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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 April 2020 (23.04.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/081886 A1

(51) International Patent Classification:
A61K 45/06 (2006.01) *A61K 31/42* (2006.01)
A61K 39/39 (2006.01) *A61K 31/58* (2006.01)
A61K 31/00 (2006.01)

(21) International Application Number:
PCT/US2019/056848

(22) International Filing Date:
18 October 2019 (18.10.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/747,398 18 October 2018 (18.10.2018) US
62/747,370 18 October 2018 (18.10.2018) US

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

(72) Inventors: **KAUFMAN, Daniel**; 3543 Greenfield Avenue, Los Angeles, California 90034 (US). **TIAN, Jide**; 3618 Keystone Avenue, #5, Los Angeles, California 90034 (US). **OLSEN, Richard**; c/o David Geffen School of Medicine, Department of Molecular and Medical Pharmacology, University of California Los Angeles, 650 Charles E. Young Dr. South, Room 23-338 CHS, Los Angeles, California 90095-1735 (US). **WALLNER, Martin**; c/o David Geffen School of Medicine, Department of Molecular and Medical Pharmacology, University of California Los Angeles, 650 Charles E. Young Dr. South, Room 23-338 CHS, Los Angeles, California 90095-1735 (US).

(74) Agent: **DUNSTON, Erin M.**; BUCHANAN INGERSOLL & ROONEY PC, P. O. Box 1404, Alexandria, Virginia 22313-1404 (US).

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

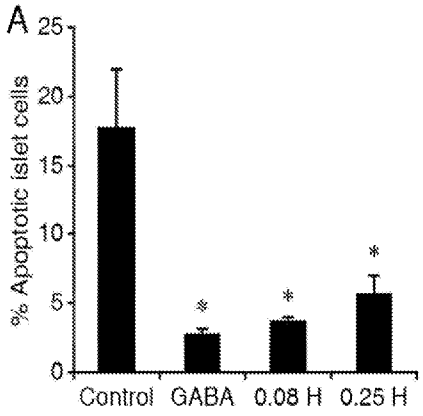
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR,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

Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

(54) Title: HOMOTAURINE FOR TREATMENT OF INFLAMMATION-RELATED DISORDERS

(57) Abstract: Blood-brain barrier-permeable $GABA_A$-R-specific agonist with an excellent safety profile. Homotaurine for limiting inflammation and inflammation-related disorders and methods of treatment of inflammation-related disorders, including MS, T1D, and RA, in subjects. Homotaurine for inducing $CD8^+$ and $CD4^+$ regulatory T cell responses in a subject. Homotaurine for inhibiting autoreactive Th17 and Th1 responses, raising C peptide levels, lowering A1C levels, promoting islet cell survival, promoting β-cell replication, and/or inhibiting inflammation in a subject.

FIGURE 6



WO 2020/081886 A1

[Continued on next page]

Published:

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

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

HOMOTAURINE FOR TREATMENT OF INFLAMMATION-RELATED DISORDERS

STATEMENT OF FEDERALLY-SPONSORED RESEARCH AND DEVELOPMENT

This invention was made with government support under Grant Number DK092480, awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Inflammation is part of the innate immune response following an insult to the body. Inflammation is typically divided between acute and chronic. Inflammatory reaction can spread throughout the systemic circulation and into the central nervous system ("CNS"). Inflammation plays a critical role in many diseases of the CNS. Depending on the stage of the disease, different immune mechanisms may predominate. Inflammatory responses in the CNS involve immune cell entry/migration, a complex interplay between resident and circulating immune cells, parenchymal cells, the cellular constituents of the CNS microvasculature, and alterations in immune cell function. In response to injury, infection, and/or disease, the CNS can generate inflammatory mediators, such as proinflammatory cytokines, prostaglandins, free radicals and complement, which can in turn induce chemokines and adhesion molecules, recruit immune cells, and activate glial cells. Chronic inflammation can affect all parts of one's body and can be a secondary component of many diseases.

Inflammatory diseases can be painful and, in some cases, progressively debilitating, which can greatly affect one's quality of life and create both societal and economic burdens. Over 1 million people in the United States are living with multiple sclerosis ("MS"). Approximately 1.25 million people in the United States are living with type 1 diabetes ("T1D"). Over 1.3 million people in the United States have rheumatoid arthritis ("RA"). It has been reported that patients with chronic inflammatory conditions in the United States spend

approximately $38,000 or more per year on additional expenditures. A study in 2010 found that annual costs for privately-insured and Medicare patients with rheumatoid arthritis were $306 million and $600 million, respectively. As many as 70,000 new cases of inflammatory bowel disease are diagnosed each year in the United States alone.

Rodent and human T cells express receptors for the nonprotein amino acid γ-aminobutyric acid ("GABA"). *See* J. Tian *et al.*, *GABA(A) receptors mediate inhibition of T cell responses*, 96 J. NEUROIMMUNOL 21-28 (1999); J. Tian *et al.*, *Gamma-aminobutyric acid inhibits T cell autoimmunity and the development of inflammatory responses in a mouse type 1 diabetes model*, 173 J. IMMUNOL 5298-5304 (2004); S. Alam *et al.*, *Human peripheral blood mononuclear cells express GABAA receptor subunits*, 43 MOL. IMMUNOL 1432-1442 (2006); S. K. Mendu *et al.*, *Different subtypes of GABA-A receptors are expressed in human, mouse and rat T lymphocytes*, 7 PLOS ONE e42959 (2012); G. J. Prud'homme *et al.*, *GABA Protects Human Islet Cells Against the Deleterious Effects of Immunosuppressive Drugs and Exerts Immunoinhibitory Effects Alone*, 96 TRANSPLANTATION 616-623 (2013). GABA is a commonly-used neurotransmitter in the CNS and its neurobiological activities in brain development and function have been extensively studied. There are two types of GABA-receptors ("GABA-Rs") that are encoded by distinct gene families and their activation induces different pathways – $GABA_A$-Rs are fast-acting chloride channels and $GABA_B$-Rs are slow-acting G-protein coupled receptors. *See* R. W. Olsen *et al.*, *Molecular biology of GABAA receptors*, 4 FASEB J 1469-1480 (1990); B. Bettler *et al.*, *Molecular structure and physiological functions of GABA(B) receptors*, 84 PHYSIOL REV 835-867 (2004). Rodent and human T cells expresses functional $GABA_A$-Rs but appear unresponsive to $GABA_B$-R-specific agonists. *See* J. Tian *et al.*, *GABA(A) receptors mediate inhibition of T cell responses*, 96 J. NEUROIMMUNOL 21-28 (1999); S. Alam *et al.*, *Human peripheral blood mononuclear cells express GABAA receptor subunits*, 43 MOL IMMUNOL 1432-1442 (2006); S. K. Mendu *et al.*, *Different subtypes of GABA-A receptors are*

expressed in human, mouse and rat T lymphocytes, 7 PLOS ONE e42959 (2012); G. J. Prud'homme *et al.*, *GABA protects human islet cells against the deleterious effects of immunosuppressive drugs and exerts immunoinhibitory effects alone*, 96 TRANSPLANTATION 616-623 (2013). In mice, GABA administration limited delayed-type hypersensitivity ("DTH") responses and inhibited or reversed disease in models of T1D (*see* Tian *et al.*, *GABA(A) receptors mediate inhibition of T cell responses*, 96 J. NEUROIMMUNOL 21-28 (1999); J. Tian *et al.*, *Gamma-aminobutyric acid inhibits T cell autoimmunity and the development of inflammatory responses in a mouse type 1 diabetes model*, 173 J. IMMUNOL 5298-5304 (2004); G. J. Prud'homme *et al.*, *GABA protects human islet cells against the deleterious effects of immunosuppressive drugs and exerts immunoinhibitory effects alone*, 96 TRANSPLANTATION 616-623 (2013); N. Soltani *et al.*, *GABA exerts protective and regenerative effects on islet beta cells and reverses diabetes*, 108 PROC. NATL. ACAD. SCI. 11692-11697 (2011); S. K. Mendu *et al., Increased GABA(A) channel subunits expression in CD8(+) but not in CD4(+) T cells in BB rats developing diabetes compared to their congenic littermates*, 48 MOL. IMMUNOL 399-407 (2011); J. Tian *et al.*, *Combined therapy with GABA and proinsulin/alum acts synergistically to restore long-term normoglycemia by modulating T-cell autoimmunity and promoting beta-cell replication in newly diabetic NOD mice*, 63 DIABETES 3128-3134 (2014)), rheumatoid arthritis (*see* J. Tian *et al.*, *Oral GABA treatment downregulates inflammatory responses in a mouse model of rheumatoid arthritis*, 44 AUTOIMMUNITY 465-470 (2011)), and limited inflammation and disease in type 2 diabetes models. *See* J. Tian *et al.*, *Oral treatment with gamma-aminobutyric acid improves glucose tolerance and insulin sensitivity by inhibiting inflammation in high fat diet-fed mice*, 6 PLOS ONE e25338 (2011); S. Sohrabipour *et al.*, *GABA dramatically improves glucose tolerance in streptozotocin-induced diabetic rats fed with high-fat diet*, EUR J PHARMACOL 2018.01.047 (2018). Studies of the mechanisms underlying those observations revealed that GABA treatment inhibited the development of autoreactive Th1 responses while

also promoting $CD4^+$ regulatory T cells ("Tregs"). These preclinical studies provided the basis for an ongoing clinical trial in which GABA is being given to individuals newly diagnosed with T1D (NCT02002130).

While autoreactive Th1 cells are thought to be primary drivers of DTH responses, T1D, and RA disease progression, autoreactive Th17 responses are thought to play a major pathogenic role in EAE and MS. See M. Rangachari *et al.*, *Using EAE to better understand principles of immune function and autoimmune pathology*, 45 J AUTOIMMUN 31-39 (2013); A. M. McGinley *et al.*, *Th17cells, gammadelta T cells and their interplay in EAE and multiple sclerosis*, J AUTOIMMUN 2018.01.001 (2018). Also, while DTH, T1D, and RA are mediated by peripheral autoimmune responses, disease relapses in experimental autoimmune encephalomyelitis ("EAE") and MS are thought be due to the spreading of T cell autoreactivity within the CNS. *See* B. L. McRae *et al.*, *Functional evidence for epitope spreading in the relapsing pathology of experimental autoimmune encephalomyelitis*, 182 J. EXP MED 75-85 (1995); O. S. Targoni *et al.*, *Frequencies of neuroantigen-specific T cells in the central nervous system versus the immune periphery during the course of experimental allergic encephalomyelitis*, 166 J. IMMUNOL 4757-4764 (2001); E. J. McMahon *et al.*, *Epitope spreading initiates in the CNS in two mouse models of multiple sclerosis*, 11 NAT MED 335-339 (2005). Orally administered GABA, however, has little to no ability to pass through the blood-brain barrier ("BBB") (*see* K. Kuriyama *et al.*, *Blood-brain barrier to H3-gamma-aminobutyric acid in normal and amino oxyacetic acid-treated animals*, 10 NEUROPHARMACOLOGY 103-108 (1971)), making it ill-suited to inhibit the spreading of T cell autoreactivities within the CNS. To circumvent that limitation, Steinman and colleagues showed that two clinically-applicable BBB-permeable anti-seizure medications that increase GABAergic tone, topiramate and vigabatrin, inhibited EAE. *See* R. Bhat *et al.*, *Inhibitory role for GABA in autoimmune inflammation*, 107 PROC NATL ACAD SCI USA 2580-2585 (2010). Topiramate, however, primarily affects other neurotransmitter systems, *e.g.*,

sodium and calcium channels, and enzymes (*see* H. S. White, *Molecular pharmacology of topiramate: managing seizures and preventing migraine*, 45 (Suppl 1) HEADACHE S48-56 (2005); R. J. Porter *et al.*, *Mechanisms of action of antiseizure drugs*, 108 HANDB CLIN NEUROL 663-681 (2012)) and vigabatrin does not bind to GABA-Rs but rather inhibits GABA transaminase (*see* R. J. Porter *et al.*, *Mechanisms of action of antiseizure drugs*, 108 HANDB CLIN NEUROL 663-681 (2012)), and both drugs have multiple adverse effects. Other studies have shown that the anti-alcohol dependence drug acamprosate, which has structural similarities with GABA, can limit EAE (*see* S. Sternberg *et al.*, *Acamprosate modulates experimental autoimmune encephalomyelitis*, 20 INFLAMMOPHARMACOLOGY 39-48 (2012)), but recent studies indicate that acamprosate does not act directly through $GABA_A$-Rs. *See* N. J. Kalk *et al.*, *The clinical pharmacology of acamprosate*, 77 BR J CLIN PHARMACOL 315-323 (2014); M. T. Reilly *et al.*, *Effects of acamprosate on neuronal receptors and ion channels expressed in Xenopus oocytes*, 32 ALCOHOL CLIN EXP RES 188-196 (2008). Benzodiazepines and barbiturates are BBB-permeable $GABA_A$-R positive allosteric modulators that can potentiate the opening of $GABA_A$-R chloride channels, but only after a $GABA_A$-R agonist opens the channel, and these drugs can be addictive.

Accordingly, there is a need for BBB-permeable $GABA_A$-R-specific agonists with excellent safety profiles that can limit inflammation in the CNS. It has now been surprisingly found, as detailed below, that homotaurine is a $GABA_A$-R-specific agonist with an excellent safety profile that can limit inflammation, including in the CNS.

Homotaurine is a natural amino acid found in algae. Homotaurine emerged as a leading candidate from a screen for compounds that physically interfered with the ability of amyloid peptide to form fibrils *in vitro*. *See* T. M. Wright *et al.*, *Tramiprosate*, 42 DRUGS TODAY 291-298 (2006); F. Gervais *et al.*, *Targeting soluble Abeta peptide with Tramiprosate for the treatment of brain amyloidosis*, 28 NEUROBIOL AGING 537-547 (2007). Subsequent studies

found that oral homotaurine can pass through the BBB and limit amyloid plaque deposition in the brain of transgenic mice that over-expressed human amyloid protein. Based on these observations, homotaurine, also known as Tramiprosate or Alzhemed, was tested in a large double-blind randomized phase III clinical trial for its ability to slow cognitive loss over 1.5 years in patients with Alzheimer's disease. *See* P. S. Aisen *et al.*, *Tramiprosate in mild-to-moderate Alzheimer's disease - a randomized, double-blind, placebo-controlled, multi-centre study (the Alphase Study)*, 7 ARCH MED SCI 102-111 (2010); S. Gauthier *et al.*, *Effect of tramiprosate in patients with mild-to-moderate Alzheimer's disease: exploratory analyses of the MRI sub-group of the Alphase study*, 13 J NUTR HEALTH AGING 550-557 (2009). While homotaurine treatment was found to *not* slow cognitive decline, it had an excellent safety profile in this long-term study. Recently, it has become appreciated that homotaurine can also act as a $GABA_A$-R-specific agonist. Homotaurine has >3-fold higher affinity for classical $GABA_A$-Rs and a longer half-life than GABA in plasma (3 hours vs. 20 minutes for GABA). *See* F. Gervais *et al.*, *Targeting soluble Abeta peptide with Tramiprosate for the treatment of brain amyloidosis*, 28 NEUROBIOL AGING 537-547 (2007); D. B. Tower & E. Roberts, *The Administration of Gamma-Aminobutyric Acid to Man: Systemic Effects and Anticonvulsant Actions*; INHIBITION IN THE NERVOUS SYSTEM AND GAMMA AMINOBUTYRIC ACID 562-578 (1960); M. V. Jones *et al.*, *Defining affinity with the GABAA receptor*, 18 J NEUROSCI 8590-8604 (1998).

