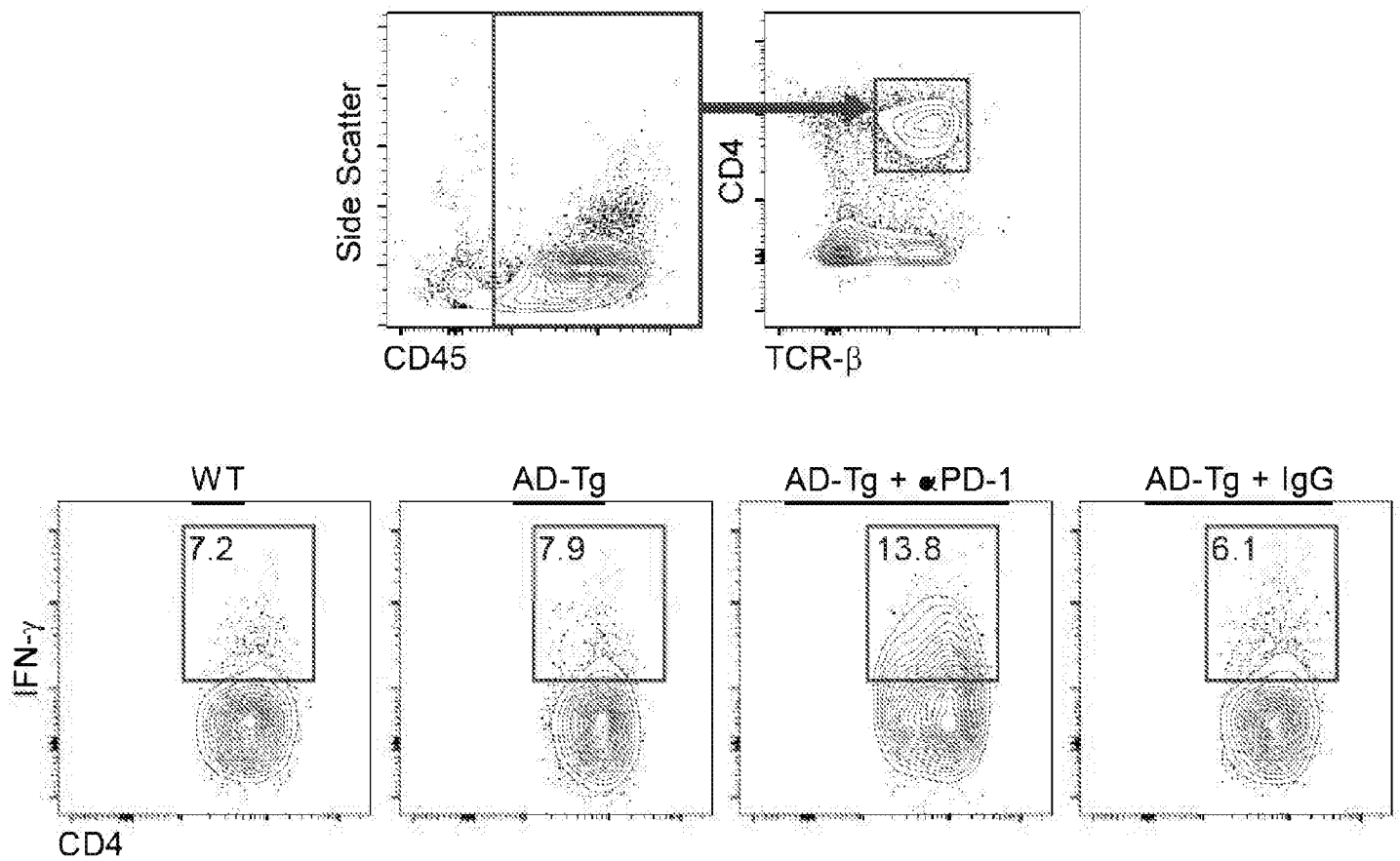


Figure 11B

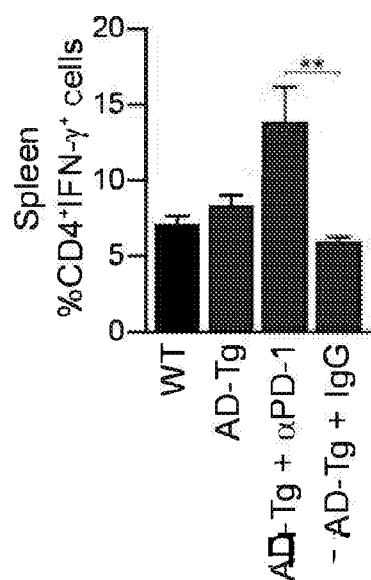
