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DESCRIPTION

FIELD OF THE INVENTION

[0001] The present invention is generally directed to antisense oligonucleotides against SMAD7 and uses thereof in treating and/or preventing diabetes and/or in promoting pancreatic islet cell survival after transplantation.

BACKGROUND

[0002] Diabetes is a metabolic disease characterized by high levels of sugar in the blood. The two most prevalent types of diabetes are Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM). Type 1, or insulin-dependent diabetes mellitus (IDDM), is a chronic autoimmune disease characterized by the extensive loss of beta cells in the pancreatic islets, which produce insulin. Type 2 diabetes, or non-insulin dependent diabetes mellitus (NIDDM), develops when muscle, fat and liver cells fail to respond normally to insulin (insulin resistance). In particular, Type 2 diabetes has become an epidemic, driven by increases in obesity and a sedentary lifestyle, and the general aging of the populations in many countries. In recent clinical practice, it has become increasingly difficult to distinguish T1DM from T2DM as many children with T1DM are overweight at diagnosis, and a considerable proportion of physician-diagnosed T2DM youth have evidence of pancreatic autoimmunity (Badaru, A. and Pihoker, C. "Type 2 diabetes in childhood: clinical characteristics and role of beta-cell autoimmunity," Curr. Diab. Rep., 2012, 12, 75-81). In 2011, more than 346 million people worldwide were affected by diabetes.

[0003] Recent studies have demonstrated that TGF-β plays a role in pancreatic islet function and diabetes development (Moritani, M. et al. "Abrogation of autoimmune diabetes in nonobese diabetic mice and protection against effector lymphocytes by transgenic paracrine TGF-β1," J. Clin. Invest., 1998, 102, 499-506; Olivieri, A. et al. "Serum transforming growth factor β1 during diabetes development in non-obese diabetic mice and humans," Clin. Exp. Immunol., 2010, 162, 407-414). For example, an islet-specific pulse of TGF-β expression for one week has been shown to delay diabetes development in NOD mice (Wållberg, M. et al. "An islet-specific pulse of TGF-β abrogates CTL function and promotes β cell survival independent of Foxp3+ T cells," J. Immunol., 2011, 186, 2543-2551), a commonly used animal model of type 1 diabetes (Roep, B. O. et al. "Satisfaction (not) guaranteed: re-evaluating the use of animal models of type 1 diabetes," Nat. Rev. Immunol., 2004, 4, 989-997). TGF-β1 was effective not only in curing diabetes in diabetic NOD mice and blocking islet destructive autoimmunity, but also in inducing islet regeneration (Luo, X. et al. "Systemic transforming growth factor-β1 gene therapy induces Foxp3+ regulatory cells, restores self-tolerance, and facilitates regeneration of beta cell function in overtly diabetic nonobese diabetic mice," Transplantation, 2005, 79, 1091-1096). Hence, therapeutic interventions along this pathway,

may not only stop the progression of the disease, but might even restore function (i.e., adequate insulin production) after onset of hyperglycemia.

[0004] The TGF-βs 1-3 are involved in a variety of biological functions including cell growth, organ development, fibrogenesis, and regulation of immune cells. TGF-β1 is the predominant form expressed in the immune system, and it is now well-recognized as a critical regulator in immune responses that can dampen T cell responses (Li, M. O. and Flavell, R. A. "TGF-beta: a master of all T cell trades," Cell, 2008, 134, 392-404). Specifically, TGF-β1 binds a heterodimeric transmembrane serine/threonine kinase receptor containing two subunits, TGF-β1 R1 and TGF-β1 R2. Upon ligand binding, the TGF-β1 R1 receptor is phosphorylated by the constitutively active TGF-β1 R2 receptor and signal is propagated to the nucleus by proteins belonging to the SMAD family. Activated TGF-β1 R1 directly phosphorylates SMAD2 and SMAD3 proteins, which then interact with SMAD4. The complex of SMAD2/SMAD3/SMAD4 translocates to the nucleus and modulates the transcription of certain genes. SMAD7 is another member of this protein family that acts as a general antagonist for TGF-β through negative-feedback mechanisms (Yan, X. and Chen, Y. G. "Smad7: not only a regulator, but also a cross-talk mediator of TGF-beta signalling," Biochem. J., 2011, 434, 1-10).

[0005] Studies have demonstrated that SMAD7 plays a role in diabetes and β-cell function. SMAD7, an intracellular protein, has been shown to interfere with binding of SMAD2/SMAD3 to the TGF-β1 R1 preventing phosphorylation and activation of these proteins, leading to inhibition of TGF-β1 mediated-signaling. Expression of SMAD7 in pancreatic β-cells has been shown to disrupt TGF-β signaling and induce reversible diabetes mellitus (Smart, N. G. et al. "Conditional expression of Smad7 in pancreatic beta cells disrupts TGF-beta signaling and induces reversible diabetes mellitus," PLoS Biol., 2006, 4, e39). Furthermore, results in NOD mice also implicate the Smad2 and TGF-β signaling pathway in activated dendritic cells in diabetogenesis, and there is evidence from human genome-wide association studies supporting a role for Smad7 in human type 1 diabetes (Hook, S. M. et al. "Smad2: A candidate gene for the murine autoimmune diabetes locus Idd21.1," J. Clin. Endocrinol. Metab., 2011, 96, E2072-E2077). Since TGF-β1 has been shown to contribute to the suppression of cytokine production, the inhibition of T cell response, and the induction of regulatory T cells (Treg) (Kawamoto, K. et al. "Transforming growth factor beta 1 (TGF-β1) and rapamycin synergize to effectively suppress human T cell responses via upregulation of FoxP3+ Tregs," Transpl. Immunol., 2010, 23, 28-33), SMAD7 modulation could also be beneficial in islet transplantation by supporting graft function, limiting toxicity, and preventing immune rejection.

[0006] Thus, there is an unmet need for new therapies in diabetes and pancreatic islet transplantation.

SUMMARY

[0007] In one aspect the present invention provides a mothers against decapentaplegic homolog 7 (SMAD7) antisense oligonucleotide selected from the group consisting of SEQ ID

NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12, for use in the treatment of diabetes by maintaining and/or restoring pancreatic β-cell function, as set out in the claims.

[0008] Also provided is a pharmaceutical composition as set out in the claims comprising a SMAD7 antisense oligonucleotide selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12, and a pharmaceutically acceptable carrier for use in the treatment of diabetes by maintaining and/or restoring pancreatic β-cell function, as set out in the claims.

[0009] Some preferred embodiments are set out in the dependent claims.

[0010] The disclosed methods are based, in part, upon the discovery of specific inhibitors of SMAD7 expression or function, *e.g.,* antisense oligonucleotides against SMAD7, that inhibit SMAD7 and therefore restore TGF-β signaling. Exemplary antisense oligonucleotides against SMAD7 include, for example, SEQ ID No:5 or SEQ ID No: 6, which may be used in a pharmaceutical composition. The disclosed SMAD7 inhibitors can be used to treat and/or prevent diabetes, *e.g.,* Type 1 diabetes, Type 2 diabetes, and gestational diabetes. The antisense oligonucleotides against SMAD7 can also be used to promote pancreatic islet cell survival after transplantation.

[0011] The foregoing aspects and embodiments of the invention may be more fully understood by reference to the following figures, detailed description and claims. As used herein, "including" means without limitation, and examples cited are non-limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012]

Fig. 1 (A) provides the nucleic acid sequence of SMAD7 (SEQ ID NO: 1) and (B) provides the amino acid sequence of SMAD7 (SEQ ID NO: 2).

Fig. 2 (A) is a graph showing the effects of TGFβ and GED-0301 (a SMAD7 antisense oligonucleotide, SEQ ID NO: 6) on NF-κB activation in HEK-Blue TNF-α sensor cells and (B) is a graph showing the effect of TGFβ and GED-0301 on NF-κB activation in HEK-Blue CD40L sensor cells challenged by TNF-α and CD40L, respectively.

Fig. 3 (A) is a panel illustrating the gating methodology used in analysis of flow cytometry results for the organ-specific uptake of fluorescein-labeled GED-0301 following administration by various routes in mice and Fig. 3 (B) shows corresponding percent uptakes (FITC+) in viable cells in the indicated organ tissues.

Fig. 4 is a multichannel high resolution microscopic image depicting sections of pancreas from a control mouse and a mouse administered fluorescein-labeled GED-0301 subcutaneously (4

hours after administration). Color legend: green - fluorescent GED-0301, grey - DAPI (a marker of cell nucleus), red - insulin (indicating insulin-producing β-cells), and blue - CD45 (indicating CD45 or leukocyte common antigen, LCA, a marker of white blood cells, the elements of the circulating blood system that comprise the cells of immunity and inflammation).

Fig. 5(A) is a graph depicting blood glucose levels in NOD mice treated with the SMAD7 antisense oligonucleotide GED-0301 (AS GED-0301), the corresponding sense control (S control), and saline (Control) (125 μg/animal, subcutaneous (s.c.), daily) following onset of hyperglycemia and Fig. 5 (B) is a corresponding Kaplan-Meier survival curve showing the percent of animals remaining diabetes-free as a function of time.

Fig. 6 is a graph comparing the results of an oral glucose tolerance test conducted in GED-0301-treated NOD mice that did not become diabetic by the end of the study (day 150) and control mice.