That homotaurine is a $GABA_A$-R-specific agonist with an excellent safety profile that can limit inflammation in the CNS is surprising at least because in the case of MS, GABA is not expected to be therapeutic because the spreading of autoimmunity occurs in the CNS (*see, e.g.*, B. L. McRae *et al.*, *Functional evidence for epitope spreading in the relapsing pathology of experimental autoimmune encephalomyelitis*, 182 J. EXP MED 75-85 (1995); O. S. Targoni *et al.*, *Frequencies of neuroantigen-specific T cells in the central nervous system versus the immune periphery during the course of experimental allergic encephalomyelitis*, 166 J. IMMUNOL 4757-

4764 (2001); E. J. McMahon *et al.*, *Epitope spreading initiates in the CNS in two mouse models of multiple sclerosis*, 11 NAT MED 335-339 (2005); R. Bhat *et al.*, *Inhibitory role for GABA in autoimmune inflammation*, 107 PROC NATL ACAD SCI USA 2580-2585 (2010)) and GABA does not penetrate the BBB. As explained above, attempts were made to circumvent that limitation, but it was found that topiramate primarily affected other neurotransmitter systems and vigabatrin does not bind to GABA-Rs and both drugs have multiple adverse effects. *See* R. Bhat *et al.*, *Inhibitory role for GABA in autoimmune inflammation*, 107 PROC NATL ACAD SCI USA 2580-2585 (2010); H. S. White, *Molecular pharmacology of topiramate: managing seizures and preventing migraine*, 45 (Suppl 1) HEADACHE S48-56 (2005); R. J. Porter *et al.*, *Mechanisms of action of antiseizure drugs*, 108 HANDB CLIN NEUROL 663-681 (2012); R. J. Porter *et al.*, *Mechanisms of action of antiseizure drugs*, 108 HANDB CLIN NEUROL 663-681 (2012). While the researchers sought to modulate immune cell GABA-Rs in brain to inhibit EAE, they chose to do so by indirectly increasing GABA action in the brain using drugs that inhibited GABA breakdown and a drug that had wide-spread actions on several receptor systems. This is because the researchers did not recognize they could directly activate GABA-Rs with homotaurine. Moreover, despite a detailed, phase III study of homotaurine for the possible effect of slowing cognitive loss in Alzheimer's patients, those in the field continued to fail to appreciate that homotaurine could be a GABA-R agonist and no CNS side-effects were found in that study (the study involved over 1,000 people, all possible side effects were required to be listed and none were mentioned, and those given homotaurine reported no complaints about CNS effects over 1.5 years of treatment). And, there was evidence that GABA could actually exacerbate EAE/MS. *See, e.g.,* S. Carmans *et al.*, *Systemic treatment with the inhibitory neurotransmitter γ-aminobutyric acid aggravates experimental autoimmune encephalomyelitis by affecting proinflammatory immune responses*, 255(1-2) J NEUROIMMUNOL 45-53 (Feb. 15, 2013). Put

differently, it was not evident in the field that there existed a GABA-R agonist that could both cross the BBB and be safe.

In view the prevalence of chronic inflammatory disorders, the impact of those disorders on patients' lives, and the economic impact to society, there is an unmet need for clinical treatments that can safely limit the progression of chronic inflammatory disorders. This is especially true for T-cell mediated autoimmune disorders such as T1D, RA, and MS in which inhibiting established autoimmune responses against the target tissue may require life-time treatment.

SUMMARY OF THE INVENTION

Provided is a blood-brain barrier-permeable $GABA_A$-R-specific agonist with an excellent safety profile that can limit inflammation-related disorders, including inflammation in the CNS.

Also provided are methods for treating inflammation-related disorders, including those of the CNS, comprising administering homotaurine, wherein said administration may be oral.

Also provided are methods for inducing $CD8^+$ and $CD4^+$ regulatory T cell responses in a subject comprising administering a safe, blood-brain barrier-permeable $GABA_A$-R-specific agonist, such as homotaurine, wherein said administration may be oral.

Also provided are methods for inhibiting autoreactive Th17 and Th1 responses in a subject, comprising administering a safe, blood-brain barrier-permeable $GABA_A$-R-specific agonist, such as homotaurine, wherein said administration may be oral.

Also provided are methods for treating type 1 diabetes in a human or animal subject in need thereof, said method comprising administering to said human or animal subject one or more immunomodulator compounds and one or more GABA-receptor agonists in an amount effective to prevent, reduce, and/or treat hyperglycemia in said human or animal subject.

Also provided are methods for raising C peptide levels in a human or animal subject in need thereof, said method comprising administering to said human or animal subject an effective amount of homotaurine, wherein said administration may be oral.

Also provided are methods for lowering A1C levels in a human or animal subject in need thereof, said method comprising administering to said human or animal subject an effective amount of homotaurine, wherein said administration may be oral.

Also provided are methods for promoting islet cell survival in a human or animal subject in need thereof, said method comprising administering to said human or animal subject an effective amount of homotaurine, wherein said administration may be oral.

Also provided are methods for promoting β-cell replication in a human or animal subject in need thereof, said method comprising administering to said human or animal subject an effective amount of homotaurine, wherein said administration may be oral.

Also provided are methods for inhibiting T cell proliferation in a human or animal subject in need thereof, said method comprising administering to said human or animal subject an effective amount of homotaurine, wherein said administration may be oral.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts that homotaurine inhibits EAE progression after the onset of symptoms in both monophasic and relapse-remitting mouse models of MS. For Figure 1A, C57Bl/6 mice were immunized with MOG_{35-55} and monitored daily for clinical signs of EAE as described in below in the Methods section. Eleven to thirteen days post-immunization all of the mice developed EAE. When the mice reached an EAE score of 1 they were randomized to receive plain water or water containing homotaurine (0.25 mg/ml). Graph shows mean EAE scores +/- SEM for mice that received plain water (circles) and homotaurine (triangles) after EAE onset. N=5 mice/group, overall p=0.01, by Kruskal-Wallis analysis. For Figure 1B, SJL mice were

immunized with $PLP_{139-151}$ and ten to twelve days later all of the mice develeoped clinical symptoms. When the mice reached an EAE score of 1 they were randomized to receive plain water or water containing homotaurine. Graph shows mean EAE scores +/-SEM for mice that received plain water (circles) or homotaurine (triangles) continuously after EAE onset. N=8 mice/group, *$p<0.05$, overall $p<0.01$. Figure 1C depicts spinal cord (left panel) and cerebellum (right panel) and in control and homotaurine-treated $PLP_{139-151}$ immunized SJL mice. Twenty-seven days after treatment, adjacent sections were stained with hematoxylin and eosin (H&E, top row) and luxol fast blue or black gold (bottom row). Arrow in H&E stained sections indicates infiltrates. Luxol fast blue and black gold staining revealed greater myelination and fine myelin fibers in homotaurine-treated mice (arrows). Left panel scale bar=1mm, right panel scale bar=250um.

Figure 2 depicts that homotaurine treatment at time of clinical EAE onset reduces the frequency of IL-17A- and IFNγ-secreting T cell responses, while increasing IL-10-secreting responses. Mice were $PLP_{139-151}$ immunized and after reaching an EAE score of 1, they received plain water or homotaurine for five days. Splenic T cells were isolated from individual mice and the percentages of IL-17A, IFNγ and IL-10 secreting T cell responses to $PLP_{139-151}$ were measured by ELISPOT. Data shown is the mean SFC ± SEM of each group of mice (n=5 per group) from two separate experiments. IL-4 responses to $PLP_{139-151}$ were at background levels (data not shown). Wells containing cells from control or homotaurine-treated mice that were incubated with medium alone had 0-5 SFC. Pairwise comparisons were performed by 2-tailed Student's t test. **$p<0.01$ vs. the controls.

Figure 3 depicts that homotaurine treatment enhances $CD4^+$ and $CD8^+$ Treg responses in mice. SJL mice were immunized with PLP_{39-151} in 50% CFA and when the mice developed EAE with a score of 1, they were randomized and provided with plain water and water containing 0.25 mg/ml homotaurine for five days. The percentages of splenic (Figure 3A)

$CD4^+Foxp3^+$, (Figure 3B, Figure 3C) $CD8\alpha^+CD122^+$ and $CD8\alpha^+CD122^+PD\text{-}1^+$ Tregs were determined by flow cytometry. The cells were gated first on living lymphocytes and $CD4^+$ or $CD8^+$ and the percentages of $CD4^+Foxp3^+$, $CD8\alpha^+CD122^+PD\text{-}1^-$ and $CD8\alpha^+CD122^+PD\text{-}1^+$ Tregs were analyzed. Data are representative flow cytometry charts or expressed as the mean ± SEM of each group of mice (n=5 per group) from two separate experiments. *p<0.05, **p<0.01 vs. the controls.

Figure 4 depicts that homotaurine promotes human islet cell replication. Human islets were cultured with, or without, the indicated dosages of homotaurine for 4 days. Data shown are the average rate of proliferation relative to that of cultures with medium alone (designated as 1). N=two independent studies with triplicate cultures. *p<0.05, **p<0.01 and ***p<0.001 for indicated homotaurine dose vs. medium alone as determined by Student T-test.

Figure 5 depicts that oral homotaurine enhances human islet β-cell replication in a xenograft model. STZ-rendered mildly hyperglycemic NOD/scid mice were transplanted with human islets under their kidney capsule. The mice were randomized and provided with water containing with BrdU, with or without homotaurine (0.08 or 0.25 mg/ml mg/ml) or GABA (6 mg/ml) for 12 days. The percentages of replicated β-cells were determined by immunofluorescent assays using anti-insulin and anti-BrdU or anti-Ki67, followed by counterstaining with DAPI. Figure 5A is a graphical representation of the percentages of $BrdU^+insulin^+$ β-cells, and Figure 5B is $Ki67^+insulin^+$ islet cells in total $insulin^+$ β-cells. Data are mean ± SEM from two independent experiments, each using islets from a human donor that were implanted into 4-9 NOD/scid mice. *P<0.05, **P<0.01 vs. the control.

Figure 6 depicts that homotaurine treatment protects human islet ß-cells from apoptosis in islet xenografts. Diabetic NOD/scid mice were implanted with human islets and treated with plain water or water containing homotaurine (0.08 or 0.25 mg/ml) or GABA (6 mg/ml) for 48 h. The percentages of apoptotic islet cells and islet ß-cells in total human islet cells were

determined by TUNEL assay and co-staining with anti-insulin as well as DAPI. At least 2,000 human islet cells in 10 fields (magnification x 400) from individual grafts were counted. Data are representative image or expressed as the mean % ± SEM for each group of mice (n = 5-7) from three separate experiments. Figure 6A shows the percent apoptotic islet cells and Figure 6B shows the percent insulin^{+} islet cells. The difference among groups was analyzed by ANOVA and post hoc Fisher's least significant difference and the difference between groups was determined Student T-test. **$p<0.01$ vs. the control.

Figure 7 depicts that homotaurine inhibits anti-CD3 stimulated splenic T cell responses. Splenic mononuclear cells were cultured with anti-CD3 and ^{3}H thymidine in the presence or absence of the indicated concentrations of homotaurine or GABA as described in Methods. *$P<0.05$, **$P<0.01$, ***$P<0.01$ vs. cultures without homotaurine or GABA added.

Figure 8 depicts a homotaurine dose-finding study and a combined homotaurine treatment with proinsulin/alum in newly diabetic NOD mice. In pilot studies, newly diabetic NOD mice were untreated (Figure 8A), or continually given homotaurine at 0.08 mg/ml (n=6) (Figure 8B), 0.25 mg/ml (n=9) (Figure 8C), or 0.75 mgs/ml (n=12) (Figure 8D) through their drinking water. Subsequently, another group of newly diabetic NOD mice received both homotaurine (0.25 mg/ml) and proinsulin/alum immunization (n=9) (Figure 8E). Data shown are longitudinal blood glucose levels for individual mice. Dashed line indicates blood glucose of 250 mg/dL. The percentage of mice in each treatment group that remained relapse free over a 45 week period are shown in Figure 8F.

Figure 9 depicts the results of combined homotaurine and low-dose anti-CD3 on hyperglycemia in severly diabetic NOD mice. Newly diabetic NOD mice with severe hyperglycemia were given homotaurine (0.25 mg/ml, n=7; Figure 9A), low-dose anti-CD3 (n=13; Figure 9B), or combined low dose anti-CD3 + homotaurine (n=25; Figure 9C). Figure 9D depicts the percentage of relapse-free mice treated with homotaurine (triangle symbol), low-

dose anti-CD3 (circle symbol), or combined low dose anti-CD3+homoaurine (square symbol) over the 25 week period. Statistical analysis indicates: Homotaurine vs. anti-CD3+homotaurine ($p=0.002$) and anti-CD3 vs. anti-CD3+homotaurine ($p=0.05$) by the log-rank test.

DETAILED DESCRIPTION OF THE INVENTION

Very few of the many drugs that can inhibit EAE successfully translate to clinical application, due to factors such as inherent differences between EAE and MS, toxicity or side-effects in humans, and supra-physiological doses that were used in the EAE studies. In regard to those issues, human T cells express $GABA_A$-Rs whose activation can limit PBMC inflammatory responses (*see* S. Alam *et al.*, *Human peripheral blood mononuclear cells express GABAA receptor subunits*, 43 MOL IMMUNOL 1432-1442 (2006); S. K. Mendu *et al.*, *Different subtypes of GABA-A receptors are expressed in human, mouse and rat T lymphocytes*, 7 PLOS ONE e42959 (2012); G. J. Prud'homme *et al.*, *GABA protects human islet cells against the deleterious effects of immunosuppressive drugs and exerts immunoinhibitory effects alone*, 96 TRANSPLANTATION 616-623 (2013)), homotaurine appears to be safe for long-term human use, and, using FDA guidelines for scaling mouse doses to the equivalent human dosage, the homotaurine dose used to inhibit EAE was 6-10-fold lower than that used in the Alzheimer's disease clinical trials.

T cells express relatively high levels of transcripts encoding the δ subunit of $GABA_A$-Rs. *See* J. Tian *et al.*, *Gamma-aminobutyric acid inhibits T cell autoimmunity and the development of inflammatory responses in a mouse type 1 diabetes model*, 173 J. IMMUNOL 5298-5304 (2004); S. Alam *et al.*, *Human peripheral blood mononuclear cells express GABAA receptor subunits*, 43 MOL IMMUNOL 1432-1442 (2006); S. K. Mendu *et al.*, *Different subtypes of GABA-A receptors are expressed in human, mouse and rat T lymphocytes*, 7 PLOS ONE e42959 (2012); G. J. Prud'homme *et al.*, *GABA Protects Human Islet Cells Against the Deleterious Effects of*

Immunosuppressive Drugs and Exerts Immunoinhibitory Effects Alone, 96 *Transplantation* 616-623 (2013). The δ subunit is of particular interest because it is found in "extrasynaptic" $GABA_A$-Rs that can be orders of magnitude more sensitive to GABA than the typical $GABA_A$-Rs subtypes that are clustered in neuronal synapses. *See* Z. Nusser *et al.*, *Segregation of different GABAA receptors to synaptic and extrasynaptic membranes of cerebellar granule cells*, 18 J. NEUROSCI 1693-1703 (1998); G. B. Richerson, *Looking for GABA in all the wrong places: the relevance of extrasynaptic GABA(A) receptors to epilepsy*, 4 EPILEPSY CURR 239-242 (2004); M. Farrant *et al.*, *The cellular, molecular and ionic basis of GABA(A) receptor signalling*, 160 PROG BRAIN RES 59-87 (2007); S. I. Storustovu *et al.*, *Pharmacological characterization of agonists at delta-containing GABAA receptors: Functional selectivity for extrasynaptic receptors is dependent on the absence of gamma2*, 316 J PHARMACOL EXP THER 1351-1359 (2006); P. Meera *et al.*, *Molecular basis for the high THIP/gaboxadol sensitivity of extrasynaptic GABA(A) receptors*, 106 J NEUROPHYSIOL 2057-2064 (2011). These δ subunits may confer T cells with sensitivity to low levels of GABA and contribute to CNS "immunological privilege." In this scenario, endogenous CNS GABA may help regulate the low-frequency, low-avidity CNS-reactive T cells that spontaneously activate under normal conditions, but it is insufficient to regulate the large number of high-avidity autoreactive T cells that are experimentally activated in EAE. As shows above, administration of a BBB-permeable high-affinity $GABA_A$-R agonist is capable of limiting CNS autoreactivity under those conditions.

These findings provide insights into the actions of $GABA_A$-Rs on lymphocytes and demonstrate that homotaurine can enhance $CD8^+$ Tregs, limit autoreactive Th17 cells, and ameliorate inflammation-related disorders of the CNS, such as Alzheimer's Disease, amyotrophic lateral sclerosis, asthma, atherosclerosis, cerebral abscess, cerebral ischaemia, Crohn's disease, encephalitis, hepatitis, inflammatory bowel disease, lupus, meningitis,

migraines, multiple sclerosis, obesity, Parkinson's disease, periodontitis, rheumatoid arthritis, sarcoidosis, stroke, tuberculosis, type 1 diabetes, ulcerative colitis, ulcers, and vasculitis.

Effective interventional therapies for T1D has been impeded by insufficient ability to safely control ß-cell autoreactivity and also promote ß-cell mass. In an effort to overcome these impediments, studies with homotaurine, a $GABA_A$-R specific against that appeared to be safe in long-term clinical trials for another indication, and whose pharmacodynamics properties could have advantages over GABA for clinical use, were conducted.

It was observed that homotaurine increased human islet cell proliferation *in vitro*, with a U-shaped dose-response curve. This pro-mitotic effect was abrogated by the $GABA_A$-R antagonist bicuculline, consistent with the notion that the proliferative effect was mediated through $GABA_A$-Rs. *In vivo*, homotaurine significantly increased ß-cell replication within human islets xenografts as measured by both BrdU/insulin and Ki67/insulin immunostaining.