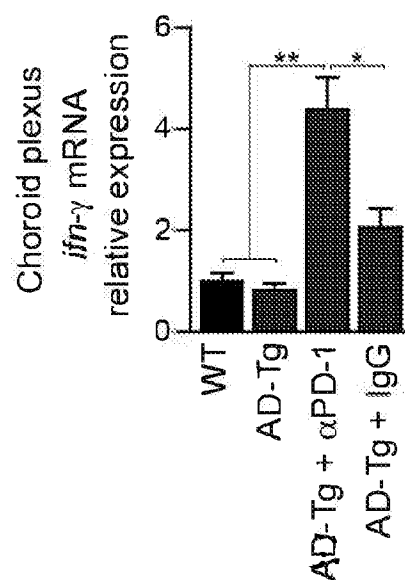
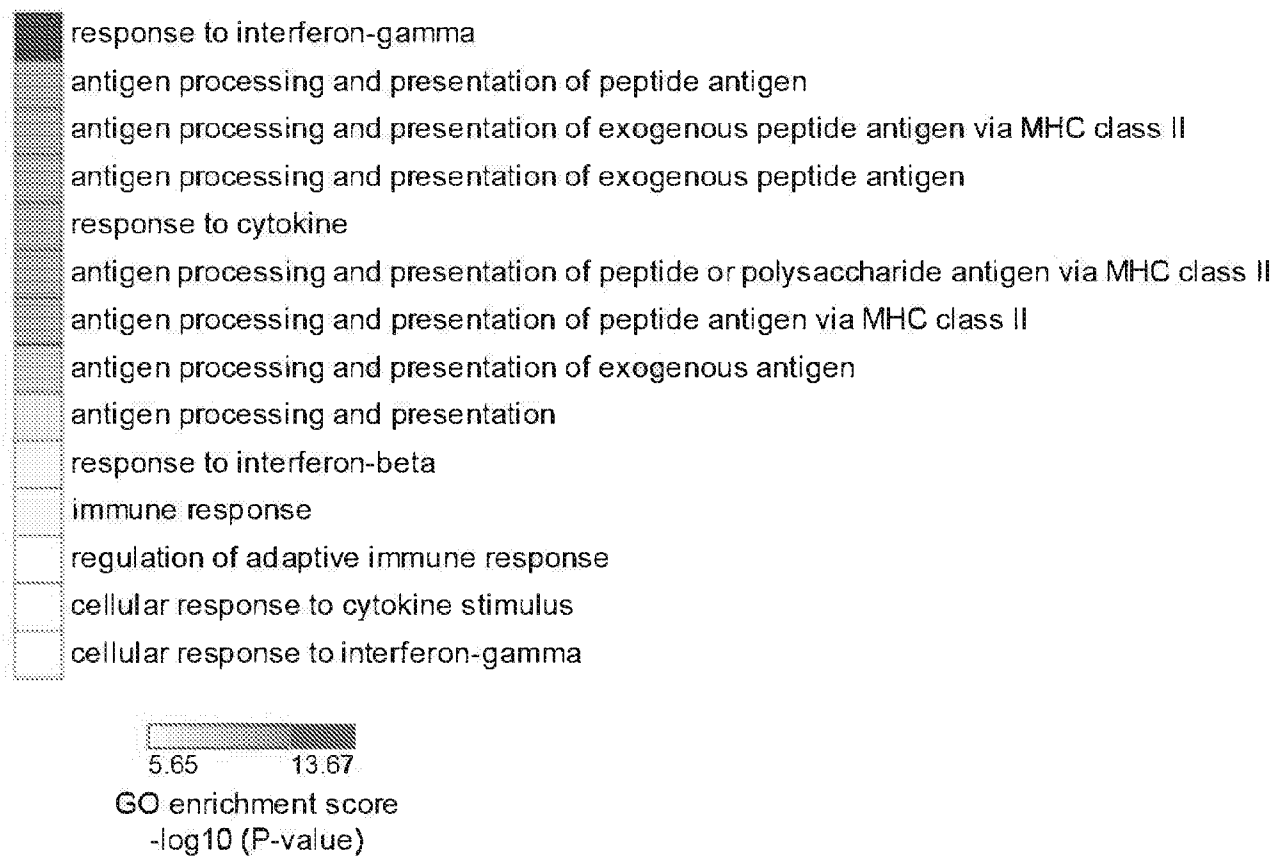