Fig. 7 shows multichannel high resolution Z-stacks of confocal microscopic images comparing representative islet-containing sections of pancreas from GED-0301-treated NOD mice that did not become diabetic by the end of the study (day 150) to those of corresponding controls. Color legend: blue - DAPI (a marker of cell nucleus), red - insulin (indicating insulin-producing β-cells), and green - glucagon (indicating glucagon-producing α-cells).

DETAILED DESCRIPTION

[0013] The present disclosure is generally directed to antisense oligonucleotides against SMAD7 and uses thereof in treating and/or preventing diabetes and/or in promoting pancreatic islet cell survival after transplantation.

Anti-SMAD7 Therapy

[0014] The disclosed methods relate to the use of a specific SMAD7 inhibitor selected from the group consisting of small binding molecules, e.g., natural and synthetic compounds, antibodies, aptamers, intramers, RNAi (double stranded RNA, siRNA) and anti-SMAD7 antisense molecules for treating and/or preventing diabetes and/or in promoting pancreatic islet cell survival after transplantation. SMAD7 inhibitors may also comprise truncated and/or mutated SMAD7 molecules which interfere with the SMAD7 and which, thereby, inhibit SMAD7 function.

[0015] For example, anti-SMAD7 therapy includes targeted therapies against SMAD7 (e.g., anti-SMAD7 antisense therapies, i.e., antisense oligonucleotide against SMAD7, and antibodies against SMAD7). Antisense oligonucleotides are short synthetic oligonucleotide sequences complementary to the messenger RNA (mRNA), which encodes for the target

protein (e.g., SMAD7). Antisense oligonucleotide sequences hybridize to the mRNA producing a double-strand hybrid that can lead to the activation of ubiquitary catalytic enzymes, such as RNase H, which degrades DNA/RNA hybrid strands thus preventing protein translation.

[0016] An anti-SMAD7 antisense oligonucleotide may target site 403, 233, 294, 295, 296, 298, 299, and/or 533 (*i.e.,* nucleotides 403, 233, 294, 295, 296, 298, 299, and 533, respectively) of the human SMAD7 mRNA (*e.g.,* of SEQ ID NO: 1) (see Fig. 1). In an exemplary embodiment, the anti-SMAD7 antisense oligonucleotide targets nucleic acids 403-423 of human SMAD7 mRNA.

[0017] In certain embodiments, an antisense oligonucleotide may be derived from the following anti-SMAD7 antisense oligonucleotide 5'-GTCGCCCCTTCTCCCCGCAGC-3' (SEQ ID NO: 3).

[0018] It is contemplated herein that an antisense oligonucleotide targeting SMAD7 may comprise a mixed-backbone wherein the cytosine residues in a CpG pair are replaced by 5'-methylcytosine (abbreviated as Me-dC). Methylphosphonate linkages may also be placed at the 5' and/or 3' ends of an antisense oligonucleotide (abbreviated as MeP). The phosphonate backbone of a contemplated anti-SMAD7 antisense oligonucleotide may optionally include 1,2, 3, 4 or more phosphorothioate bonds (e.g., phosphorothioate bonds would replace phosphodiester bonds). In an embodiment, all phosphonate bonds may be phosphorothioate bonds.

[0019] Antisense oligonucleotide therapies disclosed herein that target SMAD7 include, but are not limited to:

5'-GTXYCCCCTTCTCCCXYCAG-3' (SEQ ID NO: 4), wherein X is a nucleotide comprising a nitrogenous base selected from the group consisting of cytosine and 5-methylcytosine or a 2'-O-methylcytosine nucleoside, and wherein Y is a nucleotide comprising a nitrogenous base selected from the group consisting of guanine and 5-methylguanine or a 2'-O-methylguanine nucleoside, provided that at least one of the nucleotides X or Y comprises a methylated nitrogenous base;

5'-GTXGCCCCTTCTCCCXGCAG-3' (SEQ ID NO: 5), wherein X is 5-methyl 2'-deoxycytidine 5'-monophosphate;

5'-GTXGCCCCTTCTCCCXGCAGC-3' (SEQ ID NO: 6), wherein X is 5-methyl 2'-deoxycytidine 5'-monophosphate;

5'-ZTXGCCCCTTCTCCCXGCAZ-3' (SEQ ID NO: 7), wherein X is 5-methyl 2'-deoxycytidine 5'-monophosphate and Z is 2'-deoxyguanosine methylphosphonate;

5'-ZTXGCCCCTTCTCCCXGCAZC-3' (SEQ ID NO: 8), wherein X is 5-methyl 2'-deoxycytidine 5'-monophosphate and Z is 2'-deoxyguanosine methylphosphonate.

[0020] In a particular embodiment, contemplated SMAD7 antisense may be a sequence comprising one of:

5'-GTXGCCCCTTCTCCCXGCAG-3' (SEQ ID NO: 9), wherein X is 5-methyl 2'-deoxycytidine 5'-monophosphorothioate;

5'-GTXGCCCCTTCTCCCXGCAGC-3' (SEQ ID NO: 10), wherein X is 5-methyl 2'-deoxycytidine 5'-monophosphorothioate;

5'-ZTXGCCCCTTCTCCCXGCAZ-3' (SEQ ID NO: 11), wherein X is 5-methyl 2'-deoxycytidine 5'-monophosphate and Z is 2'-deoxyguanosine methylthiophosphonate;

5'-ZTXGCCCCTTCTCCCXGCAZC-3' (SEQ ID NO: 12), wherein X is 5-methyl 2'-deoxycytidine 5'-monophosphate and Z is 2'-deoxyguanosine methylthiophosphonate.

For example, SEQ ID NOs. 9-12 include 1, 2, 3, 4 or more phosphorothioate bonds. All O,Ophosphonate bonds of SEQ ID NOs. 9-12 may be phosphorothioate bonds.

Methods of Treatment

[0021] Disclosed herein are methods of promoting organ and/or cell, for example, pancreatic islet cell, survival after transplantation, comprising administering to a patient in need thereof an effective amount of a specific inhibitor of SMAD7 expression or function, for example, an antisense oligonucleotide against SMAD7.

[0022] Also, disclosed herein are methods of treating and/or preventing diabetes, *e.g.,* Type 1 diabetes and Type 2 diabetes, comprising administering to a patient in need thereof an effective amount of a specific inhibitor of SMAD7 expression or function, for example, an antisense oligonucleotide against SMAD7.

[0023] Diabetes includes a group of metabolic diseases characterized by high blood sugar or ketoacidosis, as well as chronic, general metabolic abnormalities arising from a prolonged high blood sugar status or a decrease in glucose tolerance. Diabetes includes both the Type 1 and Type 2 forms of the disease, gestational diabetes, and other conditions of insulin deficiency.

[0024] It will be appreciated that the disclosed methods are also applicable to treating and preventing latent autoimmune diabetes (*i.e.,* type 1.5 diabetes), metabolic imbalances, a pre-diabetic state, metabolic syndrome, a lipid or glucose related disorder, *e.g.,* hyperlipidemia and/or hypercholesterolemia, and other related disorders.

[0025] Metabolic imbalances can include any disorder or disease state or condition that are associated with an elevated plasma glucose. A metabolic imbalance, for example, comprises diabetes mellitus, gestational diabetes, genetic defects of β-cell function, genetic defects in

insulin action, diseases of the exocrine pancreas, endocrinopathies, drug or chemical-induced infections, other genetic syndromes associated with diabetes, a pre-diabetic state, and metabolic syndrome.

[0026] Metabolic syndrome is characterized by a group of metabolic risk factors in one person, as described by the American Heart Association (AHA). Metabolic syndrome is also known as metabolic syndrome X, syndrome X, insulin resistance syndrome, Reaven's syndrome, or CHAOS. The risk factors include, but are not limited to, abdominal obesity, atherogenic dyslipidemia, hypertension, insulin resistance or glucose intolerance, prothrombotic state (high fibrinogen or plasminogen activator inhibitor-1), and proinflammatory state (elevated C-reactive protein).

[0027] The terms "treat", "treatment", "treating" and the like are used herein to generally mean obtaining a desired pharmacological and/or physiological effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of partially or completely curing a disease and/or adverse effect attributed to the disease. The term "treatment" as used herein covers any treatment of a disease in a mammal, particularly a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, *i.e.* arresting its development; or (c) relieving the disease, *i.e.* causing regression of the disease.

[0028] A subject's responsiveness to treatment with an anti-SMAD7 therapy can be interpreted with respect to a control sample (described below) obtained from the subject prior to treatment. A subject may be identified as responding to treatment with an anti-SMAD7 therapy if there is a reduction in SMAD7 expression; if there is an increase in the total pancreatic insulin content; if β-cell function is maintained and/or restored (for example, as assessed via standard methods such as the intravenous glucose tolerance test, IVGTT); and/or if normoglycemic,*i.e.*, normal glucose content of the blood, is maintained and/or restored. A subject which has received an islet graft, *e.g.,* an allogenic islet graft, may be identified as responding to treatment with anti-SMAD7 therapy if there is prevention of rejection and/or prolonged survival of the islets.

[0029] A test sample may be obtained from the patient, for example, at week 1, week 2, week 4, week 8, week 10 or later after initiation of therapy to determine sensitivity to treatment. In some embodiments, the test sample may be obtained, for example, one week, two weeks, three weeks, four weeks, five weeks, six weeks, seven weeks, eight weeks, nine weeks, ten weeks, eleven weeks, twelve weeks, five months, six months, and/or one year or longer after the initiation of therapy to monitor sensitivity to treatment.