The mechanisms by which $GABA_A$-R activation promotes ß-cell replication have been studied in rodent and human islets. *See* N. Soltani *et al.*, *GABA exerts protective and regenerative effects on islet beta cells and reverses diabetes*, 108 PROC NATL ACAD SCI USA 11692-11697 (2011); M. Braun *et al.*, *Gamma-aminobutyric acid (GABA) is an autocrine excitatory transmitter in human pancreatic beta-cells*, 59 DIABETES 1694-1701 (2010); I. Purwana *et al.*, *GABA promotes human beta-cell proliferation and modulates glucose homeostasis*, 63 DIABETES 4197-4205 (2014); H. Dong *et al.*, *Gamma-aminobutyric acid up- and downregulates insulin secretion from beta cells in concert with changes in glucose concentration*, 49 DIABETOLOGIA 697-705 (2006). The activation of $GABA_A$-Rs opens a Cl^- channel, causing membrane depolarization and Ca^{2+} influx. This leads to activation of the PI3K/Akt pathway and the enhancement of ß-cell replication and survival. Although homotaurine is a $GABA_A$-R specific agonist, it promoted ß-cell replication to a similar extent as GABA, which activates both $GABA_A$-Rs and $GABA_B$-Rs.

Homotaurine treatment also significantly preserved human islet cells from apoptosis in human islet grafts. Since a large percentage of ß-cells die shortly after transplantation due to apoxia and other stressors, short-term homotaurine treatment appears to be a promising adjunct therapy to help preserve ß-cell mass following islet grafting and reduce the number of islets necessary to achieve insulin independence.

In vitro studies of homotaurine's effects on anti-CD3-stimulated T cell proliferation showed that it inhibited mitogenesis at lower concentrations than GABA, but whereas GABA's dose-response was liner, homotaurine's inhibitory actions followed a U-shaped curve. In interventional studies with newly-diabetic NOD mice, it was observed that the lowest homotaurine dose tested (0.08 mg/ml) showed a slight ability to delay disease progression compared to untreated controls. An intermediate dose of 0.25 mg/ml homotaurine reversed for a median time of 14 weeks with a few remaining normoglycemic the 46-week observation period. The higher dose of 0.75 mg/ml displayed some therapeutic effects, but to a lesser degree than that of the intermediate dose, again suggesting a U-shaped dose-response.

Homotaurine was also combined with an antigen-specific immunotherapy, proinsulin/alum immunization. Proinsulin/alum immunization on its own did not display any therapeutic effects in newly-diabetic NOD mice in a previous study. *See* J. Tian *et al.*, *Combined therapy with GABA and proinsulin/alum acts synergistically to restore long-term normoglycemia by modulating T-cell autoimmunity and promoting beta-cell replication in newly diabetic NOD mice*, 63 DIABETES 3128-3134 (2014). The combination of homotaurine with proinsulin/alum had enhanced therapeutic compared to either monotherapy; all mice became normoglycemic and the average remission period was 24 weeks compared to that of 14 weeks for homotaurine monotherapy. Of the mice treated with homotaurine+proinsulin/alum, about 5 of 9 mice relapsed before 12 weeks post-treatment, suggesting that reboosting with proinsulin/alum might be advantageous. However, 2/9 of the mice remained in remission until the end of the study at

46 weeks post-treatment.

Homotaurine in combination with low-dose anti-CD3 treatment in newly-diabetic NOD mice with severe hyperglycemia was also tested, as detailed below. While the remission rate was 27% following low-dose anti-CD3 monotherapy, the combination treatment had a remission rate of 65%, which was not statistically different from the 75% remission rate following low-dose anti-CD3+GABA. The vast majority of the mice that went into remission remained normoglycemic throughout the ensuing months. Thus, these combination treatments more than doubled the frequency of disease reversal in mice that had been severely hyperglycemic. It is notable that the increase in remission frequency following combination treatment took place within the first week following treatment. This increased response soon after treatment suggests that homotaurine acted quickly to quell autoimmune responses and/or preserve residual ß-cells. Indeed, homotaurine acted quickly to limit ß-cell apoptosis immediately following human islet xenografting (*see Figure 5*), and has rapid effects on inflammatory T cell responses in our *in vitro* assessments (*see Figure 7*).

These studies provide preclinical evidence for homotaurine's beneficial effects on inflammatory immune responses and ß-cell survival and replication. These studies indicate that homotaurine in combination with immunoregulatory and low-dose immunosuppressive agents can more effectively treat new onset T1D that the respective monotherapies.

In one embodiment, the present application relates to methods for treating an inflammation-related disorder, such as inflammation in the central nervous system, in a human or animal subject in need thereof, comprising administering to said human or animal subject an effective amount of a safe, blood-brain barrier-permeable $GABA_A$-R-specific agonist. In certain embodiments, the safe, blood-brain barrier-permeable $GABA_A$-R-specific agonist is homotaurine, wherein administration may be oral.

In certain embodiments, the inflammation-related disorder is selected from the group consisting of Alzheimer's Disease, amyotrophic lateral sclerosis, asthma, atherosclerosis, autism spectral disorders, cerebral abscess, cerebral ischaemia, cognitive disorders, Crohn's disease, deleterious immune responses to infections, encephalitis, hepatitis, inflammatory bowel disease, lupus, meningitis, migraines, multiple sclerosis, neurodegenerative diseases, neuroinflammation, neuropathic pain, obesity, paraneoplastic disorders, Parkinson's disease, periodontitis, rheumatoid arthritis, sarcoidosis, schizophrenia, stroke, traumatic brain injury, tuberculosis, type 1 diabetes, ulcerative colitis, ulcers, and vasculitis.

In certain embodiments, the inflammation-related disorder is a T-cell mediated disorder, a macrophage mediated disorder, a dendritic cell mediated disorder, and/or a microglia mediated disorder.

In certain embodiments, the T-cell mediated disorder is type 1 diabetes, rheumatoid arthritis, and/or multiple sclerosis.

In certain embodiments, the safe, blood-brain barrier-permeable $GABA_A$-R-specific agonist, such as homotaurine, is administered orally, subcutaneously, sublingually, intramuscularly, intraperitoneally, and/or transdermally.

In certain embodiments, the present application relates to methods for inducing $CD8^+$ and $CD4^+$ regulatory T cell responses in a subject in need thereof, comprising administering to said subject an effective amount of a safe, blood-brain barrier-permeable $GABA_A$-R-specific agonist. In certain embodiments, the safe, blood-brain barrier-permeable $GABA_A$-R-specific agonist is homotaurine, wherein administration may be oral.

In other embodiments, the present application relates to methods for inhibiting autoreactive Th17 and Th1 responses in a subject in need thereof, comprising administering to said subject an effective amount of a safe, blood-brain barrier-permeable $GABA_A$-R-

specific agonist. In certain embodiments, the safe, blood-brain barrier-permeable $GABA_A$-R-specific agonist is homotaurine, wherein administration may be oral.

In other embodiments, the present application relates to methods for treating type 1 diabetes in a human or animal subject in need thereof, comprising administering to said human or animal subject one or more immunomodulator compounds and one or more GABA-receptor agonists in an amount effective to prevent, reduce, and/or treat hyperglycemia in said human or animal subject. In certain embodiments, one or more immunomodulator compounds comprise one or more immunoregulators, immunostimulants, or immunosuppressants. In particular examples, the one or more immunomodulator compounds comprise one or more immunosuppressants. In certain embodiments, the one or more immunomodulator compounds are selected from the group consisting of an anti-CD3 immunotherapy compound, corticosteroids, prednisone, budesonide, prednisolone, methylprednisolone, calcineurin inhibitors, cyclosporine, tacrolimus, mTOR inhibitors, sirolimus, everolimus, IMDH inhibitors, azathioprine, leflunomide, mycophenolate, biologics, abatacept, adalimumab, anakinra, certolizumab, etanercept, golimumab, infliximab, ixekizumab, natalizumab, rituximab, secukinumab, tocilizumab, ustekinumab, vedolizumab, monoclonal antibodies, basiliximab, daclizumab, muromonab, anti-lymphocyte globin, anti-thymocyte globin, lymphocyte immune globulin, thymoglobulin, mycophenolate mofetil, mycophenolate sodium, glucocorticoids, NSAIDs / COX inhibitors (*e.g.*, aspirin, celecoxib, diclofenac, diflunisal, etodolac, ibuprofen, indomethacin, ketoprofen, ketorolac, nabumetone, naproxen, oxaprozin, piroxicam, salsalate, sulindac, and tolmetin), methotrexate, hydroxychloroquine, sulfasalazine, copaxone, and interferon β. In particular examples, the one or more immunomodulator compounds comprise an anti-CD3 immunotherapy compound. In other examples, the anti-CD3 immunotherapy compound comprises non-Fc binding anti-CD3ε FabIn certain embodiments, the one or more

immunomodulator compound comprises an anti-CD3 immunotherapy compound. In certain embodiments, the one or more GABA-receptor agonist is homotaurine. In certain embodiments, the one or more immunomodulator compound comprises an anti-CD3 immunotherapy compound and the one or more GABA-receptor agonist is homotaurine. In certain embodiments, the effective amount is lower than the effective amount for monotherapy.

In another embodiment, the GABA-receptor agonist is homotaurine and the immunomodulator is copaxone.

In another embodiment, the GABA-receptor agonist is homotaurine and the immunomodulatory is interferon β.

In other embodiments, the present application relates to methods for raising C peptide levels in a human or animal subject in need thereof, comprising administering to said human or animal subject an effective amount of homotaurine, wherein administration may be oral.

In other embodiments, the present application relates to methods for lowering A1C levels in a human or animal subject in need thereof, comprising administering to said human or animal subject an effective amount of homotaurine, wherein administration may be oral.

In other embodiments, the present application relates to methods for promoting islet cell survival in a human or animal subject in need thereof, comprising administering to said human or animal subject an effective amount of homotaurine, wherein administration may be oral.

In other embodiments, the present application relates to methods for promoting β-cell replication in a human or animal subject in need thereof, comprising administering to said human or animal subject an effective amount of homotaurine, wherein administration may be oral.

In other embodiments, the present application relates to methods for inhibiting T cell proliferation in a human or animal subject in need thereof, comprising administering to said human or animal subject an effective amount of homotaurine, wherein administration may be oral.

The invention is set forth in more detail in the following illustrative Examples and accompanying Figures, which demonstrate additional attributes and advantages of the invention. The Examples represent select embodiments of the invention and are not limiting.

EXAMPLES

Example 1 – Ability of Homotaurine to Limit Disease Progression

This example tested the ability of homotaurine to limit disease progression in the chronic model of EAE that is induced by immunizing C57Bl/6 mice with a myelin oligodendrocyte glycoprotein peptide (MOG_{35-55}). *See* I. Mendel *et al.*, *A myelin oligodendrocyte glycoprotein peptide induces typical chronic experimental autoimmune encephalomyelitis in H-2b mice: fine specificity and T cell receptor V beta expression of encephalitogenic T cells*, 25 EUR. J. IMMUNOL 1951-1959 (1995). Eleven to thirteen days after receiving the encephalitic regimen, all of the mice began to develop EAE. When an animal reached an EAE severity score of 1 it was randomly assigned to receive plain drinking water, or water containing homotaurine (0.25 mg/ml) for the rest of the observation period. This homotaurine dose was chosen based on dosing studies of homotaurine's ability to reverse disease in newly diabetic NOD mice (data not shown). Control and homotaurine-treated groups of mice drank similar amounts of water each day (≈4 ml/mouse/day). While the control group of mice quickly progressed to more severe EAE, the homotaurine treatment group of mice remained at low clinical score of EAE. *See Figure 1A*.

Example 2 – Ability of Homotaurine to Treat EAE/Multiple Sclerosis

This example tested homotaurine treatment in a relapsing-remitting model of EAE that is induced by immunizing SJL mice with a proteolipid protein peptide ($PLP_{139-151}$). *See* V.K. Tuohy *et al.*, *Identification of an encephalitogenic determinant of myelin proteolipid protein for SJL mice*, 142 J. IMMUNOL. 1523-1527 (1989). Ten to 12 days after receiving the encephalitic regimen, all of the mice began to develop EAE. When an animal reached an EAE severity score of 1 it was randomly assigned to receive plain drinking water, or water containing homotaurine (0.25 mg/ml) for the rest of the study.

Control mice displayed classical relapsing-remitting EAE. Homotaurine-treated mice had reduced mean EAE severity throughout the observation period compared to those given plain water, and eventually displayed almost complete remission. *See Figure 1B*. Histological analysis of their brains and spinal cords revealed reduced mononuclear cell infiltration and areas of myelin loss in the cerebellum and spinal cords of homotaurine-treated mice. *See Figure 1C*. Thus, treatment with homotaurine after the clinical onset of EAE inhibited disease progression in both monophasic and relapsing-remitting mouse models of MS. These results indicate that $GABA_A$-R-mediated pathways are major components of GABA's anti-inflammatory effects.

Example 3 – Homotaurine's Effect on Lymphocytes

This example examined homotaurine's effects on lymphocytes since T cells are the major mediators of EAE and MS. SJL mice were immunized with $PLP_{139-151}$ and 10 days later (when most mice had an EAE score of about 1), the mice were given plain water or water containing homotaurine (0.25 mg.ml) continuously for five days after which their splenic T cells were analyzed by ELISPOT. Consistent with previous studies of GABA treatment (*see* J. Tian *et al.*, *Gamma-aminobutyric acid inhibits T cell autoimmunity and the development of inflammatory responses in a mouse type 1 diabetes model*, 173 J. IMMUNOL

5298-5304 (2004); G. J. Prud'homme *et al.*, *GABA protects human islet cells against the deleterious effects of immunosuppressive drugs and exerts immunoinhibitory effects alone*, 96 TRANSPLANTATION 616-623 (2013); N. Soltani *et al.*, *GABA exerts protective and regenerative effects on islet beta cells and reverses diabetes*, 108 PROC. NATL. ACAD. SCI. 11692-11697 (2011); J. Tian *et al.*, *Combined therapy with GABA and proinsulin/alum acts synergistically to restore long-term normoglycemia by modulating T-cell autoimmunity and promoting beta-cell replication in newly diabetic NOD mice*, 63 DIABETES 3128-3134 (2014); J. Tian *et al.*, *Oral GABA treatment downregulates inflammatory responses in a mouse model of rheumatoid arthritis*, 44 AUTOIMMUNITY 465-470 (2011)) we observed that homotaurine treatment significantly decreased the frequency of splenic IFNγ (Th1) responses, but increased IL-10-secreting responses, to $PLP_{139-151}$. *See Figure 2.* In addition, it is now shown for the first time that $GABA_A$-R activation significantly reduces the frequency of $PLP_{139-151}$-reactive IL-17A (Th17) spot-forming colonies (SFC). *See Figure 2.* These IL-17A SFC represent antigen-specific activated/memory $CD4^+$ T cells since peptide 13mers do not stimulate $CD8^+$ T cells effectively, and it has been demonstrated in the $PLP_{139-151}$/SJL model that IL-17 secreting SFC arise from $CD4^+$ cells and not other cell types. *See* H.H. Hofstetter *et al.*, *Kinetics and organ distribution of IL-17-producing CD4 cells in proteolipid protein 139-151 peptide-induced experimental autoimmune encephalomyelitis of SJL mice*, 178 J. IMMUNOL 1372-1378 (2007). Homotaurine had no effect on Th2 frequencies (data not shown).

Example 4 – Homotaurine Enhances $CD4^+$ and $CD8^+$ Treg Responses in Mice

It is known that GABA treatment can promote $CD4^+$ Treg responses in mice. *See N.* Soltani *et al.*, *GABA exerts protective and regenerative effects on islet beta cells and reverses diabetes*, 108 PROC. NATL. ACAD. SCI. 11692-11697 (2011); J. Tian *et al.*, *Oral treatment*

with gamma-aminobutyric acid improves glucose tolerance and insulin sensitivity by inhibiting inflammation in high fat diet-fed mice, 6 PLOS ONE e25338 (2011). This example examined whether $GABA_A$-R activation might also modulate $CD8^+CD122^+PD\text{-}1^+$ regulatory T cells or IL-10-secreting Bregs, which can play crucial roles in inhibiting inflammation and T cell autoimmunity.

It was found that the percentages of splenic $CD4^+Foxp3^+$ Tregs (*see Figure 3A*) and $CD8^+CD122^+PD\text{-}1^+$, but not $CD8^+CD122^+PD\text{-}1^-$, Tregs (*see Figure 3B and Figure 3C*) in the homotaurine-treated mice were significantly higher than in controls. There was no significant difference in the frequency of splenic $CD19^+IL\text{-}10^+$ Bregs (data not shown).

Materials and Methods for Examples 1-4

Induction of EAE

Nine weeks old female C57Bl/6 or SJL mice were obtained from the Jackson Laboratory and housed in a specific pathogene-free facility with free access of food and water. C57BL/6 mice were immunized subcutaneously with MOG_{35-55} (200 ug), in 50% IFA containing *Mycobacterium tuberculosis* H37R (5 mg/ml, Difco) in multiple sites near the base of their tail on day 1 and injected intraperitoneally with pertussis toxin (200 ng/mouse) on day 1 and 2. Individual SJL mice were immunized with $PLP_{139-151}$ (100 ug/mouse), as described above, but without pertussis toxin injection. The mice were monitored for EAE onset daily: 0, no disease; 1, limp tail; 2, hind limb weakness; 3, complete hind limb paralysis; 4, quadriplegia; and 5, death. Mice that were in between the clear-cut gradations were scored intermediate in increments of 0.5. When the mice developed EAE with a score of 1 at 10-12 days post-immunization, they were randomized to receive plain water or water containing homotaurine (0.25 mg/ml, an optimal dose, based on preliminary studies of T1D prevention in NOD mice).