Figure 11C



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Figure 11D

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Figure 12A

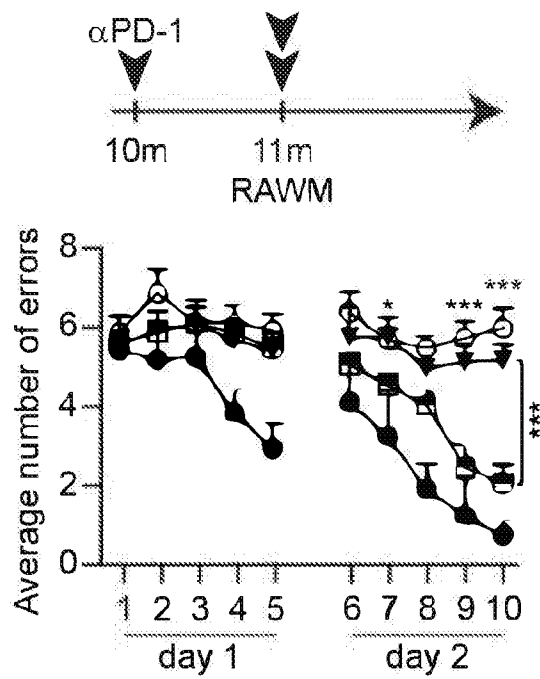
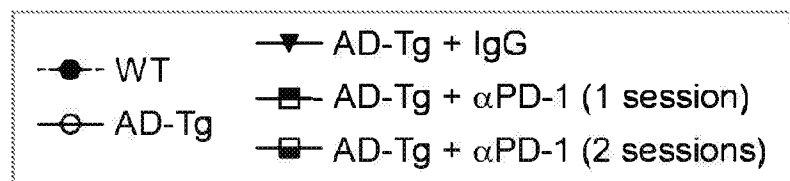
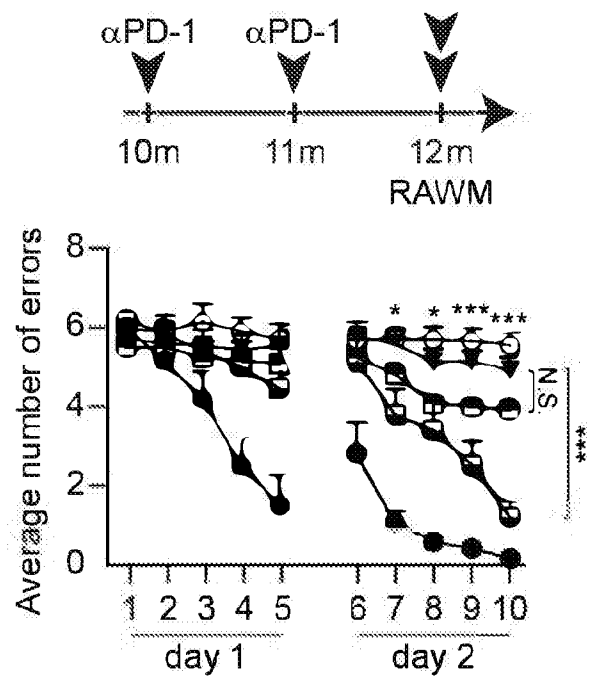
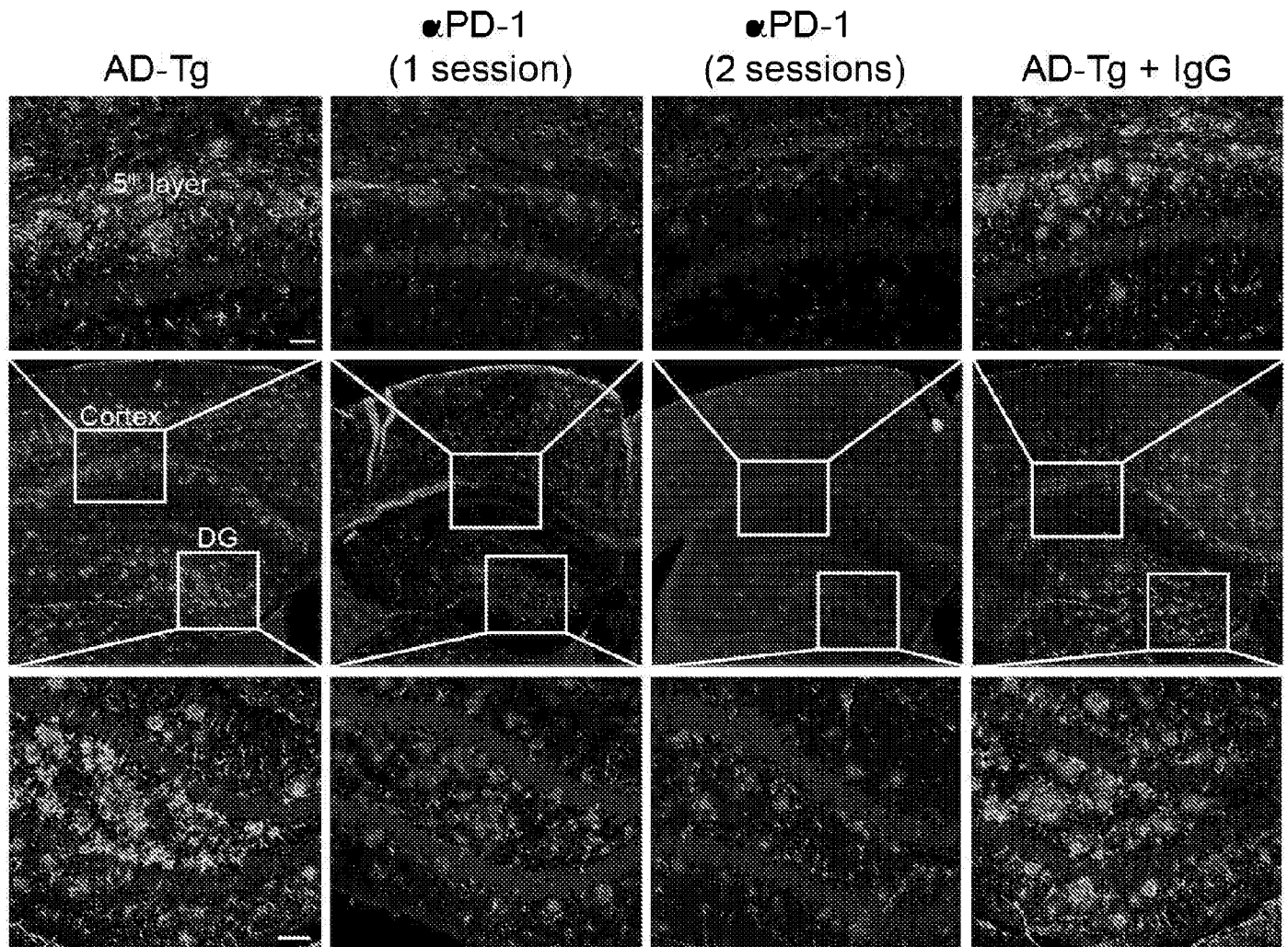