[0030] A control sample may include a sample (*e.g.,* a blood or tissue sample) obtained from the subject prior to treatment with an anti-SMAD7 therapy. The control sample provides a baseline for monitoring a subject's progress to treatment. A control sample may be obtained from the subject on the day the anti-SMAD7 therapy is first administered (*e.g.,* Day 1 of a treatment regimen). A control sample may be obtained from a subject one day prior to the start

of an anti-SMAD7 therapy (*e.g.,* Day 0 of a treatment regimen). Alternatively, a control sample may be obtained from a subject 2, 3, 4, 5, 6, 7 or more days prior to the start of an anti-SMAD7 therapy. For example, the upregulation or down regulation of certain cell samples may be measured prior to treatment (*e.g.,* a control sample), during treatment, and/or after treatment to monitor a subject's response to therapy, *e.g.,* an anti-SMAD7 therapy.

[0031] A control sample may include, for example, a sample to monitor the glucose level of a subject, wherein the sample was obtained from the subject prior to treatment with an anti-SMAD7 therapy.

[0032] A control level may be established for a subject based on long-term monitoring of certain cell populations in the subject. In such instances, it is contemplated that a subject may undergo multiple rounds of treatment with an anti-SMAD7 therapy. The amount of a certain cell population detected following multiple rounds of treatment may be compared to a prior control level for the subject to determine whether the subject has responded to therapy and/or is likely to respond to further treatment with an anti-SMAD7 therapy. A control or baseline level for a subject may be established based on an average measurement of a certain cell population determined from multiple baseline samples obtained over time (*e.g.,* obtained over the course of weeks, months, or years). Accordingly, any test or assay conducted as disclosed herein may be compared with a previous or established control level and it may not be necessary to obtain a new control sample from the subject for comparison, *e.g.,* if the subject is receiving more than one round of treatment with an anti-SMAD7 therapy.

Administration and Formulation

[0033] In some embodiments, contemplated herein are pharmaceutical compositions comprising a contemplated antisense oligonucleotide against SMAD7 as set out in the claims and a pharmaceutically acceptable carrier for use in the treatment of diabetes by maintaining and/or restoring pancreatic β-cell function. For example, the pharmaceutical composition may be administered subcutaneously. Alternatively, the pharmaceutical composition may be administered orally.

[0034] As used herein, "pharmaceutically acceptable carrier" means buffers, carriers, and excipients suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. The carrier(s) should be "acceptable" in the sense of being compatible with the other ingredients of the formulations and not deleterious to the recipient. Pharmaceutically acceptable carriers include buffers, solvents, dispersion media, coatings, isotonic and absorption delaying agents, and the like, that are compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is known in the art.

[0035] In one embodiment, a contemplated antisense oligonucleotide against SMAD7 and any

pharmaceutical composition thereof may be administered by one or several routes, including orally, topically, parenterally,e.g., subcutaneous injection, by inhalation spray or rectally. The term parenteral as used herein includes subcutaneous injections, intrapancreatic administration, intravenous, intramuscular, intraperitoneal, intrasternal injection or infusion techniques. For example, the antisense oligonucleotide against SMAD7 may be administered subcutaneously to a subject. In another example, the antisense oligonucleotide against SMAD7 may be administered orally to a subject.

[0036] Pharmaceutical compositions containing an antisense oligonucleotide against SMAD7, such as those disclosed herein, can be presented in a dosage unit form and can be prepared by any suitable method. A pharmaceutical composition should be formulated to be compatible with its intended route of administration. Useful formulations can be prepared by methods well known in the pharmaceutical art. For example, see Remington's Pharmaceutical Sciences, 18th ed. (Mack Publishing Company, 1990).

[0037] Pharmaceutical formulations preferably are sterile. Sterilization can be accomplished, for example, by filtration through sterile filtration membranes. Where the composition is lyophilized, filter sterilization can be conducted prior to or following lyophilization and reconstitution.

[0038] In an exemplary embodiment, a pharmaceutical composition for subcutaneous administration of an antisense oligonucleotide against SMAD7 comprises an antisense oligonucleotide such as that represented by SEQ ID NO: 6, or a pharmaceutically acceptable salt thereof (such as a sodium salt), and a pharmaceutically acceptable carrier.

[0039] In another embodiment, a pharmaceutical tablet formulation for oral administration of an antisense oligonucleotide against SMAD7 comprises an intragranular phase, wherein the intra-granular phase includes an antisense oligonucleotide such as that represented by SEQ ID NO: 6, or a pharmaceutically acceptable salt thereof (such as a sodium salt), and a pharmaceutically acceptable filler, and which may also include an extra-granular phase, that may include a pharmaceutically acceptable excipient such as a disintegrant. For example, a pharmaceutically acceptable tablet for oral use may comprise an intra-granular phase, comprising about 5 to about 10% by weight antisense oligonucleotide represented by SEQ ID NO 6 or a pharmaceutically acceptable salt thereof, about 40% by weight mannitol, about 8% by weight microcrystalline cellulose, about 5% by weight hydropropylmethyl cellulose, and about 2% by weight sodium starch glycolate; an extra-granular phase comprising about 17% by weight microcrystalline cellulose, about 2% by weight sodium starch glycolate, about 0.4% by weight magnesium stearate; and an enteric coating over the tablet, comprising about 13% by weight AcyrlEZE® (see, e.g., PCT Publication No. WO/2010/054826).

[0040] Exemplary formulations include dosage forms that include or consist essentially of about 35 mg to about 500 mg of an antisense oligonucleotide against SMAD7. For example, formulations that include about 35 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, 100 mg, 110 mg, 120 mg, 130 mg, 140 mg, 150 mg, 160 mg, 170 mg, 180 mg, 190 mg, 200 mg, or 250

mg of an antisense oligonucleotide against SMAD7 are contemplated herein. In one embodiment, a formulation may include about 40 mg, 80 mg, or 160 mg of an antisense oligonucleotide against SMAD7. The amount administered will depend on variables such as the type and extent of disease or indication to be treated, the overall health of the patient, the *in vivo* potency of the antibody, the pharmaceutical formulation, and the route of administration. The initial dosage can be increased beyond the upper level in order to rapidly achieve the desired blood-level or tissue level. Alternatively, the initial dosage can be smaller than the optimum, and the dosage may be progressively increased during the course of treatment. Human dosage can be optimized, *e.g.,* in a conventional Phase I dose escalation study designed to run from 40 mg to 160 mg. Dosing frequency can vary, depending on factors such as route of administration, dosage amount and the disease being treated. Exemplary dosing frequencies are once per day, once per week and once every two weeks. In some embodiments, dosing is once per day for 7 days. A preferred route of administration is subcutaneous.

[0041] In some aspects, methods provided herein may further include administering at least one other agent that is directed to treatment of diseases and disorders disclosed herein. Contemplated other agents may be co-administered (*e.g.,* sequentially or simultaneously).

[0042] Agents contemplated include immunosuppressive agents including glucocorticoids, cytostatics, antibodies, agents acting on immunophilins, interferons, opioids, TNF binding proteins, mycophenolate, and small biological agents. For example, contemplated immunosuppressive agents include, but are not limited to: tacrolimus, cyclosporine, pimecrolimus, sirolimus, everolimus, mycophenolic acid, fingolimod, dexamethasone, fludarabine, cyclophosphamide, methotrexate, azathioprine, leflunomide, teriflunomide, anakinra, anti-thymocyte globulin, anti-lymphocyte globulin, muromonab-CD3, afutuzumab, rituximab, teplizumab, efalizumab, daclizumab, basiliximab, adalimumab, infliximab, and etanercept.

[0043] Agents contemplated include drug therapies for regulating blood sugar levels including oral therapies with hypoglycemic agents and/or oral anti-diabetic agents, injectable therapies, and the like. Non-drug therapies for regulating blood sugar level include, but are not limited to, diatetic and/or exercise control measures.

[0044] Oral drug therapies for regulating blood sugar levels include hypoglycemic agents that may include, but are not limited to: Acarbose, Acetohexamide, Chlorpropamide, Darglitazone Sodium, Glimepiride, Glipizide, Glyburide, Repaglinide, Troglitazone, Tolazamide, and Tolbutamide.

[0045] Oral drug therapies for regulating blood sugar levels include antidiabetic agents that may include but are not limited to: Acarbose, Acetohexamide, Buformin, Butoxamine Hydrochloride, Camiglibose, Chlorpropamide, Ciglitazone, Englitazone Sodium, Etoformin Hydrochloride, Gliamilide, Glibornuride, Glicetanile Gliclazide Sodium, Gliflumide, Glipizide, Glucagon, Glyburide, Glyhexamide, Glymidine Sodium, Glyoctamide, Glyparamide, Insulin

Isophane, Insulin Human Zinc, Extended Insulin, Insulin Lispro, Linogliride, Linogliride Fumarate, Metformin, Methyl Palmoxirate, Palmoxirate Sodium, Pioglitazone Hydrochloride, Pirogliride Tartrate, Proinsulin Human, Repaglinide, Seglitide Acetate, Tolazamide, Tolbutamide, Tolpyrramide, Troglitazone, and Zopolrestat.