Histology

Mice were perfused with 4% paraformaldehyde in 0.1M phosphate buffer, fixed overnight at 4C, cryoprotected and frozen. The brain and spinal cord cryostat sections (10 and 20 um) were directly mounted on slides or collected into PBS with 0.06% NaN3. The sections were mounted onto Superfrost+ slides and stained with H&E or Black Gold II. Some mouse brain and spinal cord tissues were paraffin-embedded after fixation and stained with H&E or luxol fast blue. The sections were imaged under a light microscope.

ELISPOT Assays for IFN, IL-17A, and IL-10

SJL mice were immunized with 100 ug $PLP_{139-151}$ in 50% IFA containing 5 mg/ml Mycobacterium tuberculosis H37R. When the mice developed EAE with a score of 1, the mice were randomized and received plain water or water containing 0.25 mg/ml homotaurine for 5 days. Their splenic mononuclear cells were isolated and the frequency of IFNγ, IL-17A, IL-4 and IL-10-secreting T cells responding to antigens were determined by ELISPOT as described previously (*see* J. Tian *et al.*, *Combined therapy with GABA and proinsulin/alum acts synergistically to restore long-term normoglycemia by modulating T-cell autoimmunity and promoting beta-cell replication in newly diabetic NOD mice*, 63 DIABETES 3128-3134 (2014)) with the addition that rat anti-mouse interleukin IL-17 (4 μg/ml, clone TC-11-18H10, BD Biosciences) and biotin-conjugated anti-IL-17 (0.5 μg/ml; Pharmingen, clone TC-11-8H4.1) were used as capture and detection antibodies for IL-17A, respectively. Briefly, splenic mononuclear cells (10^6/well) were stimulated in duplicate with PLP139-151 (15 μg/ml), positive control PPD (5 μg/ml) or mouse serum albumin (50 μg/ml, Sigma) in HL-1 medium for 24 or 48 h. The spot forming colonies (SFC) in each well were counted in a blinded manner.

FACS

When $PLP_{139-151}$ immunized mice developed EAE with a score of 1, they were randomized to receive plain water or water containing 0.25 mg/ml homotaurine for 5 days.

Splenic mononuclear cells (10^6/tube) from the control and homotaurine-treated groups were treated with 1 μg anti-CD16/anti-CD32 and stained with FITC-anti-CD8α, APC-anti-CD122 and PE-anti-PD-1. After being washed, the frequency of $CD8^+CD122^+$ and $CD8^+CD122^+PD\text{-}1^+$ regulatory T cells was determined by flow cytometry. In addition, splenic mononuclear cells (10^6/tube) were stained with FITC-anti-CD4, fixed, permeabilized and intracellularly stained with PE-anti-Foxp3. The frequency of $CD4^+Foxp3^+$ Tregs was determined by flow cytometry. Moreover, splenic mononuclear cells (10^6/tube) were stimulated with 50 nM PMA and 1 μM ionomycine (Sigma) for 2 h and in the presence of monensin for another 3h. The cells were stained with FITC-anti-CD19, fixed, permeabilized and intracellularly stained with PE-anti-IL-10. The cells with isotype controls served as the negative controls. The frequency of $CD19^+IL\text{-}10^+$ Bregs was determined by flow cytometry.

Statistics

EAE scores were evaluated using Kruskal-Wallis test. Pairwise comparisons were performed by 2-tailed Student's t test. $P < 0.05$ was considered significant.

Example 5 – Homotaurine Promotes Islet Cell Replication *In Vitro*

Cultured human islets were treated with or without different concentrations of homotaurine for 4 days and the proliferation of islet cells was determined by ^{3}H-thymidine incorporation. Treatment with 0.1-3 mM homotaurine significantly increased the proliferation index of the human islet cells (*see Figure 4*). The dose-response curve followed an inverted U-shape, with 0.3 mM homotaurine resulting in the highest proliferation index. The addition of bicuculline (50 μM, a $GABA_A$-R antagonist) to the human islet cultures almost completely inhibited homotaurine's ability to stimulate proliferation across the tested homotaurine dose range (*see Figure 4*) indicating that homotaurine-stimulated islet cell proliferation was highly

dependent on $GABA_A$-R activation. Hence, homotaurine-mediated activation of $GABA_A$-Rs promotes human islet cell proliferation *in vitro*.

Example 6 – Homotaurine Promotes β-Cell Replication in Human Islet Xenografts

To assess the nature of the replicating islet cells, a human xenograft model was used and ß-cell replication at the single cell level was immunohistologically examined. NOD/scid mice were rendered diabetic with STZ and then implanted with ~2000 human islets under their kidney capsule. The graft recipients were then provided with water containing BrdU with, or without, homotaurine (at 0.08 or 0.25 mgs/ml) or positive control GABA (6 mgs/ml) for 12 days. All islet graft recipients, including the controls that received plain water, rapidly became normoglycemic and maintained normoglycemia throughout the experiment. There was no significant difference in food and water consumption, as well as body weight, between these groups of mice (data not shown).

Immunohistochemical analysis of the grafted islets revealed that oral homotaurine treatment at both 0.08 and 0.25 mgs/ml significantly increased the frequency of $BrdU^+insulin^+$ and $Ki67^+insulin^+$ islet ß-cells relative to that in human islets from the control mice that received plain water. *See Figure 5*. There was no significant difference in the frequency of $BrdU^+insulin^+$ or $Ki67^+insulin^+$ islet ß-cells between groups of mice treated with GABA or either dose of homotaurine. Thus, the $GABA_A$-R-specific homotaurine can promote human islet ß-cell replication *in vivo* at levels similar to that of GABA (which activates both $GABA_A$ R and $GABA_B$-Rs).

Example 7 – Homotaurine Treatment Improves Human Islet Cell Survival Following Islet Transplantation

The process of human islet isolation and implantation cause a wide range of stressors that lead to the apoptosis of a large proportion of grafted islet cells within a few days

following implantation. *See* J. A. Emamaullee *et al.*, *Factors influencing the loss of beta-cell mass in islet transplantation*, 16 CELL TRANSPLANT 1-8 (2007); A. M. Davalli *et al.*, *A selective decrease in the beta cell mass of human islets transplanted into diabetic nude mice*, 59 TRANSPLANTATION 817-820 (1995). Whether homotaurine administration could limit islet cell apoptosis in human islet xenografts was examined.

Human islets were implanted under the kidney capsule STZ-rendered hyperglycemic NOD/scid mice and recipients placed on plain water, or water containing homotaurine (0.08 or 0.25 mg/ml) or positive control GABA (6 mg/ml). After two days, the implanted kidneys were removed and tissue sections were stained by TUNEL and anti-insulin antibodies. Treatment with 0.08 or 0.25 mg/ml homotaurine significantly reduced the percentages of apoptotic islet cells and increased the frequency of insulin$^+$ ß-cells in human islet grafts. *See Figure 6A.* On average, GABA-treated animals had less frequent apoptotic islet cells and a higher percentage of insulin$^+$ cells than that in homotaurine recipients, but these differences were not statistically significant.

Example 8 – Homotaurine Inhibits Anti-CD3-Stimulated T Cell Proliferation

The ability of homotaurine to limit T cell proliferation in murine splenic T cell cultures that were stimulated with anti-CD3 was examined. As a positive control, GABA was tested over a dose-range. GABA significantly inhibited T cell proliferation beginning at 0.1 mM and this inhibitory effect increased in a dose-dependent manner up to 3 mM (*see Figure 7*), as in previous *in vitro* studies. *See* J. Tian *et al.*, *GABA(A) receptors mediate inhibition of T cell responses*, 96 J NEUROIMMUNOL 21-28 (1999); G. J. Prud'homme *et al.*, *GABA protects human islet cells against the deleterious effects of immunosuppressive drugs and exerts immunoinhibitory effects alone*, 96 TRANSPLANTATION 616-623 (2013). In contrast, homotaurine displayed a "U-shaped" dose-response, with maximal inhibition

occurring at 0.1 mM. The maximal inhibitory effect of homotaurine at 0.1 mM was of similar magnitude to that of GABA at a 3-10-fold higher concentration (0.3-1.0 mM).

Example 9 – Homotaurine Reverses Hyperglycemia in Newly-Diabetic NOD Mice

A dose finding study was performed with homotaurine dissolved in the drinking water at 0, 0.08, 0.25 or 0.75 mg/ml to determine the therapeutic potential in NOD mice. None of the mouse groups under study differed in their water or food consumption or body weights over the course of the study (data not shown). Newly-diabetic NOD mice that were untreated rapidly progressed to severe hyperglycemia within 1 week. *See Figure 8A.* Treatment with homotaurine at 0.08 mg/ml delayed disease progression for a very brief period (mean of 2.2 weeks). *See Figure 8B.* Treatment with homotaurine at 0.25 mg/ml restored normoglycemia in all mice. *See Figure 8C.* Most of these mice became hyperglycemic again within 6 weeks of treatment but a few mice displayed extended remission of 14 to 46 weeks (the end of the study), leading to a mean remission period of 14 weeks for all mice. Newly-diabetic mice treated with higher dosage homotaurine (0.75 mg/ml) also displayed some delay in disease progression (*see Figure 8D*), but this high dosage was on average less effective that the intermediate 0.25 mg/ml dose. Thus, oral homotaurine monotherapy at an appropriate dose can quickly correct hyperglycemia but most of the treated mice become hyperglycemic again within a short period.

Example 10 – A Combined Homotaurine with Proinsulin/Alum Therapy More Effectively Reverses Hyperglycemia Than Either Monotherapy in Newly-Diabetic NOD Mice

Proinsulin/alum immunization alone was previously shown to have little to no therapeutic effect in newly-diabetic NOD mice. *See* J. Tian *et al., Combined therapy with GABA and proinsulin/alum acts synergistically to restore long-term normoglycemia by modulating T-cell autoimmunity and promoting beta-cell replication in newly-diabetic NOD*

mice, 63 DIABETES 3128-3134 (2014). We tested whether the combination of oral homotaurine (0.25 mg/ml) and proinsulin/alum immunization could increase the frequency or length of disease remission in newly diabetic NOD mice. All mice receiving the combination therapy displayed a period of disease remission (*see Figure 8E*). The mice receiving the combination therapy had a mean remission period of 24 weeks, which was an increase of 10 weeks over the mean remission period of homotaurine monotherapy, although this difference was not statistically significant. The percentage of relapse-free mice in all groups is shown in Figure 8F.

Example 11 – Combined Treatment with Homotaurine and Anti-CD3 Reverses Hyperglycemia in Newly-Diabetic NOD Mice

In prior studies, a low-dose anti-CD3 treatment protocol (35 μg anti-CD3 on three consecutive days) was found to reverse hyperglycemia in about a third of newly-diabetic NOD mice with severe hyperglycemia at entry into the study (blood glucose>350 mg/dL). We tested whether combining homotaurine with anti-CD3 would provide improved results. Newly-severely-hyperglycemic NOD mice were treated with low-dose anti-CD3 and placed on plain water or water containing homotaurine (0.25 mg/ml). A control group received homotaurine alone.

Homotaurine monotherapy was unable to induce remission at this later stage of disease (*see Figure 9A*). Low-dose ant-CD3 treatment alone led to disease remission in about 31% of treated animals, although it generally took several weeks for remission to occur (*see Figure 9B, 9D*).

The combination of low-dose anti-CD3 and homotaurine doubled the remission rate (to 64%) compared to that of anti-CD3 treatment alone (p=0.05 vs. low-dose anti-CD3 monotherapy) and 82% of these mice remained in remission throughout the 25 week observation period (*see Figure 9C, 9D*). Thus, the combination of low-dose anti-CD3 and

homotaurine had increased ability to restore and maintain normoglycemia after the development of severe hyperglycemia in NOD mice.

Similarly, addition of GABA treatment to the anti-CD3 regimen resulted in significantly greater disease remission than anti-CD3 monotherapy. *See Table 1.* While a greater percentage of anti-CD3+GABA treated mice responded to therapy compared to those given anti-CD3+homotaurine, there was no statistical difference between these groups. *See Table 1.*

Table 1: Frequency of disease remission following combined subclinical-dose anti-CD3 and GABA agonist treatment vs. low dose anti-CD3 monotherapy

Group	% Responders	P value
Anti-CD3	31%	N/A
Anti-CD3 + homotaurine	64%	0.05
Anti-CD3 + GABA	75%	0.02

An IPGT test was performed on the mice which remained in remission 25 weeks after initiating treatment. We observed no significant difference between mice that received anti-CD3 alone, and those which received combined therapy, as might be expected since both these groups of mice were normoglycemic. Immunohistological analysis of pancreata from severely diabetic NOD mice that responded to low-dose anti-CD3 monotherapy revealed their islets that had just a few insulin$^+$ cells when examined 25 weeks after the initiation of treatment. These nonfunctional islets were essentially insulitis-free. The vast majority of islets in pancreata from mice that had been started on combined therapy 25 weeks earlier were also almost devoid of insulin$^+$ cells, except that we observed rare islets that had many ß-cells. These functional islets had a surrounding peri-insulitis and islet membrane damage was evident.

Example 12 – Combined Treatment with Homotaurine and Anti-CD3 Increases the Frequency of CD4+ and CD8+ Tregs in the Spleens and Pancreatic Lymph Nodes of Severely Diabetic NOD Mice

Previous studies have shown that treatment with anti-CD3 depletes effector T cells while preserving splenic $CD4^+Foxp3^+$ Tregs in NOD mice (Penaranda, C., Q. Tang, and J. A. Bluestone. 2011. Anti-CD3 therapy promotes tolerance by selectively depleting pathogenic cells while preserving regulatory T cells. *J Immunol* 187: 2015-2022). Studies with homotaurine in the EAE mouse model demonstrated that homotaurine monotherapy increased the frequency of splenic $CD4^+Foxp3^+$ and $CD8^+CD122^+PD\text{-}1^+$ Tregs in SJL mice (Tian, J., H. Dang, M. Wallner, R. Olsen, and D. L. Kaufman. 2018. Homotaurine, a safe blood-brain barrier permeable GABAA-R-specific agonist, ameliorates disease in mouse models of multiple sclerosis. *Sci Rep* 8: 16555). To begin to elucidate the potential mechanisms underlying the therapeutic action of combined homotaurine and anti-CD3 we first characterized the percentages of splenic $CD4^+Foxp3^+$ and $CD8^+CD122^+PD\text{-}1^+$ Tregs in mice 25 weeks after treatment with anti-CD3 alone or combined anti-CD3+homotaurine. We observed that the percentages of splenic $CD4^+Foxp3^+$ and $CD8^+CD122^+PD\text{-}1^+$ Tregs in the mice given combined therapy were significantly higher than that of the mice given anti-CD3 monotherapy ($p<0.001$ for both). There was no significant difference in the frequency of splenic $CD8^+CD122^+PD\text{-}1^-$ T cells between these two groups of mice. Thus, combined treatment with homotaurine and anti-CD3 significantly increased the frequency of both $CD4^+$ and $CD8^+$ Tregs in the spleen in comparison to anti-CD3 monotherapy in severely diabetic NOD mice.

Analysis of pancreatic lymph node (PLN) mononuclear cells in additional groups of NOD mice that had been treated with vehicle alone (control), homotaurine alone, low-dose of anti-CD3 alone, or homotaurine+anti-CD3 at 15-18 weeks in age and studied at 3 weeks later showed that treatment with homotaurine or anti-CD3 monotherapies significantly increased

the frequencies of $CD4^+CD25^+Foxp3^+$ and $CD8^+CD122^+PD\text{-}1^+$ cells in the PLN, relative to control groups. Notably, the combination treatment further elevated the average frequencies of $CD4^+CD25^+Foxp3^+$ cells and $CD8^+CD122^+PD\text{-}1^+$ cells in the PLN above that observed from the monotherapies. This increase was significant for $CD4^+CD25^+Foxp3^+$ cells in comparison to homotaurine monotherapy. Thus, combined therapy also elevated the average frequencies of $CD4^+$ and $CD8^+$ Tregs within the target tissue/PLN of NOD mice.

Materials and Methods for Examples 5-12

Chemicals

Homotaurine, GABA, bicuculline, and 5-bromo-2-deoxyuridine ("BrdU") were purchased from Sigma-Aldrich.

Islet cell proliferation assay

Fresh human islets were obtained from the Integrated Islet Distribution Program ("IIDP"). The islets (50-75 IEQ/well) were treated in triplicate with, or without, the indicated dosages of homotaurine or GABA in CMRL medium (0.1% glucose, Gibco) containing 10% human AB-type sera (MP Biomedicals, Santa Ana, USA) and 1.5 μCi/ml of ^{3}H-thymidine in the presence or absence of the competitive $GABA_A$-R antagonist bicuculline (50 uM) for 4 days. Islets cultured in medium alone served as controls. The ^{3}H-thymidine uptake in individual wells was measured by β-counter. Data were analyzed by the proliferation index formula: CPM of experimental wells / CPM of controls.