Figure 12B



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Figure 13A



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Figure 13B

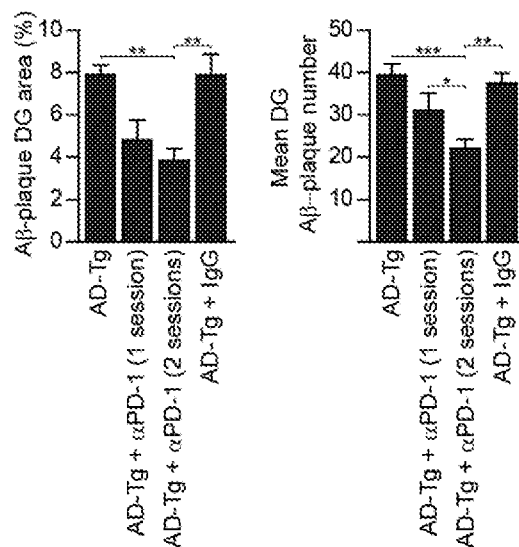


Figure 13C

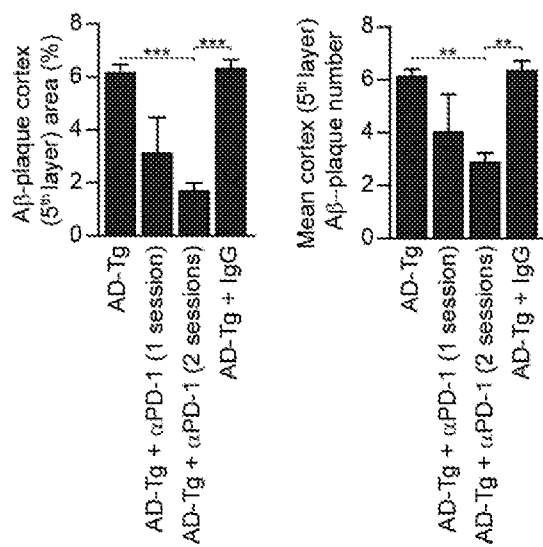
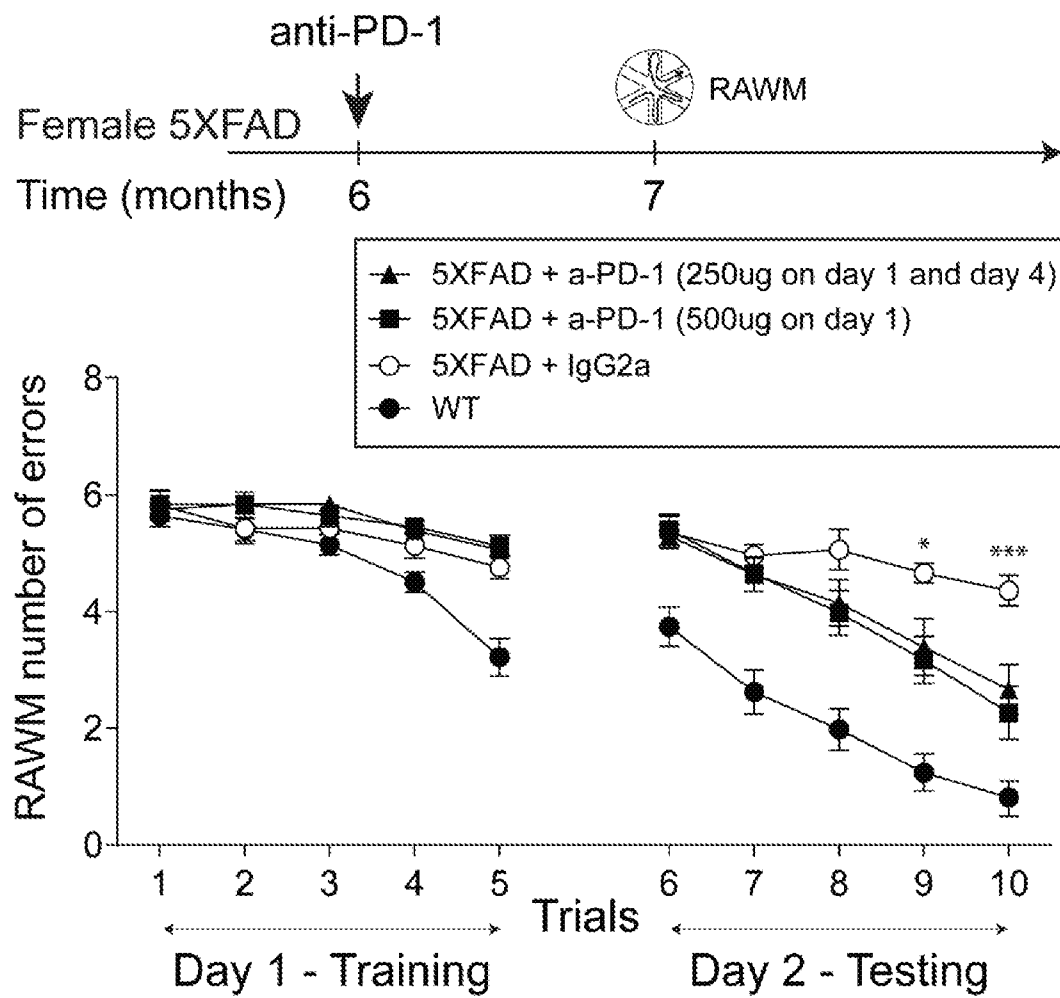