[0046] Injectable therapies for regulating blood sugar levels may include, but are not limited to fast-acting insulin, long-acting insulin, and related insulin. Fast-acting insulin includes regular insulin, Prompt Insulin Zinc Suspension, and Semilente® insulin, Humalog® Injection, Humulin® R, Iletin II, Novolin R, Purified Pork Regular Insulin, and Velosulin BR Human Insulin. Long-acting insulin includes Protamine Zinc Insulin Suspension, Extended Insulin Zinc Suspension, Ultralente® Insulin, and Humulin® U. Other insulin includes Isophane Insulin Suspension, NPH insulin, isophane insulin; Insulin Zinc Suspension Lente® Insulin, Human Insulin Isophane Suspension/Human Insulin Injection, Humulin® 50/50, Humulin® 70/30, and Novolin® 70/30.

EXAMPLES

[0047] The invention is further illustrated by the following examples. The examples are provided for illustrative purposes only, and are not to be construed as limiting the scope or content of the invention in any way.

Example 1: Effect of SMAD7 Antisense on NF-κB Activation

[0048] HEK-Blue™ (InvivoGen) TNF-α/IL-1β sensor cells are designed to detect bioactive TNF-α and IL-1β by monitoring the activation of the NF-κB pathway. These cells were generated by stable transfection of human embryonic kidney HEK293 cells with a SEAP reporter gene under the control of the IFN-β minimal promoter fused to five NF-κB and five AP-1 binding sites. HEK-Blue™ (InvivoGen) CD40L sensor cells are used to measure the bioactivity of CD154 (CD40L) through the secretion of embryonic alkaline phosphatase (SEAP) upon NF-κB activation following CD40 stimulation. These cells were generated by stable transfection of HEK293 cells with the human CD40 gene and an NF-κB-inducible SEAP construct. Therefore, HEK-Blue™ cells measure the bioactivity of TNFα/CD40L through the secretion of embryonic alkaline phosphatase (SEAP) upon NF-κB activation following TNFR/CD40 stimulation. Tumor necrosis factor alpha (TNF-α) is a known cytokine involved in systemic inflammation and the regulation of immune cells. CD40L is a co-stimulatory protein involved in T-cell activation and the development of effective immune responses, and is thought to be involved in the development of autoimmune diseases including T1DM (Margolles-Clark, E. et al. "Small molecule costimulatory blockade: organic dye inhibitors of the CD40-CD154 interaction," J. Mol. Med., 2009, 87, 1133-1143).

[0049] HEK Blue TNFα and CD40L sensor cells (Invivogen) (50,000/well) were incubated with

GED-0301 antisense (AS) or control sense (S) oligonucleotide (OGN; 4 μg/mL) for 6 h in the presence of lipofectamine (LPF) then media for 18 h followed by challenge by TNFα (Ing/mL) or CD40L (25 ng/mL) in the presence or absence of TGF-β (200 ng/mL; 24 h, DMEM P/S 2% serum). GED-0301 is an SMAD7 antisense oligonucleotide (GTXGCCCCTTCTCCCXGCAGC, wherein X is 5-methyl-2'-deoxycytidine 5' monophosphate (5-Me-dC) (SEQ ID NO: 6)). Secretion of SEAP induced by TNFα or CD40L in these cells was quantified using QUANTI-Blue™, a colorimetric enzyme assay provided by the manufacturer and specifically developed for this purpose with a medium that changes to a purple-blue color in the presence of SEAP, which can be quantified by reading OD at 625-655 nm. Data are the average of three independent experiments with quadruplicates per plate using values normalized to the stimulated response (TNFα or CD40L alone) in untreated control cells.

[0050] The effects of TGF-β and GED-0301 on NF-κB activation in HEK-Blue cells by TNFα and CD40L, respectively are shown in Figs. 2A and 2B. Results show that both TNFα and CD40L produced considerable NF-κB activation (as measured by SEAP expression) and these were both suppressed by TGF-β. This suppression was considerably enhanced further by treatment with the SMAD7 antisense oligonucleotide GED-0301 indicating that this treatment can enhance and/or restore the effects of TGF-β. In cell culture systems, TGF-β can either promote or inhibit NF-κB activation depending on the cell type used (Hong, S. et al. "Smad7 sensitizes tumor necrosis factor induced apoptosis through the inhibition of antiapoptotic gene expression by suppressing activation of the nuclear factor-kappaB pathway," Cancer Res., 2007, 67, 9577-9583 and references therein). TGF-β was a negative regulator of NF-κB in B cells and in human intestinal lamina propria mononuclear cells (LPMC). The results for transfected HEK cells are similar to those observed for LPMC, where it has been shown that TGF-β1 negatively regulates NF-κB activation in normal LPMC (as pretreatment with TGF-β1 suppressed TNF-α-induced NF-κB activation), and that treatment of IBD LPMC with a specific Smad7 antisense resulted in inhibition of NF-κB activation (Monteleone, G. et al. "A failure of transforming growth factor-β1 negative regulation maintains sustained NF-κB activation in gut inflammation," J. Biol. Chem., 2004, 279, 3925-3932).

[0051] To confirm that GED-0301 can penetrate the assayed cells, HEK Blue sensor cells (50,000/well) were incubated with fluorescein-labeled GED-0301 AS OGN for 48 h in the presence or absence of lipofectamine (LPF, Invitrogen) and then analyzed for cellular uptake. Microscope imaging confirmed that the HEK Blue sensor cells which were incubated with fluorescein-labeled GED-0301 in the presence of lipofectamine displayed uptake of the fluorescein-labeled GED-0301 better than cells not treated with lipofectamine.

Example 2: Distribution Study of SMAD7 Antisense in Mice

[0052] Regular B6 mice (6 wk, males; Jackson Lab.) with multiple low-dose streptozotocin-induced diabetes (STZ, a β-cell specific toxin; 40 mg/kg i.p. for 5 consecutive days) were administered fluorescein-labeled GED-0301 antisense oligonucleotides (TriLink) by three different routes: oral (p.o.), intraperitoneal (i.p.), and subcutaneous (s.c.) (125 μg/mouse = 5

mg/kg; p.o. by oral gavage using 500 μL bicarbonate solution, pH 9.5 to protect from gastric degradation). At two different time-points (4 h and 24 h), organs were collected and processed from two different animals (one for microscopy, one for flow cytometry). The following organs were collected: pancreas, liver, kidney, spleen, thymus, intestine, brain, blood, pancreatic lymph nodes, brachial lymph node, gut-associated lymphoid tissue. For flow cytometry analysis, organs were digested using collagenase D (Roche), passed through a cell strainer (70 μm; BD Falcon), re-suspended in HBSS, and stained for live cells (LIVE/DEAD® fixable violet dead cell stain kit; Invitrogen) and for leukocytes (anti-mouse CD45 APC; eBioscience). They were then analyzed for fluorescein content in viable cells by using a BD LSR II Flow Cytometer (BD Biosciences, San Jose, CA) and the software FlowJo version 7.2.2 (Ashland, OR). Fig 3A shows an example to illustrate the gating methodology used to analyze the flow cytometry results. Fig. 3B includes illustrative flow cytometry results obtained for the corresponding percent uptakes in viable cells in specific organs, and they demonstrate significant antisense oligonucleotide uptake (percent FITC+) by the pancreas, pancreatic lymph nodes, and other organs (both in leukocytes, CD45+/FITC+, and in other cells, CD45-/FITC+), especially following s.c. administration. Some organs such as the brain showed no significant uptake. These results confirm that GED-0301 can be delivered to specific organs using clinically acceptable administration routes including subcutaneous administration.

[0053] Multichannel high resolution microscopic images(Fig. 4) confirmed the distribution of GED-0301 into the pancreas of mouse following subcutaneous administration of the fluorescently labeled antisense oligonucleotide in agreement with the flow cytometry results (Fig. 3B). Color legend: green - fluorescent GED0301, grey - DAPI (a marker of cell nucleus), red - insulin (indicating insulin-producing β-cells), and blue - CD45 (indicating CD45 or leukocyte common antigen, LCA, a marker of white blood cells, the elements of the circulating blood system that comprise the cells of immunity and inflammation)

Example 3: *In Vivo* Studies with SMAD7 Antisense

[0054] Further *in vivo* studies can be used to establish the effect of the treatment in animal models.

Hyperglycemia Reversal Study of SMAD7 Antisense in NOD Mice

[0055] Non-obese diabetic (NOD) mice, *e.g.,* NOD mice were used for an intervention-type diabetes prevention trial with treatment started only upon onset of hyperglycemia. NOD mice are a commonly used animal model of type 1 diabetes (Roep, B. O. et al. Satisfaction (not) guaranteed: re-evaluating the use of animal models of type 1 diabetes. Nat. Rev. Immunol., 2004, 4, 989-997), and they have been used to evaluate a large number of possible treatments (Shoda, L. K. et al. "A comprehensive review of interventions in the NOD mouse and implications for translation," Immunity, 2005, 23, 115-126). Eight week old prediabetic

female animals were procured (Taconic), and starting from week 10 (following acclimatization), glycosuria was monitored twice a week. In animals that turned positive, blood glucose levels (glycemia) were monitored three times a week. Animals with elevated glucose levels (nonfasting glycemia>200 mg/dL) on two consecutive days, received one sustained-release insulin pellet implant (LinBit, LinShin Canada, Inc.) to avoid severe hyperglycemia and exhaustion/over-work of the remaining β-cells, and they were started on treatment. Treatments were administered s.c., since this route is clinically relevant and the distribution studies described above indicated it to be effective in targeting the pancreas, the pancreatic LN, and other organs of possible interest. Animals were assigned to one of three treatment arms following a predefined rotating pattern in the order of their hyperglycemia development. Treatment (GED-0301 = Smad7 AS OGN; 125 µg/mouse) and corresponding controls (S OGN and saline only) were administered daily. Animals were monitored twice weekly and assessed for their ability to maintain normoglycemia. Following the disappearance of the effects of the implanted insulin pellets (approximately 25 days), animals with three consecutive glucose readings of >250 mg/dL were considered as diabetic and sacrificed with representative organs (pancreas, PLN) collected. In animals that did not become diabetic, daily treatment was stopped after 10 weeks, and they were monitored for an additional 12 weeks (up to a total of 150 days) to establish whether there is a lasting effect. At the end, all animals were sacrificed and representative organs (pancreas, PLN, and others) collected.