Analysis of human ß-cell replication *in* vivo

NOD/scid mice were injected intraperitoneally with STZ to induce diabetes and implanted with about 2000 human islets under their kidney capsule. The mice were randomized and treated for 12 days with plain water containing 0.8 mg/ml of BrdU as the control, or water containing the same dose of BrdU and homotaurine (0.08 or 0.25 mg/ml), or

positive control GABA (6 mg/ml). At the end of treatment, the percentages of $BrdU^+insulin^+$ and $Ki67^+insulin^+$ β-cells in at least 2000 islet cells of 10 fields (magnification x 400) of each islet graft were determined by immunofluorescence in a blinded manner. *See* J. Tian *et al.*, *gamma-Aminobutyric acid regulates both the survival and replication of human beta-cells*, 62 DIABETES 3760-3765 (2013).

Analysis of human β-cell apoptosis *in vivo*

STZ-rendered diabetic NOD/scid mice were implanted with about 2000-3000 human islets under their kidney capsule. The mice were randomized and given plain water, or water containing homotaurine (0.08 or 0.25 mg/ml), or positive control GABA (6 mg/ml). Forty-eight hours later, the percentages of $insulin^+$ β-cells or $TUNEL^+$ apoptotic islet cells in total islet cells within the grafts of individual recipients were determined by immunofluorescence in a blinded manner. *See* J. Tian *et al.*, *gamma-Aminobutyric acid regulates both the survival and replication of human beta-cells*, 62 DIABETES 3760-3765 (2013).

T cell proliferation assays

Mononuclear splenic cells (3x105/ well) from naive mice were incubated with 1 anti-CD3 (1 ug/ml, 2c11 clone, Pharmingen) with the indicated dose of GABA or homotaurine for 48 h. During the last 8 h of incubation, ^{3}H-thymidine (1 μC/well) was added into each well to determine T cell proliferation. The data are presented the mean ^{3}H-thymidine incorporation relative to that in control cultures with without homotaurine or GABA

T1D intervention studies in newly diabetic NOD mice

NOD mice (Taconic Farms, Germantown) were housed in a specific pathogen-free facility. Only female NOD mice were used.

Homotaurine monotherapy dose studies

Blood glucose levels were monitored 2-3 times weekly using a One Touch Ultrasensitive monitor and those with two consecutive daily blood glucose levels between

250-300 mg/dL were entered into the study. The mice were randomly assigned to groups that continually received water containing 0, 0.08, 0.25, or 0.75 mg/ml homotaurine, pH 7.2 through their drinking water. Each mouse consumed on average about 4-5 ml of water per day. Water bottles were changed every five days. Treated mice with two consecutive blood glucose readings below 250 mg/dL were considered to be in remission after which two consecutive blood glucose readings >250 mg/dL was considered disease relapse.

Combined homotaurine and proinsulin/alum treatment

Newly-diabetic mice (blood glucose 250-300 mg/dL) received 100 μg proinsulin (kindly provided by Eli Lilly, Indianapolis) complexed with alum (Pierce, Rockford, IL)) intraperitoneally. The same day, the animals were placed on water containing a low-dose of homotaurine (0.08 mg/ml) which was continued for the length of the study. The mice were immunized once more with proinsulin/alum ten days after the first vaccination. Treated mice were monitored for disease remission and relapse as described above.

Combined homotaurine and low-dose anti-CD3 treatment

Treatment was initiated when the NOD mice had blood glucose levels >350 mg/dL. Based on pilot studies, the suboptimal anti-CD3 dose described by von Herrath and colleagues (*see* D. Bresson *et al.*, *Anti-CD3 and nasal proinsulin combination therapy enhances remission from recent-onset autoimmune diabetes by inducing Tregs*, 116 J CLIN INVEST 1371-1381 (2006)) was further reduced to three consecutive daily treatments of 35 μg anti-CD3 (hamster anti-CD3ε 2C11 F(ab')2 fragment, BioXCell, West Lebanon, NH) intravenously. At the time of the first anti-CD3 treatment, the animals were randomized to receive plain water, or water containing homotaurine (0.25 mg/ml) or GABA (6 mg/ml) which was continued for the length of the study. Treated mice with two consecutive blood glucose readings below 250 mg/dL were considered to be in remission after which two consecutive blood glucose readings >250 mg/dL was considered to be disease relapse.

REFERENCES

The invention described herein may be embodied in other specific forms without departing from the spirit or essential characteristics of the invention. The foregoing embodiments are illustrative and not limiting. Additionally, the publications cited herein are incorporated by reference in their entireties for all purposes.

1 Tian, J., Chau, C., Hales, T. G. & Kaufman, D. L. GABA(A) receptors mediate inhibition of T cell responses. *J Neuroimmunol* **96**, 21-28 (1999).

2 Tian, J. *et al.* Gamma-aminobutyric acid inhibits T cell autoimmunity and the development of inflammatory responses in a mouse type 1 diabetes model. *J Immunol* **173**, 5298-5304 (2004).

3 Alam, S., Laughton, D. L., Walding, A. & Wolstenholme, A. J. Human peripheral blood mononuclear cells express GABAA receptor subunits. *Mol Immunol* **43**, 1432-1442 (2006).

4 Mendu, S. K., Bhandage, A., Jin, Z. & Birnir, B. Different subtypes of GABA-A receptors are expressed in human, mouse and rat T lymphocytes. *PLoS One* 7, e42959 (2012).

5 Prud'homme, G. J. *et al.* GABA Protects Human Islet Cells Against the Deleterious Effects of Immunosuppressive Drugs and Exerts Immunoinhibitory Effects Alone. *Transplantation* **96**, 616-623, doi:10.1097/TP.0b013e31829c24be (2013).

6 Olsen, R. W. & Tobin, A. J. Molecular biology of GABAA receptors. *Faseb J* **4**, 1469-1480 (1990).

7 Bettler, B., Kaupmann, K., Mosbacher, J. & Gassmann, M. Molecular structure and physiological functions of GABA(B) receptors. *Physiol Rev* **84**, 835-867 (2004).

8 Prud'homme, G. J. *et al.* GABA protects human islet cells against the deleterious effects of immunosuppressive drugs and exerts immunoinhibitory effects alone. *Transplantation* **96**, 616-623, doi:10.1097/TP.0b013e31829c24be (2013).

9 Soltani, N. *et al.* GABA exerts protective and regenerative effects on islet beta cells and reverses diabetes. *Proc Natl Acad Sci U S A* **108**, 11692-11697, doi:10.1073/pnas.1102715108 (2011).

10 Mendu, S. K. *et al.* Increased GABA(A) channel subunits expression in CD8(+) but not in CD4(+) T cells in BB rats developing diabetes compared to their congenic littermates. *Mol Immunol* **48**, 399-407, doi:10.1016/j.molimm.2010.08.005 (2011).

11 Tian, J., Dang, H., Nguyen, A. V., Chen, Z. & Kaufman, D. L. Combined therapy with GABA and proinsulin/alum acts synergistically to restore long-term normoglycemia by modulating T-cell autoimmunity and promoting beta-cell replication in newly diabetic NOD mice. *Diabetes* **63**, 3128-3134, doi:10.2337/db13-1385 (2014).

12 Tian, J., Yong, J., Dang, H. & Kaufman, D. L. Oral GABA treatment downregulates inflammatory responses in a mouse model of rheumatoid arthritis. *Autoimmunity* **44**, 465-470 (2011).

13 Tian, J. *et al.* Oral treatment with gamma-aminobutyric acid improves glucose tolerance and insulin sensitivity by inhibiting inflammation in high fat diet-fed mice. *PLoS One* **6**, e25338, doi:10.1371/journal.pone.0025338 (2011).

14 Sohrabipour, S., Reza Sharifi, M., Talebi, A., Sharifi, M. & Soltani, N. GABA dramatically improves glucose tolerance in streptozotocin-induced diabetic rats fed with high-fat diet. *Eur J Pharmacol*, doi:10.1016/j.ejphar.2018.01.047 (2018).

15 Rangachari, M. & Kuchroo, V. K. Using EAE to better understand principles of immune function and autoimmune pathology. *J Autoimmun* **45**, 31-39, doi:10.1016/j.jaut.2013.06.008 (2013).

16 McGinley, A. M., Edwards, S. C., Raverdeau, M. & Mills, K. H. G. Th17cells, gammadelta T cells and their interplay in EAE and multiple sclerosis. *J Autoimmun*, doi:10.1016/j.jaut.2018.01.001 (2018).

17 McRae, B. L., Vanderlugt, C. L., Dal Canto, M. C. & Miller, S. D. Functional evidence for epitope spreading in the relapsing pathology of experimental autoimmune encephalomyelitis. *J Exp Med* **182**, 75-85 (1995).

18 Targoni, O. S. *et al.* Frequencies of neuroantigen-specific T cells in the central nervous system versus the immune periphery during the course of experimental allergic encephalomyelitis. *J Immunol* **166**, 4757-4764. (2001).

19 McMahon, E. J., Bailey, S. L., Castenada, C. V., Waldner, H. & Miller, S. D. Epitope spreading initiates in the CNS in two mouse models of multiple sclerosis. *Nat Med* **11**, 335-339 (2005).

20 Kuriyama, K. & Sze, P. Y. Blood-brain barrier to H3-gamma-aminobutyric acid in normal and amino oxyacetic acid-treated animals. *Neuropharmacology* **10**, 103-108 (1971).

21 Bhat, R. *et al.* Inhibitory role for GABA in autoimmune inflammation. *Proc Natl Acad Sci U S A* **107**, 2580-2585 (2010).

22 White, H. S. Molecular pharmacology of topiramate: managing seizures and preventing migraine. *Headache* **45 Suppl 1**, S48-56, doi:10.1111/j.1526-4610.2005.4501006.x (2005).

23 Porter, R. J., Dhir, A., Macdonald, R. L. & Rogawski, M. A. Mechanisms of action of antiseizure drugs. *Handb Clin Neurol* **108**, 663-681, doi:10.1016/B978-0-444-52899-5.00021-6 (2012).

24 Sternberg, Z. *et al.* Acamprosate modulates experimental autoimmune encephalomyelitis. *Inflammopharmacology* **20**, 39-48, doi:10.1007/s10787-011-0097-1 (2012).

25 Kalk, N. J. & Lingford-Hughes, A. R. The clinical pharmacology of acamprosate. *Br J Clin Pharmacol* **77**, 315-323, doi:10.1111/bcp.12070 (2014).

26 Reilly, M. T. *et al.* Effects of acamprosate on neuronal receptors and ion channels expressed in Xenopus oocytes. *Alcohol Clin Exp Res* **32**, 188-196, doi:10.1111/j.1530-0277.2007.00569.x (2008).

27 Wright, T. M. Tramiprosate. *Drugs Today (Barc)* **42**, 291-298 (2006).

28 Gervais, F. *et al.* Targeting soluble Abeta peptide with Tramiprosate for the treatment of brain amyloidosis. *Neurobiol Aging* **28**, 537-547 (2007).

29 Aisen, P. S. *et al.* Tramiprosate in mild-to-moderate Alzheimer's disease - a randomized, double-blind, placebo-controlled, multi-centre study (the Alphase Study). *Arch Med Sci* **7**, 102-111 (2010).

30 Gauthier, S. *et al.* Effect of tramiprosate in patients with mild-to-moderate Alzheimer's disease: exploratory analyses of the MRI sub-group of the Alphase study. *J Nutr Health Aging* **13**, 550-557 (2009).

31 Tower, D. B. & Roberts, E., *The Administration of Gamma-Aminobutyric Acid to Man: Systemic Effects and Anticonvulsant Actions*; INHIBITION IN THE NERVOUS SYSTEM AND GAMMA AMINOBUTYRIC ACID 562-578 (Pergamon Press, New York, 1960).

32 Jones, M. V., Sahara, Y., Dzubay, J. A. & Westbrook, G. L. Defining affinity with the GABAA receptor. *J Neurosci* **18**, 8590-8604 (1998).

33 Mendel, I., Kerlero de Rosbo, N. & Ben-Nun, A. A myelin oligodendrocyte glycoprotein peptide induces typical chronic experimental autoimmune encephalomyelitis in H-2b mice: fine specificity and T cell receptor V beta expression of encephalitogenic T cells. *Eur J Immunol* **25**, 1951-1959, doi:10.1002/eji.1830250723 (1995).

34 Tuohy, V. K., Lu, Z., Sobel, R. A., Laursen, R. A. & Lees, M. B. Identification of an encephalitogenic determinant of myelin proteolipid protein for SJL mice. *J Immunol* **142**, 1523-1527 (1989).

35 Hofstetter, H. H., Toyka, K. V., Tary-Lehmann, M. & Lehmann, P. V. Kinetics and organ distribution of IL-17-producing CD4 cells in proteolipid protein 139-151 peptide-induced experimental autoimmune encephalomyelitis of SJL mice. *J Immunol* **178**, 1372-1378 (2007).

36 Nusser, Z., Sieghart, W. & Somogyi, P. Segregation of different GABAA receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. *J Neurosci* **18**, 1693-1703 (1998).

37 Richerson, G. B. Looking for GABA in all the wrong places: the relevance of extrasynaptic GABA(A) receptors to epilepsy. *Epilepsy Curr* **4**, 239-242, doi:10.1111/j.1535-7597.2004.46008.x (2004).

38 Farrant, M. & Kaila, K. The cellular, molecular and ionic basis of GABA(A) receptor signalling. *Prog Brain Res* **160**, 59-87, doi:10.1016/S0079-6123(06)60005-8 (2007).

39 Storustovu, S. I. & Ebert, B. Pharmacological characterization of agonists at delta-containing GABAA receptors: Functional selectivity for extrasynaptic receptors is

dependent on the absence of gamma2. *J Pharmacol Exp Ther* **316**, 1351-1359, doi:10.1124/jpet.105.092403 (2006).

40 Meera, P., Wallner, M. & Otis, T. S. Molecular basis for the high THIP/gaboxadol sensitivity of extrasynaptic GABA(A) receptors. *J Neurophysiol* **106**, 2057-2064, doi:10.1152/jn.00450.2011 (2011).

41. Soltani N, Qiu H, Aleksic M, Glinka Y, Zhao F, Liu R, Li Y, Zhang N, Chakrabarti R, Ng T, Jin T, Zhang H, Lu WY, Feng ZP, Prud'homme GJ, Wang Q: GABA exerts protective and regenerative effects on islet beta cells and reverses diabetes. Proc Natl Acad Sci U S A 2011;108:11692-11697

42. Tian J, Dang H, Nguyen AV, Chen Z, Kaufman DL: Combined therapy with GABA and proinsulin/alum acts synergistically to restore long-term normoglycemia by modulating T-cell autoimmunity and promoting beta-cell replication in newly diabetic NOD mice. Diabetes 2014;63:3128-3134

43. Niazi M, Silberg DG, Miller F, Ruth M, Holmberg AA: Evaluation of the pharmacokinetic interaction between lesogaberan (AZD3355) and esomeprazole in healthy subjects. Drugs R D 2010;10:243-251

44. Boeckxstaens GE, Beaumont H, Hatlebakk JG, Silberg DG, Bjorck K, Karlsson M, Denison H: A novel reflux inhibitor lesogaberan (AZD3355) as add-on treatment in patients with GORD with persistent reflux symptoms despite proton pump inhibitor therapy: a randomised placebo-controlled trial. Gut 2011;60:1182-1188

45. Shaheen NJ, Denison H, Bjorck K, Karlsson M, Silberg DG: Efficacy and safety of lesogaberan in gastro-oesophageal reflux disease: a randomised controlled trial. Gut 2013;62:1248-1255

46. Atkinson MA, von Herrath M, Powers AC, Clare-Salzler M: Current concepts on the pathogenesis of type 1 diabetes--considerations for attempts to prevent and reverse the disease. Diabetes Care 2015;38:979-988

47. Staeva TP, Chatenoud L, Insel R, Atkinson MA: Recent lessons learned from prevention and recent-onset type 1 diabetes immunotherapy trials. Diabetes 2013;62:9-17

48. Tian J, Chau C, Hales TG, Kaufman DL: GABA(A) receptors mediate inhibition of T cell responses. J Neuroimmunol 1999;96:21-28

49. Prud'homme GJ, Glinka Y, Hasilo C, Paraskevas S, Li X, Wang Q: GABA protects human islet cells against the deleterious effects of immunosuppressive drugs and exerts immunoinhibitory effects alone. Transplantation 2013;96:616-623

50. Tian J, Lu Y, Zhang H, Chau CH, Dang HN, Kaufman DL: Gamma-aminobutyric acid inhibits T cell autoimmunity and the development of inflammatory responses in a mouse type 1 diabetes model. J Immunol 2004;173:5298-5304