Figure 13D



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Figure 14



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Figure 15A

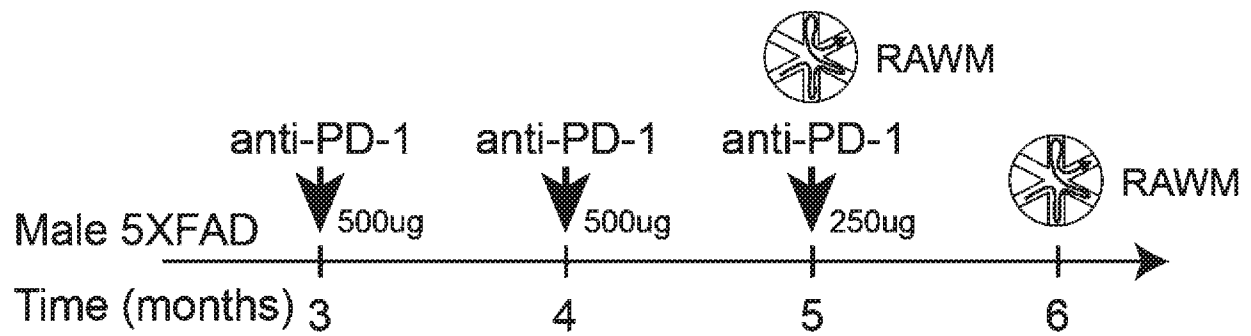


Figure 15B

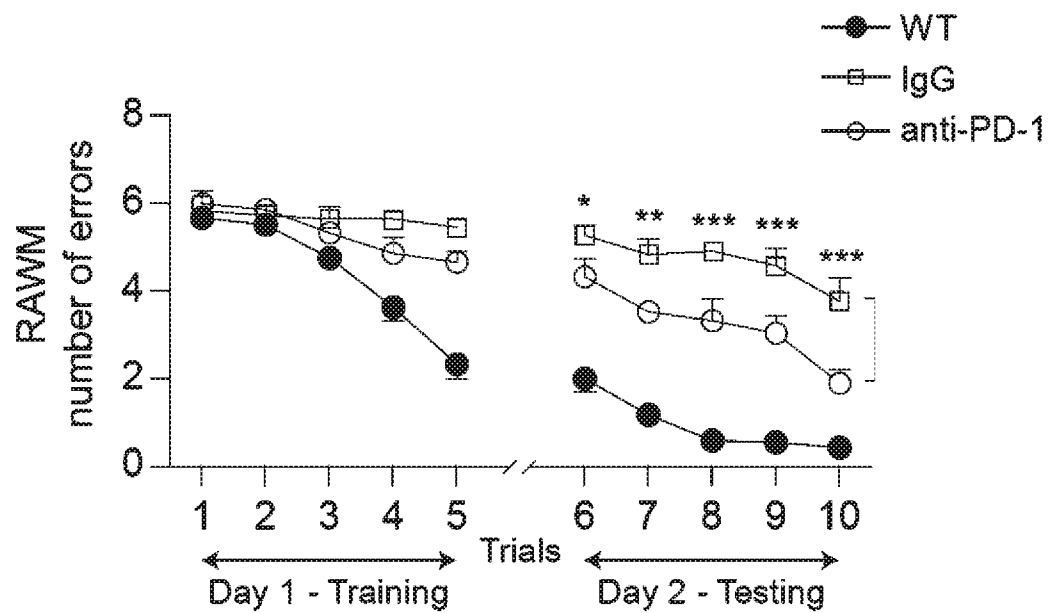
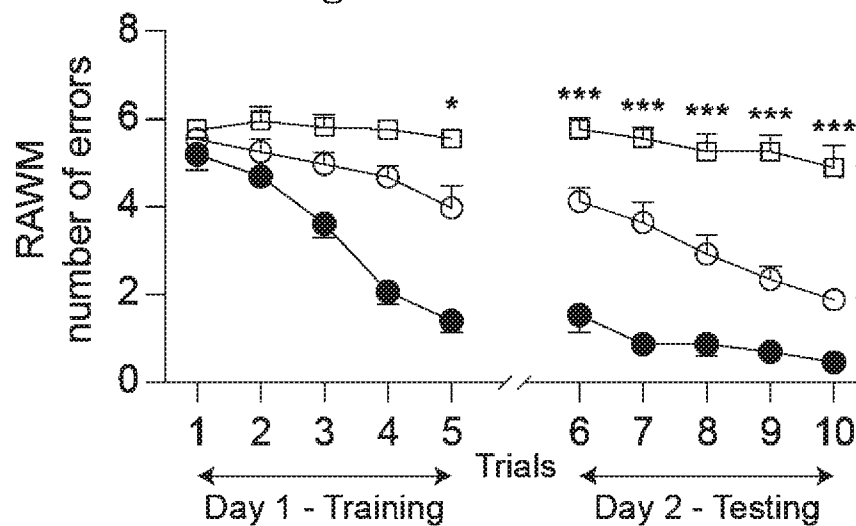