[0056] Results from the study are shown in Fig. 5 with blood glucose levels in Fig. 5A and a corresponding Kaplan-Meier survival curve showing the percent of animals remaining diabetes-free as a function of time in Fig. 5B. Treatment was initiated after the onset of hyperglycemia when β-cell damage had already occurred. Accordingly, severe hyperglycemia can develop very quickly. All animals treated with saline or sense oligonucleotide control developed diabetes (n = 7 and 6, respectively), as indicated by their hyperglycemia (blood glucose >250 mg/dL) following the exhaustion of the LinBit implant with sustained release insulin by day 20-25. For the animals treated with the active GED-0301 antisense oligonucleotide, 6 out of the 11 treated did not develop hyperglycemia and they remained diabetes-free up to the end of the study (day 150), long after the treatment had been stopped (day 70).

[0057] To verify the glucose response of non-diabetic animals at the end of the study (day 150), an oral glucose tolerance test (OGTT) was performed in several GED-0301-treated as well as control mice. A glucose dose of 150 mg was administered by oral gavage to mice fasted for 16h and blood samples were collected at predefined time intervals for up to 150 minutes. Results shown in Fig. 6 confirm that the glucose response, and hence insulin-secreting ability, was comparable to that of healthy animals. Blood glucose levels returned to normal within 150 minutes of the oral challenge in all animals.

[0058] High-resolution confocal microscopic imaging of pancreas sections from GED-0301-treated NOD mice that did not become diabetic by the end of the study (day 150) was performed to confirm the presence of insulin-producing β-cells. Fig. 7 shows a series of multichannel Z-stack images of representative islet-containing sections of pancreas from GED-

0301-treated mice and corresponding controls. Different colors (columns) are as follows: blue is DAPI (a marker of cell nucleus), red is insulin (indicating insulin-producing β-cells), and green is glucagon (indicating glucagon-producing α-cells). The scale bar indicates 100 μm. Whereas normal, non-diabetic control animals had normal islets containing both insulin- and glucagon-producing cells (top row), NOD mice that became diabetic during the study lost the insulin-producing β-cells from their islets (bottom row; from a sense oligonucleotide-treated mouse that did not revert to normoglycemia). GED-0301 antisense (AS) treated animals that did not become diabetic following treatment initiated at the onset of hyperglycemia had several islets that lost their insulin-producing cells (second row from top showing the presence of glucagon, but not insulin), but they also had islets with conserved insulin-producing cells (third row from top). Hence, GED-0301 treatment initiated at the onset of hyperglycemia seemed to be able to counter the effects of autoimmune attack in NOD mice and preserve insulin-producing β-cells in sufficient numbers to maintain normal blood glucose response and avoid the onset of hyperglycemia.

Study of SMAD7 Antisense in Islet Transplantation

[0059] Restoration of TGF-β signaling in the local microenvironment has shown promise in experimental allogeneic islet transplantation models, including in the presence of an underlying autoimmune disease. Treatment with GED-0301 can be done to establish the effect of an antisense targeting SMAD7 on the survival and/or tolerance to allogeneic islet grafts. Specifically, the effect of GED-0301 in preventing rejection and/or prolonging survival of islets in a model of allogeneic transplantation in rodents rendered diabetic by the administration of a beta cell-specific toxin (STZ) can be determined. Primary endpoints can include islet allograft survival, activation markers on T lymphocytes, dendritic cells, frequency and suppressive function of Treg and other suppressor cells as a correlation with Smad7 expression/modulation in the transplantation site.

Study of SMAD7 Antisense and Low-Grade Inflammation in Type 2 Diabetes

[0060] As low-grade systemic and local inflammation characterizes prediabetes and type 2 diabetes, the potential role of SMAD7 in the development of the low-grade inflammation within the pancreatic islet can be determined. Furthermore, the role of SMAD7 in islet vascular complications in diabetes can be studied.

Study of SMAD7 Antisense in Diabetic Nephropathy

[0061] In human diabetic nephropathy and other proteinuric glomerular diseases, Smad7 is strongly upregulated. This upregulation occurs primarily in podocytes, the glomerular cells responsible for the integrity of the glomerular filtration barrier and for the prevention of

albuminuria, an independent predictor of cardiovascular outcome in the general population. Targeting of TGF-beta has been the topic of an intense translational effort (trial with pirfenidone). In these efforts, results have been unexpectedly negative for the cure of diabetic nephropathy and other chronic kidney diseases. Restoring a proper TGF-β signaling by Smad7 inhibition may be a promising and novel approach to treat these conditions. Established experimental models of diabetic nephropathy developed by the NIH Consortium on Diabetic Complications can be utilized to generate preliminary *in vivo* data on soft and hard renal outcome (albuminuria, glomerular filtration rate, glomerulosclerosis). Subsequent clinical studies can be developed in the Diabetic Nephropathy clinic at the Diabetes Research Institute.

Study of SMAD7 Antisense on Glucose Stimulated Insulin Secretion

[0062] Mice with pancreatic β-cells with Smad7 overexpression are characterized by impairment of insulin release and increased fasting glucose. Therefore, Smad7 inhibition could result in the improvement of pancreatic β-cell function. Glucose-stimulated insulin-release experiments in human islets genetically manipulated to express different level of SMAD7 can be performed. In further studies, human patients can be studied to determine the role of Smad7 inhibition in glucose intolerance in pre-diabetic subjects.

REFERENCES CITED IN THE DESCRIPTION

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1

Patentkrav

1. Mothers against decapentaplegic homolog 7 (SMAD7) antisense-oligonukleotid valgt fra gruppen bestående af SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ
5 ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, og SEQ ID NO: 12, til anvendelse til behandling af diabetes ved at bevare og/eller genoprette pankreatisk β-cellefunktion.

2. SMAD7-antisense-oligonukleotid til anvendelse ifølge krav 1, hvor SMAD7-
10 antisenseoligonukleotidet er SEQ ID NO: 10, og hvor alle phosphodiesterbindinger af oligonukleotidet af SEQ ID NO: 10 er phosphorthioatbindinger.

3. SMAD7-antisense-oligonukleotid til anvendelse ifølge krav 1 eller 2, hvor antisenseoligonukleotidet er til parenteral indgivelse.
15
4. SMAD7-antisense-oligonukleotid til anvendelse ifølge krav 3, hvor antisense-oligonukleotidet er til subkutan indgivelse.

5. Farmaceutisk sammensætning omfattende et SMAD7-antisense-oligonukleotid
20 valgt fra gruppen bestående af SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, og SEQ ID NO: 12, og en farmaceutisk acceptabel bærer til anvendelse til behandling af diabetes ved at bevare og/eller genoprette pankreatisk β-cellefunktion.

25 **6.** Farmaceutisk sammensætning til anvendelse ifølge krav 5, hvor SMAD7-antisense-oligonukleotidet er valgt fra gruppen bestående af SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, og SEQ ID NO: 12, eller et farmaceutisk acceptabelt salt deraf, og den farmaceutiske sammensætning er til parenteral indgivelse.
30
7. Farmaceutisk sammensætning til anvendelse ifølge krav 6, hvor den farmaceutiske sammensætning er til subkutan indgivelse.

8. Farmaceutisk sammensætning til anvendelse ifølge krav 5, endvidere omfattende:

en intra-granulær fase omfattende SMAD7-antisense-oligonukleotidet, hvor SMAD7-antisense-oligonukleotidet er valgt fra gruppen bestående af SEQ

5   ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, og SEQ ID NO: 12, eller et farmaceutisk acceptabelt salt deraf; og

et farmaceutisk acceptabelt fyldstof,

hvor den farmaceutiske sammensætning er en farmaceutisk

10   tabletformulering til oral indgivelse.

9. Farmaceutisk sammensætning til anvendelse ifølge krav 8, endvidere omfattende en ekstra-granulær fase omfattende en farmaceutisk acceptabel excipiens.

15

10. Farmaceutisk sammensætning til anvendelse ifølge krav 9, hvor den farmaceutisk acceptable excipiens er et sprængemiddel.

11. Farmaceutisk sammensætning til anvendelse ifølge et hvilket som helst af

20 kravene 6 til 8, hvor det farmaceutisk acceptable salt af SMAD7-antisense-oligonukleotidet er et natriumsalt.