51. Mendu SK, Akesson L, Jin Z, Edlund A, Cilio C, Lernmark A, Birnir B: Increased GABA(A) channel subunits expression in CD8(+) but not in CD4(+) T cells in BB rats developing diabetes compared to their congenic littermates. Mol Immunol 2011;48:399-407

52. Tian J, Yong J, Dang H, Kaufman DL: Oral GABA treatment downregulates inflammatory responses in a mouse model of rheumatoid arthritis. Autoimmunity 2011;44:465-470

53. Bhat R, Axtell R, Mitra A, Miranda M, Lock C, Tsien RW, Steinman L: Inhibitory role for GABA in autoimmune inflammation. Proc Natl Acad Sci U S A 2010;107:2580-2585

54. Tian J, Dang HN, Yong J, Chui WS, Dizon MP, Yaw CK, Kaufman DL: Oral treatment with gamma-aminobutyric acid improves glucose tolerance and insulin sensitivity by inhibiting inflammation in high fat diet-fed mice. PLoS One 2011;6:e25338

55. Sohrabipour S, Reza Sharifi M, Talebi A, Sharifi M, Soltani N: GABA dramatically improves glucose tolerance in streptozotocin-induced diabetic rats fed with high-fat diet. Eur J Pharmacol 2018;

56. Shang W, Si X, Zhou Z, Strappe P, Blanchard C: Wheat bran with enriched gamma-aminobutyric acid attenuates glucose intolerance and hyperinsulinemia induced by a high-fat diet. Food Funct 2018;9:2820-2828

57. Braun M, Wendt A, Buschard K, Salehi A, Sewing S, Gromada J, Rorsman P: GABAB receptor activation inhibits exocytosis in rat pancreatic beta-cells by G-protein-dependent activation of calcineurin. J Physiol 2004;559:397-409

58. Wendt A, Birnir B, Buschard K, Gromada J, Salehi A, Sewing S, Rorsman P, Braun M: Glucose inhibition of glucagon secretion from rat alpha-cells is mediated by GABA released from neighboring beta-cells. Diabetes 2004;53:1038-1045

59. Braun M, Ramracheya R, Bengtsson M, Clark A, Walker JN, Johnson PR, Rorsman P: Gamma-aminobutyric acid (GABA) is an autocrine excitatory transmitter in human pancreatic beta-cells. Diabetes 2010;59:1694-1701

60. Rorsman P, Berggren PO, Bokvist K, Ericson H, Mohler H, Ostenson CG, Smith PA: Glucose-inhibition of glucagon secretion involves activation of GABAA-receptor chloride channels. Nature 1989;341:233-236

61. Taneera J, Jin Z, Jin Y, Muhammed SJ, Zhang E, Lang S, Salehi A, Korsgren O, Renstrom E, Groop L, Birnir B: gamma-Aminobutyric acid (GABA) signalling in human pancreatic islets is altered in type 2 diabetes. Diabetologia 2012;55:1985-1994

62. Tian J, Dang H, Chen Z, Guan A, Jin Y, Atkinson MA, Kaufman DL: gamma-Aminobutyric acid regulates both the survival and replication of human beta-cells. Diabetes 2013;62:3760-3765

63. Purwana I, Zheng J, Li X, Deurloo M, Son DO, Zhang Z, Liang C, Shen E, Tadkase A, Feng ZP, Li Y, Hasilo C, Paraskevas S, Bortell R, Greiner DL, Atkinson M, Prud'homme GJ, Wang Q: GABA promotes human beta-cell proliferation and modulates glucose homeostasis. Diabetes 2014;63:4197-4205

64. Gu XH, Kurose T, Kato S, Masuda K, Tsuda K, Ishida H, Seino Y: Suppressive effect of GABA on insulin secretion from the pancreatic beta-cells in the rat. Life Sci 1993;52:687-694

65. Brice NL, Varadi A, Ashcroft SJ, Molnar E: Metabotropic glutamate and GABA(B) receptors contribute to the modulation of glucose-stimulated insulin secretion in pancreatic beta cells. Diabetologia 2002;45:242-252

66. Martin P, Massol J, Pichat P, Puech AJ: Decreased central GABA B receptor binding sites in diabetic rats. Neuropsychobiology 1988;19:146-148

67. Tian J, Dang H, D.L. K: Combining Antigen-Based Therapy with GABA Treatment Synergistically Prolongs Survival of Transplanted ß-Cells in Diabetic NOD Mice. PLoS One 2011;6(9): e25337

68. Bailey SJ, Ravier MA, Rutter GA: Glucose-dependent regulation of gamma-aminobutyric acid (GABA A) receptor expression in mouse pancreatic islet alpha-cells. Diabetes 2007;56:320-327

69. Ben-Othman N, Vieira A, Courtney M, Record F, Gjernes E, Avolio F, Hadzic B, Druelle N, Napolitano T, Navarro-Sanz S, Silvano S, Al-Hasani K, Pfeifer A, Lacas-Gervais S, Leuckx G, Marroqui L, Thevenet J, Madsen OD, Eizirik DL, Heimberg H,

Kerr-Conte J, Pattou F, Mansouri A, Collombat P: Long-Term GABA Administration Induces Alpha Cell-Mediated Beta-like Cell Neogenesis. Cell 2016:31523-31529

70. Li J, Casteels T, Frogne T, Ingvorsen C, Honore C, Courtney M, Huber KV, Schmitner N, Kimmel RA, Romanov RA, Sturtzel C, Lardeau CH, Klughammer J, Farlik M, Sdelci S, Vieira A, Avolio F, Briand F, Baburin I, Majek P, Pauler FM, Penz T, Stukalov A, Gridling M, Parapatics K, Barbieux C, Berishvili E, Spittler A, Colinge J, Bennett KL, Hering S, Sulpice T, Bock C, Distel M, Harkany T, Meyer D, Superti-Furga G, Collombat P, Hecksher-Sorensen J, Kubicek S: Artemisinins Target GABAA Receptor Signaling and Impair alpha Cell Identity. Cell 2017;168:86-100 e115

71. van der Meulen T, Lee S, Noordeloos E, Donaldson CJ, Adams MW, Noguchi GM, Mawla AM, Huising MO: Artemether Does Not Turn alpha Cells into beta Cells. Cell Metab 2018;27:218-225 e214

72. Otomo E, Araki G, Mori A, Kurihara M: Clinical evaluation of GABA in the treatment of cerebrovascular disorders. Multi-center double-blind study in comparison with pyrithioxine and placebo. Arzneimittelforschung 1981;31:1511-1523

73. Loeb C, Benassi E, Bo GP, Cocito L, Maffini M, Scotto P: Preliminary evaluation of the effect of GABA and phosphatidylserine in epileptic patients. Epilepsy Res 1987;1:209-212

74. Tower DB, Roberts E: Inhibition in the Nervous System and GABA. New York, Pergamon Press, 1960, p. 562-578

75. Li J, Zhang Z, Liu X, Wang Y, Mao F, Mao J, Lu X, Jiang D, Wan Y, Lv JY, Cao G, Zhang J, Zhao N, Atkinson M, Greiner DL, Prud'homme GJ, Jiao Z, Li Y, Wang Q: Study of GABA in Healthy Volunteers: Pharmacokinetics and Pharmacodynamics. Front Pharmacol 2015;6:260

76. Kuriyama K, Sze PY: Blood-brain barrier to H3-gamma-aminobutyric acid in normal and amino oxyacetic acid-treated animals. Neuropharmacology 1971;10:103-108

77. Blandino-Rosano M, Chen AY, Scheys JO, Alejandro EU, Gould AP, Taranukha T, Elghazi L, Cras-Meneur C, Bernal-Mizrachi E: mTORC1 signaling and regulation of pancreatic beta-cell mass. Cell Cycle 2012;11:1892-1902

78. Gervais F, Paquette J, Morissette C, Krzywkowski P, Yu M, Azzi M, Lacombe D, Kong X, Aman A, Laurin J, Szarek WA, Tremblay P: Targeting soluble Abeta peptide with Tramiprosate for the treatment of brain amyloidosis. Neurobiol Aging 2007;28:537-547

79. Wright TM: Tramiprosate. Drugs Today (Barc) 2006;42:291-298

80. Aisen PS, Gauthier S, Ferris SH, Saumier D, Haine D, Garceau D, Duong A, Suhy J, Oh J, Lau WC, Sampalis J: Tramiprosate in mild-to-moderate Alzheimer's disease - a randomized, double-blind, placebo-controlled, multi-centre study (the Alphase Study). Arch Med Sci 2010;7:102-111

81. Gauthier S, Aisen PS, Ferris SH, Saumier D, Duong A, Haine D, Garceau D, Suhy J, Oh J, Lau W, Sampalis J: Effect of tramiprosate in patients with mild-to-moderate Alzheimer's disease: exploratory analyses of the MRI sub-group of the Alphase study. J Nutr Health Aging 2009;13:550-557

82. Caltagirone C, Ferrannini L, Marchionni N, Nappi G, Scapagnini G, Trabucchi M: The Potential Protective Effect of Tramiprosate (Homotaurine) against Alzheimer Disease: a Review. Aging Clin Exp Res 2012;

83. Chatenoud L, Thervet E, Primo J, Bach JF: Anti-CD3 antibody induces long-term remission of overt autoimmunity in nonobese diabetic mice. Proc Natl Acad Sci U S A 1994;91:123-127

84. Chatenoud L: CD3-specific antibody-induced active tolerance: from bench to bedside. Nat Rev Immunol 2003;3:123-132

85. Penaranda C, Tang Q, Bluestone JA: Anti-CD3 therapy promotes tolerance by selectively depleting pathogenic cells while preserving regulatory T cells. J Immunol 2011;187:2015-2022

86. Chatenoud L, Primo J, Bach JF: CD3 antibody-induced dominant self tolerance in overtly diabetic NOD mice. J Immunol 1997;158:2947-2954

87. Herold KC, Hagopian W, Auger JA, Poumian-Ruiz E, Taylor L, Donaldson D, Gitelman SE, Harlan DM, Xu D, Zivin RA, Bluestone JA: Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. N Engl J Med 2002;346:1692-1698

88. Keymeulen B, Vandemeulebroucke E, Ziegler AG, Mathieu C, Kaufman L, Hale G, Gorus F, Goldman M, Walter M, Candon S, Schandene L, Crenier L, De Block C, Seigneurin JM, De Pauw P, Pierard D, Weets I, Rebello P, Bird P, Berrie E, Frewin M, Waldmann H, Bach JF, Pipeleers D, Chatenoud L: Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. N Engl J Med 2005;352:2598-2608

89. Herold KC, Gitelman SE, Ehlers MR, Gottlieb PA, Greenbaum CJ, Hagopian W, Boyle KD, Keyes-Elstein L, Aggarwal S, Phippard D, Sayre PH, McNamara J, Bluestone JA, Ab ATEST: Teplizumab (anti-CD3 mAb) treatment preserves C-peptide responses in patients with new-onset type 1 diabetes in a randomized controlled trial: metabolic and immunologic features at baseline identify a subgroup of responders. Diabetes 2013;62:3766-3774

90. Bresson D, Togher L, Rodrigo E, Chen Y, Bluestone JA, Herold KC, von Herrath M: Anti-CD3 and nasal proinsulin combination therapy enhances remission from recent-onset autoimmune diabetes by inducing Tregs. J Clin Invest 2006;116:1371-1381

91. Emamaullee JA, Shapiro AM: Factors influencing the loss of beta-cell mass in islet transplantation. Cell Transplant 2007;16:1-8

92. Davalli AM, Ogawa Y, Ricordi C, Scharp DW, Bonner-Weir S, Weir GC: A selective decrease in the beta cell mass of human islets transplanted into diabetic nude mice. Transplantation 1995;59:817-820

93. Dong H, Kumar M, Zhang Y, Gyulkhandanyan A, Xiang YY, Ye B, Perrella J, Hyder A, Zhang N, Wheeler M, Lu WY, Wang Q: Gamma-aminobutyric acid up- and downregulates insulin secretion from beta cells in concert with changes in glucose concentration. Diabetologia 2006;49:697-705.

What is claimed is:

1. A method for treating an inflammation-related disorder in a human or animal subject in need thereof, said method comprising administering to said human or animal subject an effective amount of a safe, blood-brain barrier-permeable $GABA_A$-R-specific agonist.

2. The method of Claim 1, wherein said safe, blood-brain barrier-permeable $GABA_A$-R-specific agonist is homotaurine.

3. The method of Claim 1 or Claim 2, wherein said inflammation-related disorder is selected from the group consisting of Alzheimer's Disease, amyotrophic lateral sclerosis, asthma, atherosclerosis, autism spectral disorders, cerebral abscess, cerebral ischaemia, cognitive disorders, Crohn's disease, deleterious immune responses to infections, encephalitis, hepatitis, inflammatory bowel disease, lupus, meningitis, migraines, multiple sclerosis, neurodegenerative diseases, neuroinflammation, neuropathic pain, obesity, paraneoplastic disorders, Parkinson's disease, periodontitis, rheumatoid arthritis, sarcoidosis, schizophrenia, stroke, traumatic brain injury, tuberculosis, type 1 diabetes, ulcerative colitis, ulcers, and vasculitis.

4. The method of any one of Claims 1-3, wherein said inflammation-related disorder is a T-cell mediated disorder, a macrophage mediated disorder, a dendritic cell mediated disorder, and/or a microglia mediated disorder.

5. The method of Claim 4, wherein said T-cell mediated disorder is selected from the group consisting of type 1 diabetes, rheumatoid arthritis, and multiple sclerosis.

6. The method of any one of Claims 2-5, wherein said homotaurine is administered orally, subcutaneously, sublingually, intramuscularly, intraperitoneally, and/or transdermally.

7. The method of Claim 6, wherein said homotaurine is administered orally.

8. A method for reducing inflammation in the central nervous system of a human or animal subject in need thereof, said method comprising administering to said human or animal subject an effective amount of a safe, blood-brain barrier-permeable $GABA_A$-R-specific agonist.

9. The method of Claim 8, wherein said safe, blood-brain barrier-permeable $GABA_A$-R-specific agonist is homotaurine.

10. The method of Claim 8 or Claim 9, wherein said inflammation-related disorder is selected from the group consisting of Alzheimer's Disease, amyotrophic lateral sclerosis, asthma, atherosclerosis, autism spectral disorders, cerebral abscess, cerebral ischaemia, cognitive disorders, Crohn's disease, deleterious immune responses to infections, encephalitis, hepatitis, inflammatory bowel disease, lupus, meningitis, migraines, multiple sclerosis, neurodegenerative diseases, neuroinflammation, neuropathic pain, obesity, paraneoplastic disorders, Parkinson's disease, periodontitis, rheumatoid arthritis, sarcoidosis, schizophrenia, stroke, traumatic brain injury, tuberculosis, type 1 diabetes, ulcerative colitis, ulcers, and vasculitis.

11. The method of any one of Claims 8-10, wherein said inflammation-related disorder is a T-cell mediated disorder, a macrophage mediated disorder, a dendritic cell mediated disorder, and/or a microglia mediated disorder.

12. The method of Claim 11, wherein said T-cells mediated disorder is selected from the group consisting of type 1 diabetes, rheumatoid arthritis, and multiple sclerosis.

13. The method of any one of Claims 9-12, wherein said homotaurine is administered orally, subcutaneously, sublingually, intramuscularly, intraperitoneally, and/or transdermally.

14. The method of Claim 13, wherein said homotaurine is administered orally.

15. A method for inducing $CD8^+$ and $CD4^+$ regulatory T cell responses in a subject in need thereof, said method comprising administering to said subject an effective amount of a safe, blood-brain barrier-permeable $GABA_A$-R-specific agonist.

16. The method of Claim 15, wherein said safe, blood-brain barrier-permeable $GABA_A$-R-specific agonist is homotaurine.

17. The method of Claim 16, wherein said homotaurine is administered orally.

18. A method for inhibiting autoreactive Th17 and Th1 responses in a subject in need thereof, said method comprising administering to said subject an effective amount of a safe, blood-brain barrier-permeable $GABA_A$-R-specific agonist.

19. The method of Claim 18, wherein said safe, blood-brain barrier-permeable $GABA_A$-R-specific agonist is homotaurine.

20. The method of Claim 19, wherein said homotaurine is administered orally.

21. A method for treating type 1 diabetes in a human or animal subject in need thereof, said method comprising administering to said human or animal subject one or more immunomodulator compounds and one or more GABA-receptor agonists in an amount effective to prevent, reduce, and/or treat hyperglycemia in said human or animal subject.

22. The method of Claim 21, wherein said one or more immunomodulator compound comprises an anti-CD3 immunotherapy compound, copaxone, and/or interferon β.

23. The method of Claim 21, wherein said one or more GABA-receptor agonist is homotaurine.

24. The method of Claim 21, wherein said one or more immunomodulator compound comprises an anti-CD3 immunotherapy compound, copaxone, and/or interferon β and wherein said one or more GABA-receptor agonist is homotaurine.

25. The method of Claim 24, wherein said effective amount is lower than the effective amount for monotherapy.

26. A method for raising C peptide levels in a human or animal subject in need thereof, said method comprising administering to said human or animal subject an effective amount of homotaurine.