Figure 15C



INTERNATIONAL SEARCH REPORT

International application No
PCT/IL2016/050750

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/395 A61K38/00 A61K39/00
ADD.

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)
A61K

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)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	OLEG BUTOVSKY ET AL: "Selective ablation of bone marrow-derived dendritic cells increases amyloid plaques in a mouse Alzheimer's disease model", EUROPEAN JOURNAL OF NEUROSCIENCE, vol. 26, no. 2, 10 July 2007 (2007-07-10), pages 413-416, XP055211364, ISSN: 0953-816X, DOI: 10.1111/j.1460-9568.2007.05652.x page 414 - page 416; figure 1 ----- -/--	1,8-12, 16,20,21



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

26 October 2016

Date of mailing of the international search report

04/11/2016

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Fax: (+31-70) 340-3016

Authorized officer

Fellows, Edward

INTERNATIONAL SEARCH REPORT

International application No

PCT/IL2016/050750

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SCHWARTZ MICHAL ET AL: "Breaking peripheral immune tolerance to CNS antigens in neurodegenerative diseases: Boosting autoimmunity to fight-off chronic neuroinflammation", JOURNAL OF AUTOIMMUNITY, LONDON, GB, vol. 54, 8 September 2014 (2014-09-08), pages 8-14, XP029081825, ISSN: 0896-8411, DOI: 10.1016/J.JAUT.2014.08.002 tables 2,3	1,8-12, 16,20,21
X	----- ANGELOV D N ET AL: "THERAPEUTIC VACCINE FOR ACUTE AND CHRONIC MOTOR NEURON DISEASES: IMPLICATIONS FOR AMYOTROPHIC LATERAL SCLEROSIS", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, NATIONAL ACADEMY OF SCIENCES, US, vol. 100, no. 8, 15 April 2003 (2003-04-15), pages 4790-4795, XP009045397, ISSN: 0027-8424, DOI: 10.1073/PNAS.0530191100 page 4790 - page 4794; figure 2	1,8-12, 16,20,21
X	----- DEREK A. WAINWRIGHT ET AL: "Targeting Tregs in Malignant Brain Cancer: Overcoming IDO", FRONTIERS IN IMMUNOLOGY, vol. 4, 1 January 2013 (2013-01-01), XP055211742, ISSN: 1664-3224, DOI: 10.3389/fimmu.2013.00116 the whole document	1-24
X	----- AVIDAN H ET AL: "Vaccination with autoantigen protects against aggregated [beta]-amyloid and glutamate toxicity by controlling microglia: Effect of CD4<+>CD25<+> T cells", EUROPEAN JOURNAL OF IMMUNOLOGY, WILEY - V C H VERLAG GMBH & CO. KGAA, DE, vol. 34, no. 12, 1 December 2004 (2004-12-01), pages 3434-3445, XP002525982, ISSN: 0014-2980, DOI: 10.1002/EJI.200424883 the whole document	1-24
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INTERNATIONAL SEARCH REPORT

International application No

PCT/IL2016/050750

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YUJIE LIU ET AL: "Inhibition of p300 impairs Foxp3+ T regulatory cell function and promotes antitumor immunity", NATURE MEDICINE, vol. 19, no. 9, 18 August 2013 (2013-08-18), pages 1173-1177, XP055212950, ISSN: 1078-8956, DOI: 10.1038/nm.3286 the whole document -----	1-24