12. Farmaceutisk sammensætning til anvendelse ifølge et hvilket som helst af kravene 5-11, hvor SMAD7-antisense-oligonukleotidet er SEQ ID NO: 10, og hvor

25 alle phosphodiesterbindinger af oligonukleotidet af SEQ ID NO: 10 er phosphorthioatbindinger.

13. SMAD7-antisense-oligonukleotid til anvendelse ifølge et hvilket som helst af kravene 1 til 4, eller den farmaceutiske sammensætning til anvendelse ifølge et

30 hvilket som helst af kravene 5 til 12, hvor diabetesen er valgt fra gruppen bestående af Type 1 diabetes, Type 2 diabetes og svangerskabsdiabetes.

14. SMAD7-antisense-oligonukleotid til anvendelse ifølge krav 1, hvor SMAD7-antisense-oligonukleotidet er SEQ ID NO: 10, hvor to eller flere af

internukleosidbindingerne af SEQ ID NO: 10 er phosphorthioatbindinger.

15. Farmaceutisk sammensætning til anvendelse ifølge krav 5, hvor SMAD7-antisense-oligonukleotidet er SEQ ID NO: 10, hvor to eller flere af

5 internukleosidbindingerne af SEQ ID NO: 10 er phosphorthioatbindinger.

DRAWINGS

FIG. 1A

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   1 ggcacgagcg gagagccgcg cagggcgcgg gccgcgcggg gtggggcagc cggagcgcag
  61 gccccgatc cccggcgggc gccccgggc cccgcgcgg gccccggcct cggggagact
 121 ggcgcatgcc acggagcgcc cctcgggccg ccgccgctcc tgcccggcc cctgctgctg
 181 ctgctgtcgc ctgcgcctgc tgcccaact cggcgcccga cttcttcatg gtgtgcggag
 241 gtcatgttcg ctccttagca ggcaaacgac tttctcctc gcctcctcgc cccgcatgtt
 301 caggaccaaa cgatctgcgc tcgtccggcg tctctggagg agccgtgcgc ccggcggcga
 361 ggacgaggag gagggcgcag ggggaggtgg aggaggaggc gagctgcggg gagaaggggc
 421 gacggacagc cgagcgcatg gggccggtgg cggcggcccg ggcagggctg gatgctgcct
 481 gggcaaggcg gtgcgaggtg ccaaaggtca ccaccatccc cacccgccaa ccgcgggcgc
 541 cggcgcggcc gggggcgccg aggcggatct gaaggcgctc acgcactcgg tgctcaagaa
 601 actgaaggag cggcagctgg agctgctgct ccaggccgtg gagtcccgcg gcgggacgcg
 661 caccgcgtgc ctcctgctgc ccggccgcct ggactgcagg ctgggccggg gggcgcccgc
 721 cggcgcgcag cctgcgcagc cgccctcgtc ctactcgctc cccctcctgc tgtgcaaagt
 781 gttcaggtgg ccggatctca ggcattcctc ggaagtcaag aggctgtgtt gctgtgaatc
 841 ttacgggaag atcaaccccg agctggtgtg ctgcaacccc catcacctta gccgactctg
 901 cgaactagag tctcccccc ctccttactc cagataccc atggattttc tcaaaccaac