27. A method for lowering A1C levels in a human or animal subject in need thereof, said method comprising administering to said human or animal subject an effective amount of homotaurine.

28. A method for promoting islet cell survival in a human or animal subject in need thereof, said method comprising administering to said human or animal subject an effective amount of homotaurine.

29. A method for promoting β-cell replication in a human or animal subject in need thereof, said method comprising administering to said human or animal subject an effective amount of homotaurine.

30. A method for inhibiting T cell proliferation in a human or animal subject in need thereof, said method comprising administering to said human or animal subject an effective amount of homotaurine.

31. The method according to any one of Claims 21-30, wherein said administration is oral.

32. The method according to any one of Claims 1-21, 23, and 25-31, wherein copaxone, and/or interferon β is also administered.

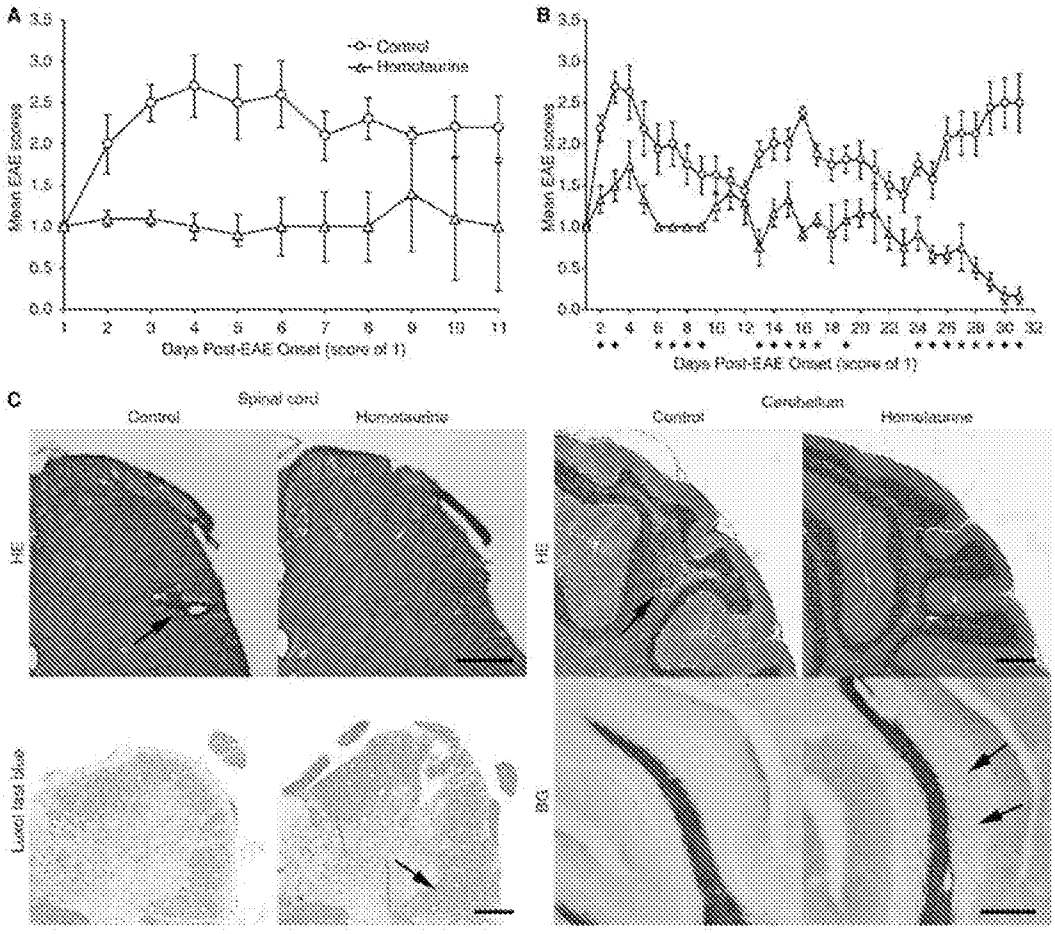

A
Control
Homotaurine
Mean EAE scores
Days Post-EAE Onset (score of 1)
B
Mean EAE scores
Days Post-EAE Onset (score of 1)
C
Spinal cord
Control
Homotaurine
Cerebellum
Control
Homotaurine
H&E
Luxol fast blue
H&E
Luxol fast blue

FIGURE 1

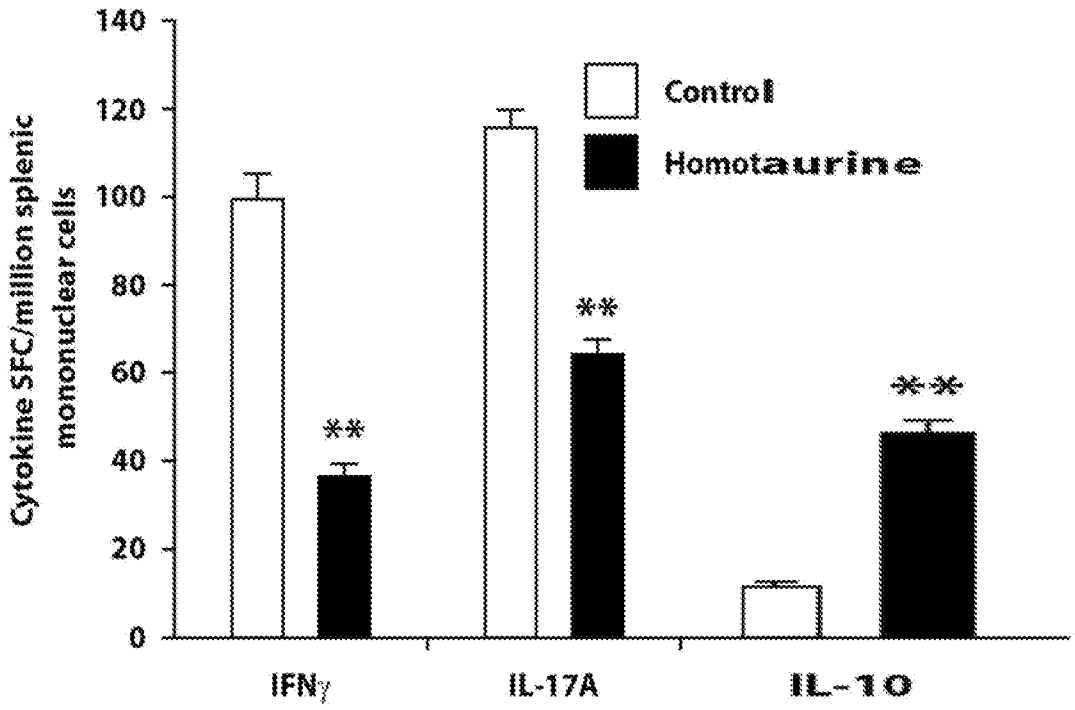

Cytokine SFC/million splenic mononuclear cells
140
120
100
80
60
40
20
0
Control
Homotaurine
**
**
**
IFNγ
IL-17A
IL-10

FIGURE 2

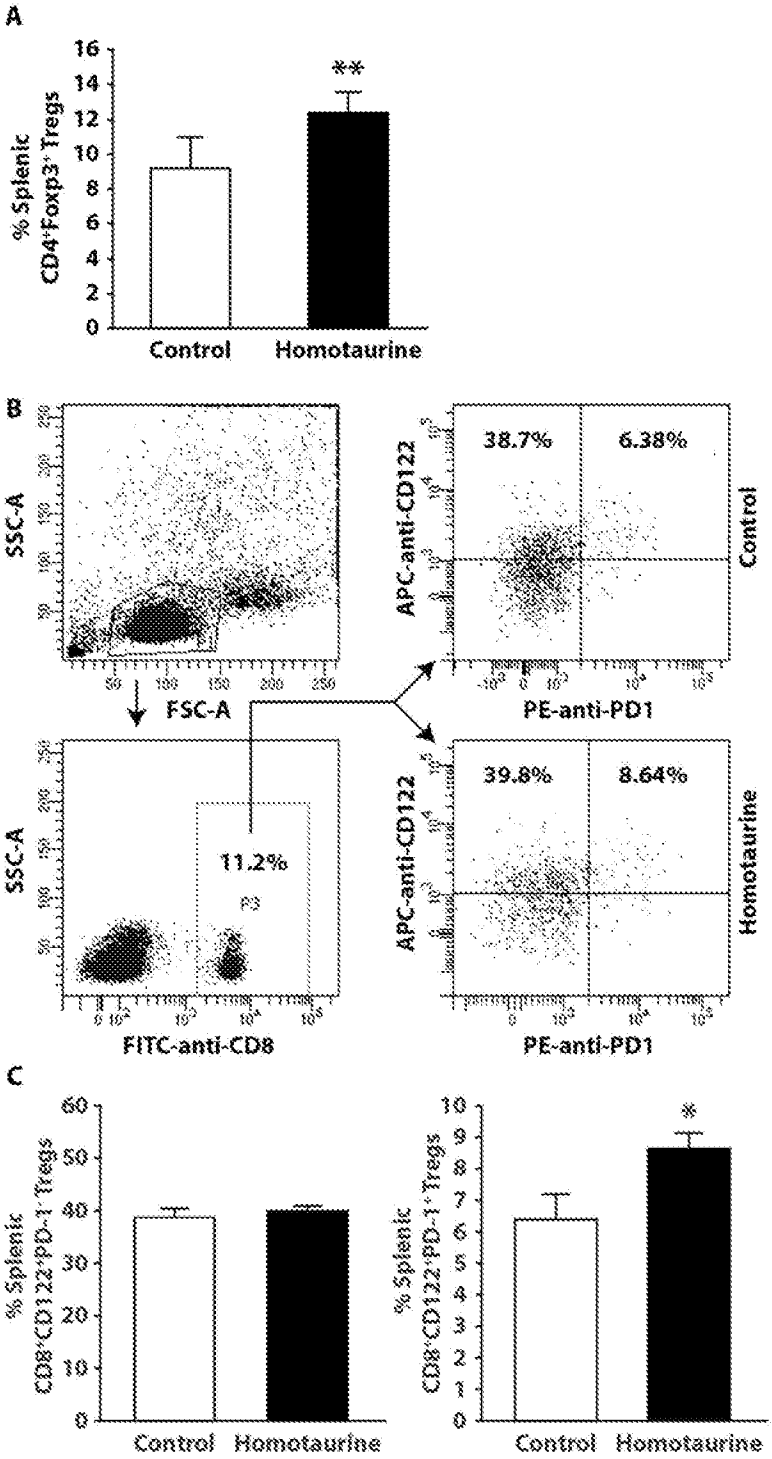

A
% Splenic CD4+Foxp3+ Tregs
16
14
12
10
8
6
4
2
0
**
Control
Homotaurine
B
SSC-A
FSC-A
38.7%
6.38%
APC-anti-CD122
PE-anti-PD1
Control
11.2%
SSC-A
FITC-anti-CD8
39.8%
8.64%
APC-anti-CD122
PE-anti-PD1
Homotaurine
C
% Splenic CD8+CD122+PD-1+ Tregs
60
50
40
30
20
10
0
Control
Homotaurine
% Splenic CD8+CD122+PD-1+ Tregs
10
9
8
7
6
5
4
3
2
1
0
*
Control
Homotaurine

FIGURE 3

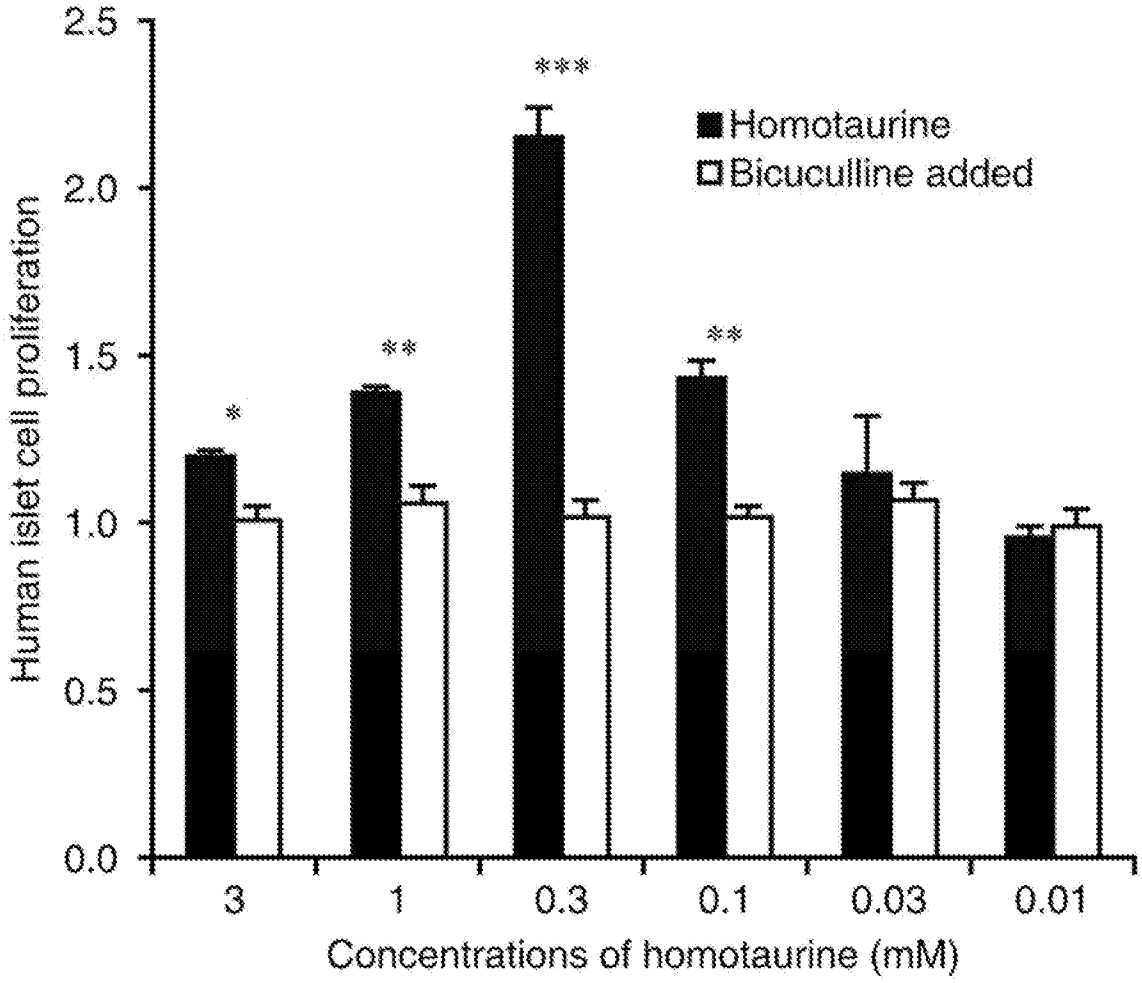

2.5
2.0
1.5
1.0
0.5
0.0
Human islet cell proliferation
*
**
***
**
Homotaurine
Bicuculline added
3
1
0.3
0.1
0.03
0.01
Concentrations of homotaurine (mM)

FIGURE 4

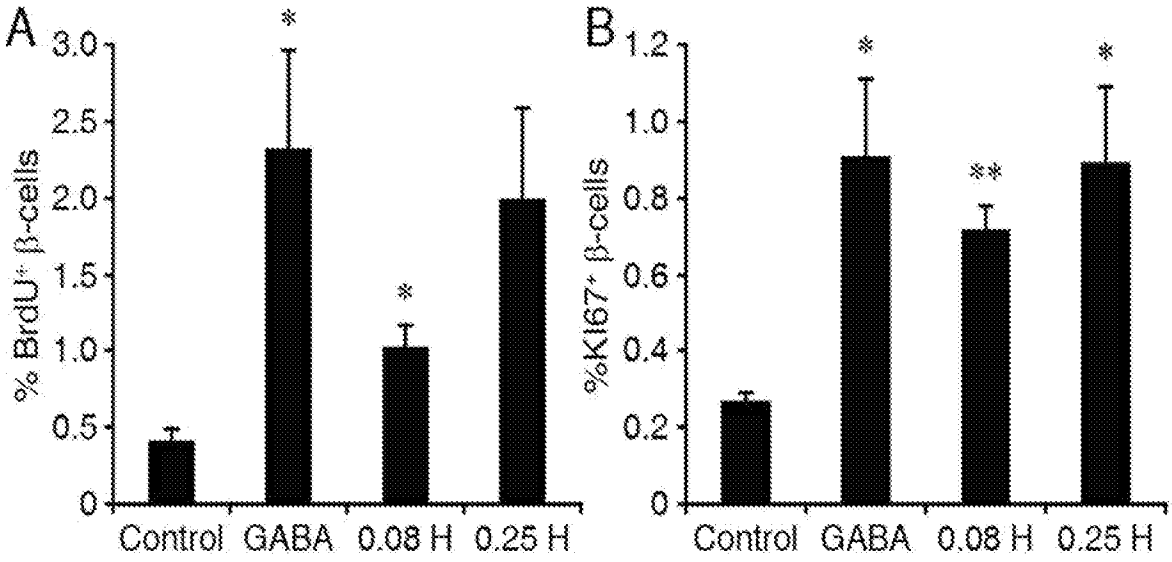

A
% BrdU+ β-cells
3.0
2.5
2.0
1.5
1.0
0.5
0
*
*
Control
GABA
0.08 H
0.25 H
B
%KI67+ β-cells
1.2
1.0
0.8
0.6
0.4
0.2
0
*
**
*
Control
GABA
0.08 H
0.25 H