 961 tgcagactgt ccagatgctg tgccttcctc cgctgaaaca gggggaacga attatctggc
1021 ccctgggggg ctttcagatt cccaacttct tctggagcct ggggatcggt cacactggtg
1081 cgtggtggca tactgggagg agaagacgag agtggggagg ctctactgtg tccaggagcc
1141 ctctctggat atcttctatg atctacctca ggggaatggc ttttgcctcg gacagctcaa
1201 ttcggacaac aagagtcagc tggtgcagaa ggtgcggagc aaaatcggct ggggcatcca
1261 gctgacgcgg gaggtggatg gtgtgtgggt gtacaaccgc agcagttacc ccatcttcat
1321 caagtccgcc acactggaca accgggactc caggacgctg ttggtacaca aggtgttccc
1381 cggtttctcc atcaaggctt cgactacga gaaggcgtac agcctgcagc ggcccaatga
1441 ccacgagttt atgcagcagc cgtggacggg ctttaccgtg cagatcagct ttgtgaaggg
1501 ctggggtcag tgctacaccc gccagttcat cagcagctgc ccgtgctggc tagaggtcat
1561 cttcaacagc cggtagccgc gtgcggaggg gacagagcgt gagctgagca ggccacactt
1621 caaactactt tgctgctaat attttcctcc tgagtgcttg cttttcatgc aaactctttg
1681 gtcgtttttt ttttgtttgt tggttggttt tcttcttctc gtcctcgttt gtgttctgtt
1741 ttgtttcgct ctttgagaaa tagcttatga aaagaattgt tgggggtttt tttggaagaa
1801 ggggcaggta tgatcggcag gacaccctga taggaagcag ggaagcagaa atccaagcac
1861 caccaaacac agtgtatgaa ggggggcggt catcatttca cttgtcagga gtgtgtgtga
1921 gtgtgagtgt gcggctgtgt gtgcacgcgt gtgcaggagc ggcagatggg gagacaacgt
1981 gctctttgtt ttgtgtctct tatggatgtc cccagcagag aggtttgcag tcccaagcgg
2041 tgtctctcct gcccttgga cacgctcagt ggggcagagg cagtacctgg gcaagctggc
2101 ggctggggtc ccagcagctg ccaggagcac ggctctgtcc ccagcctggg aaagcccctg
2161 cccctcctct ccctcatcaa ggacacgggc ctgtccacag gcttctgagc agcgagcctg
2221 ctagtggccg aaccagaacc aattattttc atccttgtct tattcccttc ctgccagccc
2281 ctgccattgt agcgtctttc ttttttggcc atctgctcct ggatctccct gagatgggct
2341 tcccaagggc tgcaggggca gccccctcac agtattgctc acccagtgcc ctctccctc
2401 agcctctccc ctgcctgccc tggtgacatc aggttttttcc cggacttaga aaaccagctc
2461 agcactgct gctcccatcc tgtgtgttaa gctctgctat taggccagca agcggggatg
2521 tccctgggag ggacatgctt agcagtcccc ttccctccaa gaaggatttg gtccgtcata
2581 acccaaggta ccatcctagg ctgacaccta actcttcttt catttcttct acaactcata
2641 cactcgtatg atacttcgac actgttctta gctcaatgag catgtttaga ctttaacata
2701 agctatttt ctaactacaa aggtttaaat gaacaagaga agcattctca ttggaaattt