FIGURE 5

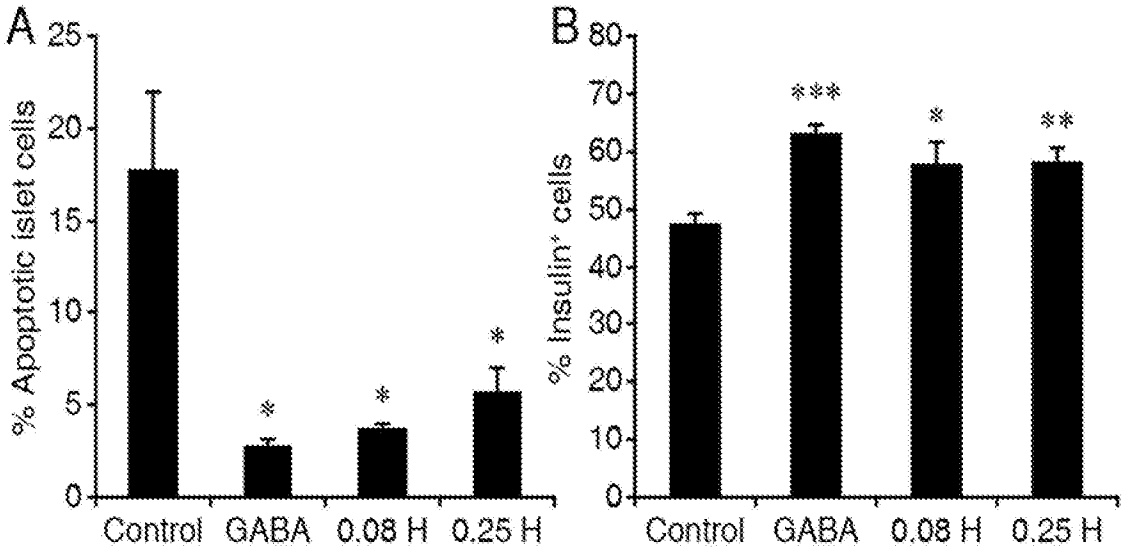

A
% Apoptotic islet cells
25
20
15
10
5
0
*
*
*
Control
GABA
0.08 H
0.25 H
B
% Insulin' cells
80
70
60
50
40
30
20
10
0
***
*
**
Control
GABA
0.08 H
0.25 H

FIGURE 6

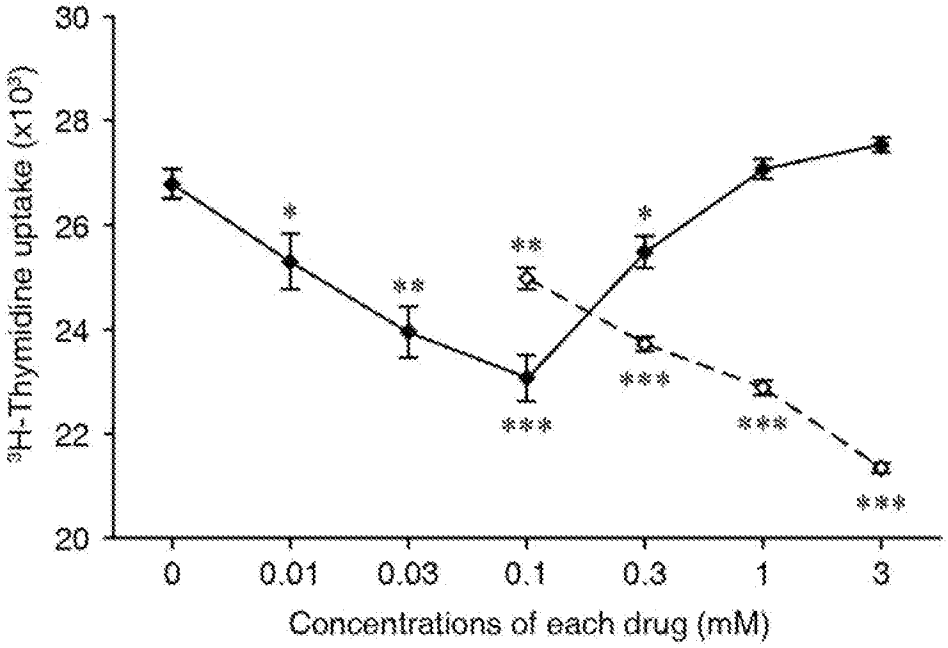

30
28
26
24
22
20
^{3}H-Thymidine uptake (x10^3)
*
**
***
**
*
***
***
***
0
0.01
0.03
0.1
0.3
1
3
Concentrations of each drug (mM)

FIGURE 7

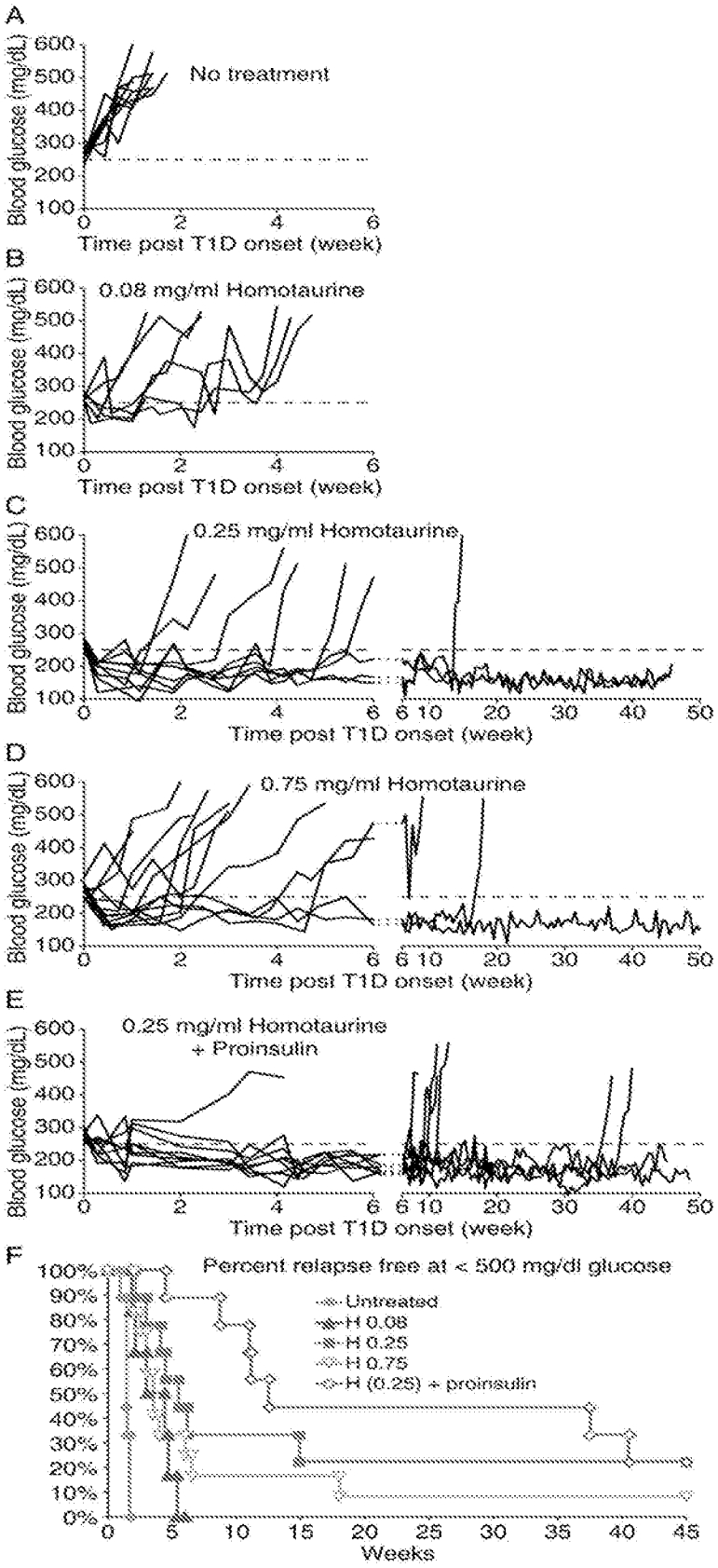

A
No treatment
Blood glucose (mg/dL)
Time post T1D onset (week)
B
0.08 mg/ml Homotaurine
C
0.25 mg/ml Homotaurine
D
0.75 mg/ml Homotaurine
E
0.25 mg/ml Homotaurine
+ Proinsulin
F
Percent relapse free at < 500 mg/dl glucose
Untreated
H 0.08
H 0.25
H 0.75
H (0.25) + proinsulin
Weeks

FIGURE 8

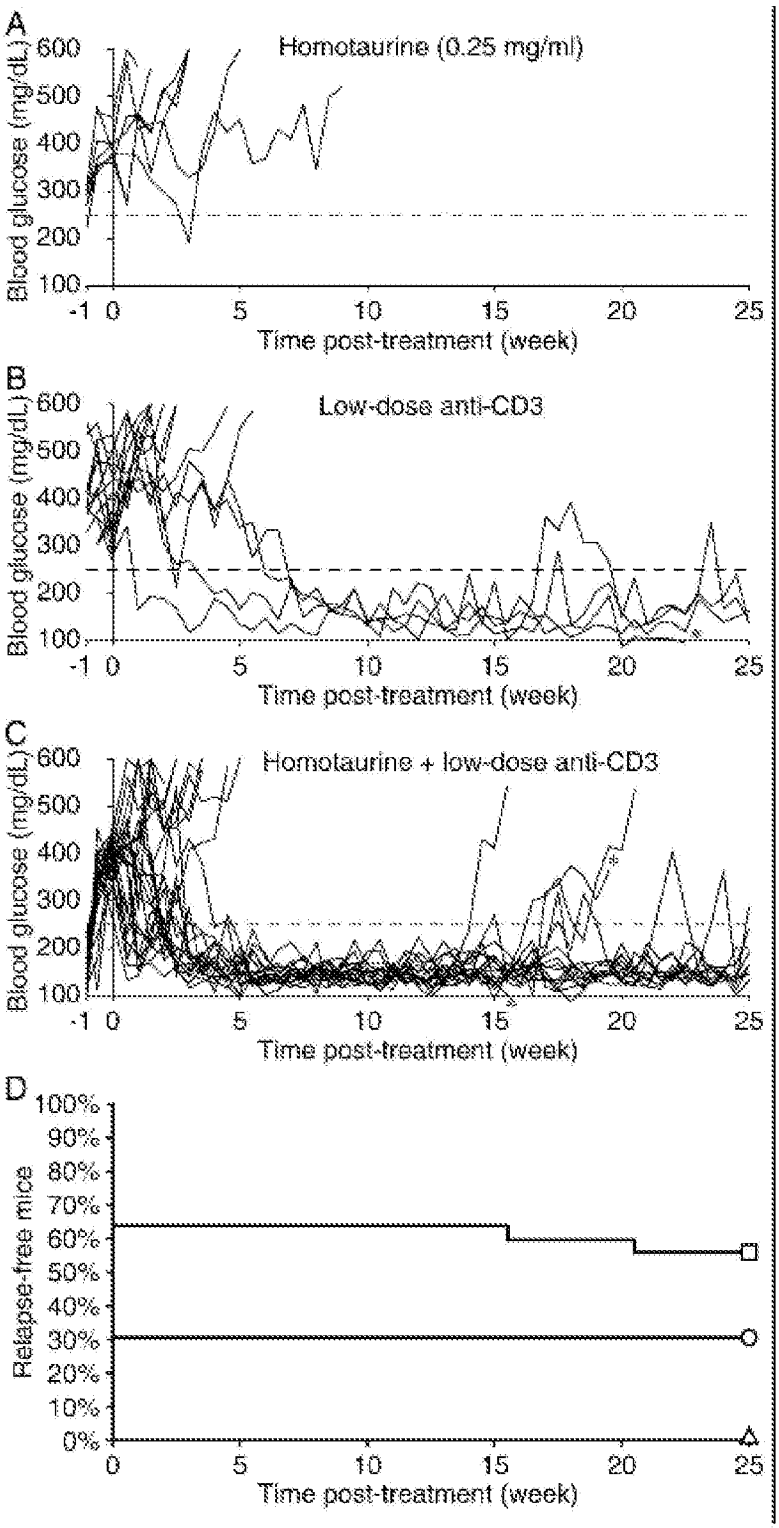

A
Homotaurine (0.25 mg/ml)
Blood glucose (mg/dL)
600
500
400
300
200
100
-1 0 5 10 15 20 25
Time post-treatment (week)
B
Low-dose anti-CD3
Blood glucose (mg/dL)
Time post-treatment (week)
C
Homotaurine + low-dose anti-CD3
Blood glucose (mg/dL)
Time post-treatment (week)
D
Relapse-free mice
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0 5 10 15 20 25
Time post-treatment (week)

FIGURE 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/56848

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 45/06, A61K 39/39, A61K 31/00, A61K 31/42, A61K 31/58 (2019.01)

CPC - A61K 31/197, A61K 31/4465, A61K 38/2013, A61K 39/0008, A61K 31/515

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2013/0251671 A1 (KAUFMAN et al.) 26 September 2013 (26.09.2013) Abstract; Claim 1; Claim 2; Claim 12; para [0005]; para [0016]; para [0063-0064]; para [0069]; para [0114]	1-3, 8-10, 15-17
Y		18-20
Y	BHAT et al., Inhibitory role for GABA in autoimmune inflammation. Proc Natl Acad Sci U S A. 9 Feb. 2010, Vol. 107, No.6, pp: 2580-2585. Abstract; p2583, col 1, para 1	18-20

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

* Special categories of cited documents:

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

"D" document cited by the applicant in the international application

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 February 2020	27 FEB 2020
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Lee Young Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/56848

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-7, 11-14, 32
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
----Please see continuation in first extra sheet ----------

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, 8-10, 15-20

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US 19/56848

Continuation of Box No. III. Observations where unity of invention is lacking.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-3, 8-10, 15-20, directed to a method for treating an inflammation-related disorder, reducing inflammation in the central nervous system, inducing CD8+ and CD4+ regulatory T cell responses and inhibiting autoreactive Th17 and Th1 responses in a human or animal subject.

Group II, claims 21-25, 31 (in part), directed to a method for treating type 1 diabetes in a human or animal subject in need thereof.

Group III, claims 26-30, 31 (in part), directed to a method for raising C peptide levels, lowering A1C levels, promoting islet cell survival, promoting Beta-cell replication, and inhibiting T cell proliferation in a human or animal subject.

The inventions listed as Groups I-III do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special technical features:

Group I has the special technical feature of treating an inflammation-related disorder by administering a GABAA-R specific agonist, that is not required by Groups II-III.

Group II has the special technical feature of treating type 1 diabetes by administering one or more immunomodulator compounds and one or more GABA-receptor agonist, that is not required by Groups I or III.

Group III has the special technical feature of method for improving clinical aspects associated with diabetes by administering homotaurine, that is not required by Groups I or II.

Common technical features:

Groups I-III share the common technical feature of: treating a disease in a human or animal subject by administering a GABAA-R specific agonist, homotaurine. However, this shared technical feature does not represent a contribution over prior art, because this shared technical feature is anticipated by US 2013/0251671 A1 to Kaufman et al., (hereinafter 'Kaufman').

Kaufman teaches treating a disease such as diabetes by administering a GABAA-R specific agonist, homotaurine (Abstract - 'In certain embodiments methods are provided for the therapeutic or prophylactic amelioration of one or more symptoms or disorders associated with metabolic syndrome. In various embodiments the methods involve administering to a subject in need thereof, a GABA receptor agonist, in an amount sufficient to ameliorate said one or more symptoms. In certain embodiments methods are provided for the prophylaxis or treatment of type I diabetes and related pathologies that involve the use of GABA or GABA agonists in combination with certain other compounds (e.g., one more antigens (e.g., GAD) that have a therapeutic effect in type I diabetes and/or an anti-CD3 antibody, an anti-CD20 antibody, exendin-4, and/or or a pro-insulin therapeutic).'; Claim 12 - 'The method of claim 1, wherein said GABA or GABA receptor agonist comprises a compound selected from the group consisting of....Homotaurine'; Claim 6 - 'A method of slowing or stopping the progression from a pre-diabetic condition in a mammal or from a non-diabetic condition in a mammal at risk for type II diabetes to type II diabetes,').

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I-III inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

NOTE, continuation of number 4 above: claims 4-7, 11-14, 32 are held unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).