2761 agcattgtag tgctttgaga gagaaaggac tcctgaaaaa aaacctgaga tttattaaag
2821 aaaaaaatgt attttatgtt atatataaat atattattac ttgtaaatat aaagacgttt
2881 tataagcatc attatttatg tattgtgcaa tgtgtataaa caagaaaaat aaagaaaaga
2941 tgcactttgc tttaatataa atgcaaataa caaatgccaa attaaaaaag ataaacacaa
3001 gattggtgtt ttttcctatg ggtgttatca cctagctgaa tgttttttcta aaggagttta
3061 tgttccatta aacgattttt aaaatgtaca cttgaaaaaa aaaaaaaaa a
```
{SEQ ID NO: 1}

FIG. 1B

MFRTKRSALVRRLWRSRAPGGEDEEEGAGGGGGGGELRGEGATD
SRAHGAGGGGPGRAGCCLGKAVEGAKGHHHPHPPAAGAGAAGGAEADLKALTHSVLKK
LKERQLELLLQAVESRGGTRTACLLLPGRLDCRLGPGAPAGAQPAQPPSSYSLPLLLC
KVFRWPDLRHSSEVKRLCCCESYGKINPELVCCNPHHLSRLCELESPPPPYSRYPMDF
LKPTADCPDAVPSSAETGGTNYLAPGGLSDSQLLLEPGDRSHWCVVAYWEEKTRVGRL
YCVQEPSLDIFYDLPQGNGFCLGQLNSDNKSQLVQKVRSKIGCGIQLTREVDGVWVYN
RSSYPIFIKSATLDNFDSRTLLVHKVFPGFSIKAFDYEKAYSLQRPNDHEFMQQPWTG
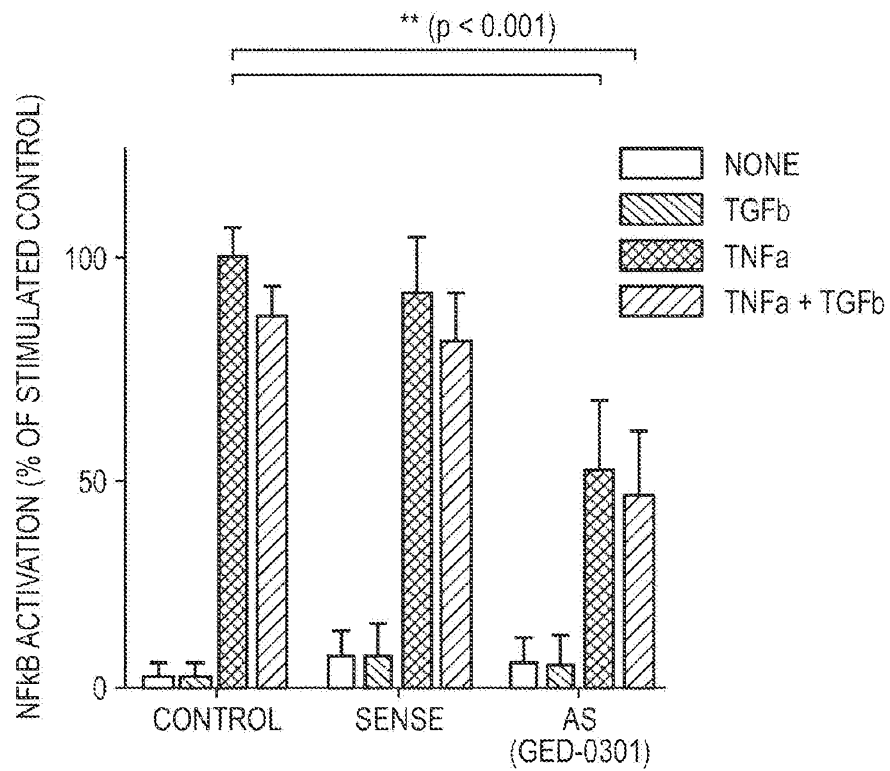FTVQISFVKGWGQCYTRQFISSCPCWLEVIFNSR
(SEQ ID NO:2)

FIG. 2A

EFFECT OF SMAD7 AS & TGFβ ON NF-κB ACTIVATION
IN CD40L-STIMULATED HEK-BLUE SENSOR CELLS
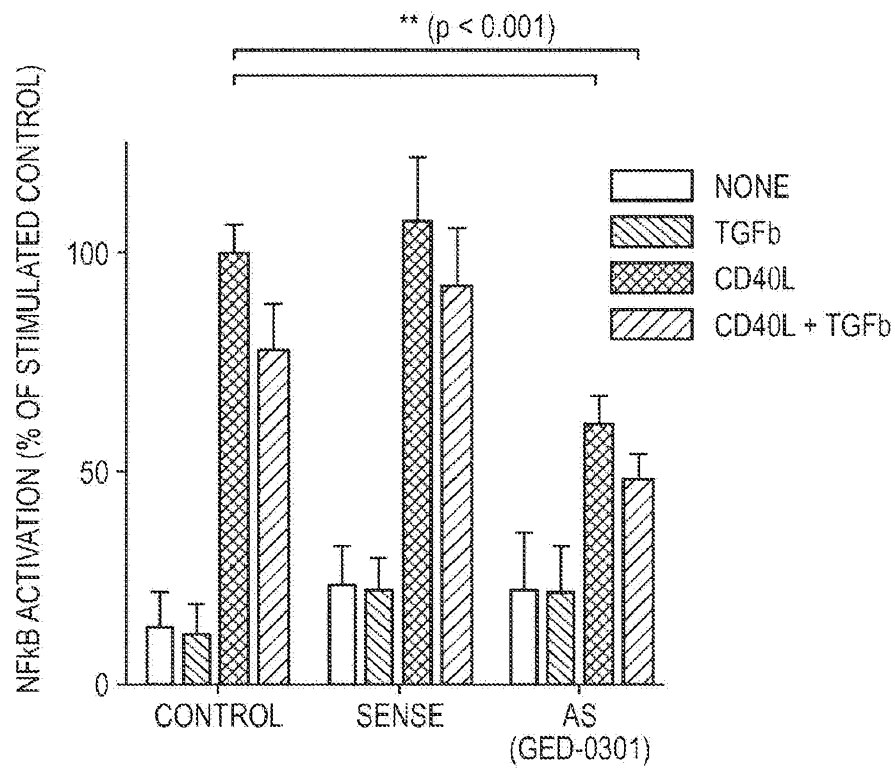
FIG. 2B

OLIGONUCLEOTIDE DISTRIBUTION STUDY; FACS ANALYSIS - METHOD
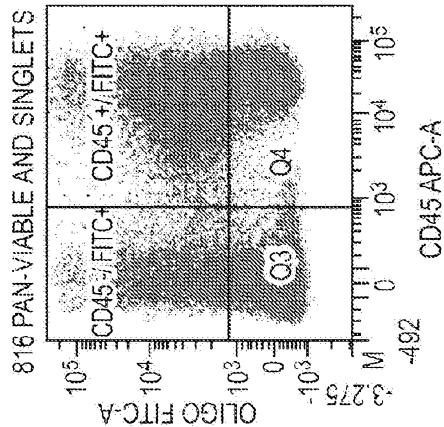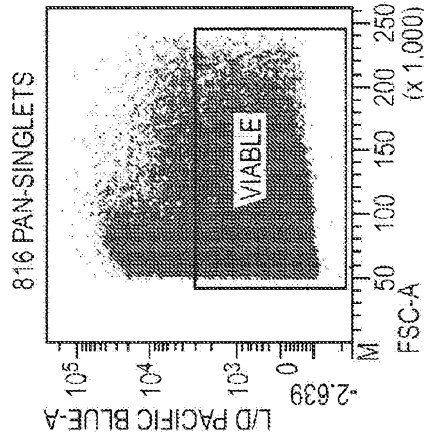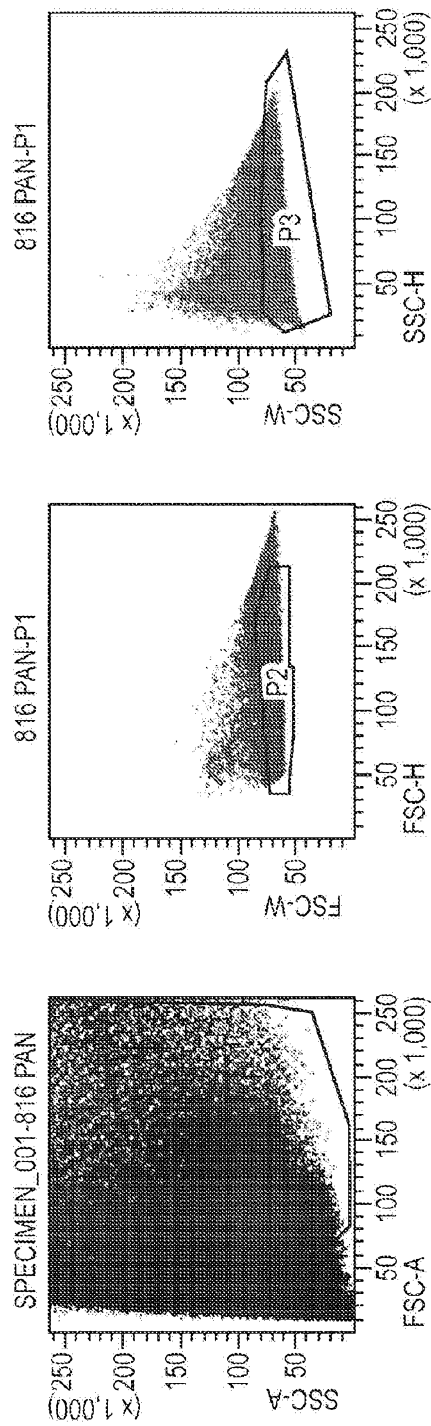
TUBE: 816 PAN			
POPULATION	# EVENTS	% PARENT	% TOTAL
☐ All Events	410,080	####	100.0
☐ P1	142,475	34.7	34.7
☐ P2	305,172	74.4	74.4
☐ P3	288,728	70.4	70.4
☐ SINGLETS	128,763	31.4	31.4
☐ VIABLE	206,679	50.4	50.4
☐ VIABLE AND SINGLETS	110,269	26.9	26.9
☒ CD 45- /FITC+	7,480	6.8	1.8
☒ CD 45+ /FITC+	26,857	24.4	6.5
☒ Q3	50,605	45.9	12.3
☒ Q4	25,327	23.0	6.2

FIG. 3A

OLIGONUCLEOTIDE DISTRIBUTION STUDY: RESULTS IN VIABLE CELLS (%, 4H)
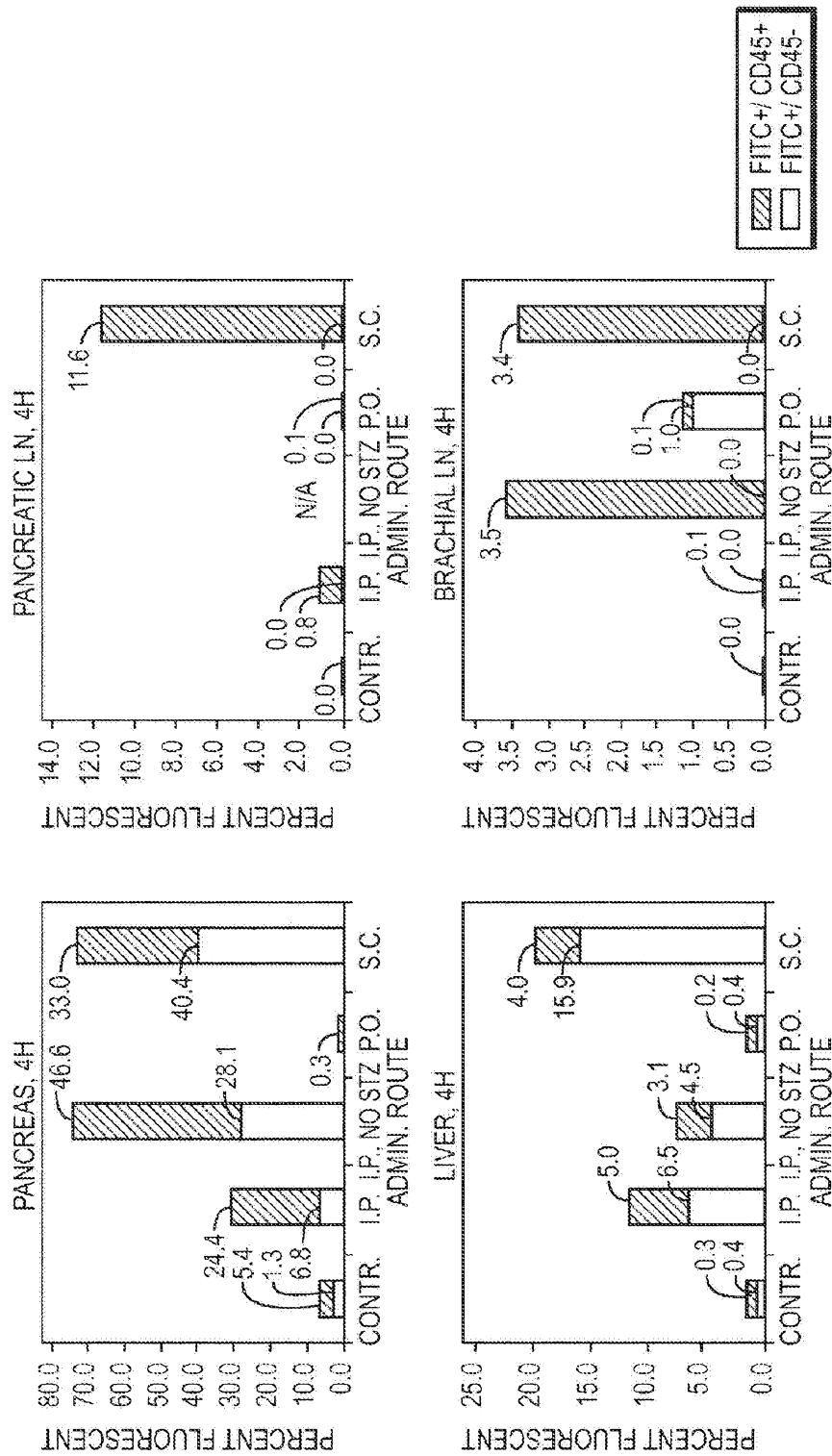
FIG. 3B

OLIGONUCLEOTIDE DISTRIBUTION STUDY: RESULTS IN VIABLE CELLS (%, 4H)
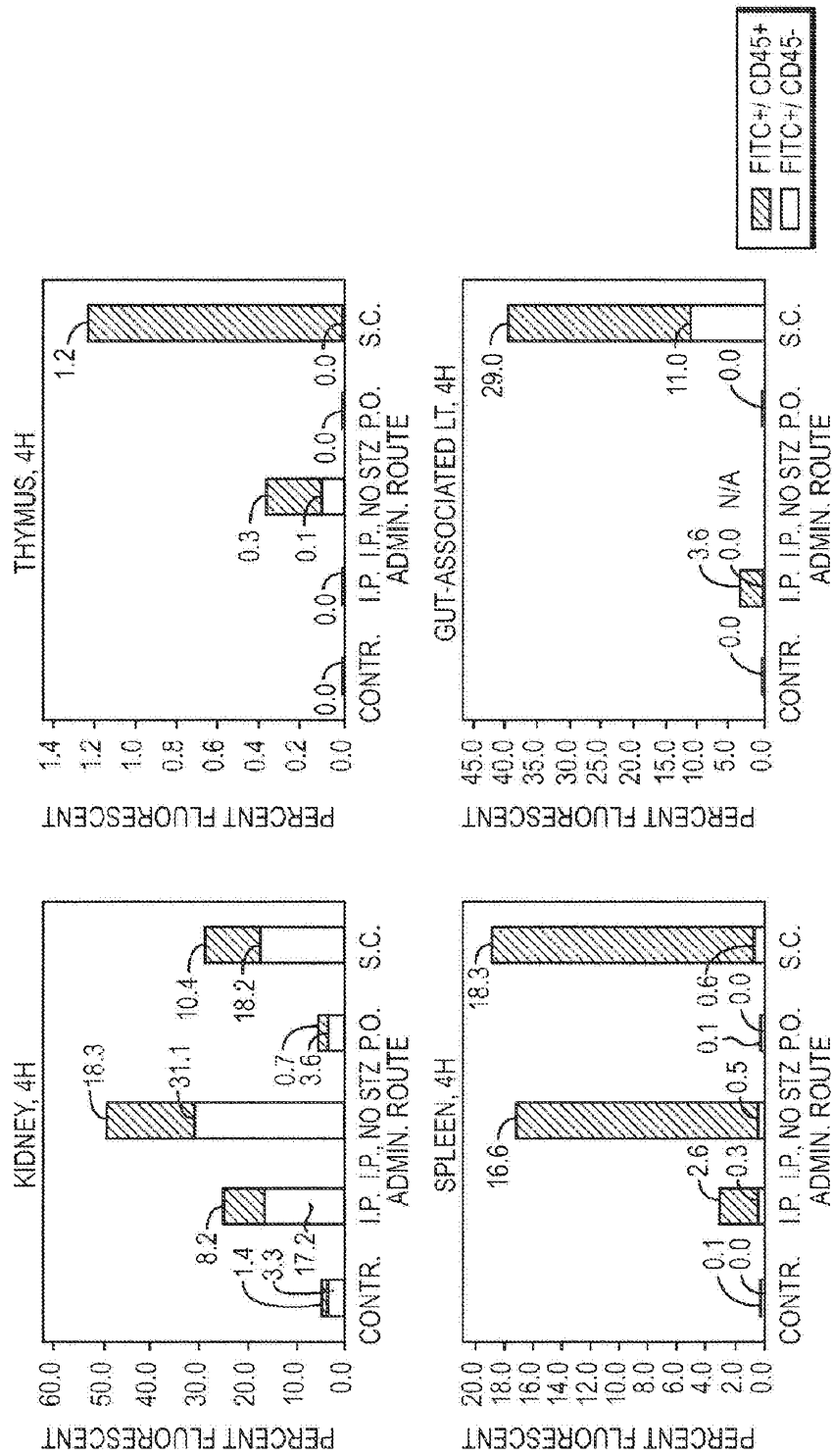
FIG. 3B

FLUORESCENT ANTISENSE OLIGONUCLEOTIDE IN MOUSE PANCREAS
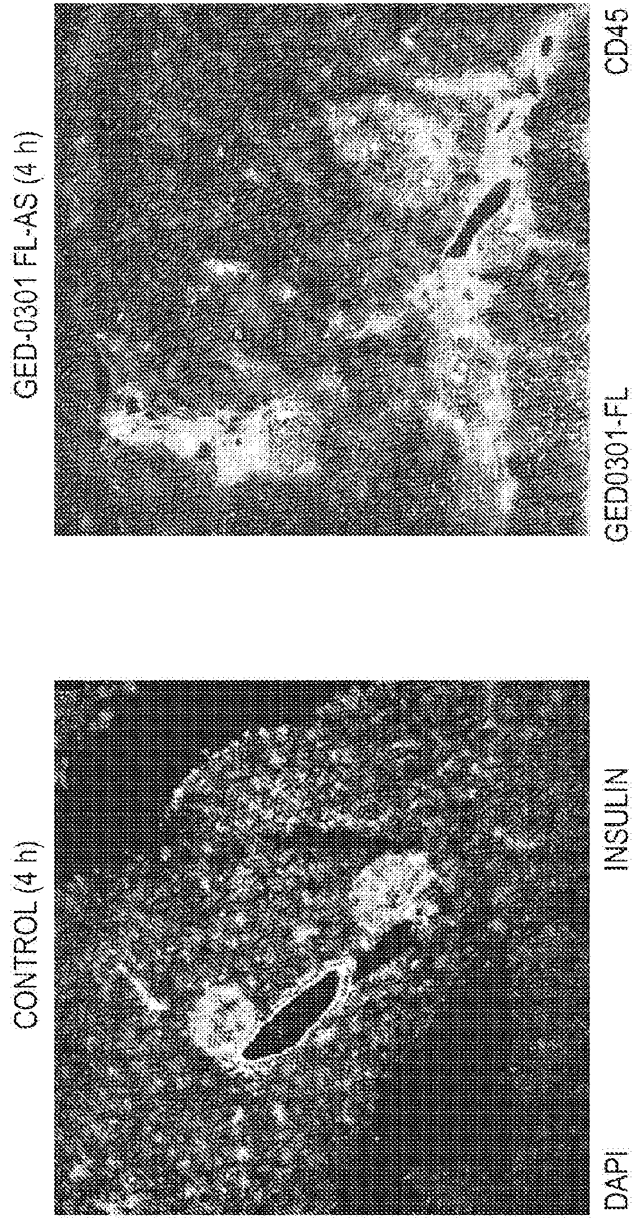AFTER SUBCUTANEOUS INJECTION

CONTROL (4 h)

GED-0301 FL-AS (4 h)

DAPI

INSULIN

GED0301-FL

CD45

FIG. 4

GED-0301 INTERVENTION STUDY IN HYPERGLYCEMIC NOD MICE. BLOOD GLUCOSE LEVELS
SMAD7 ANTISENSE GED-0301-NOD INTERVENTION STUDY
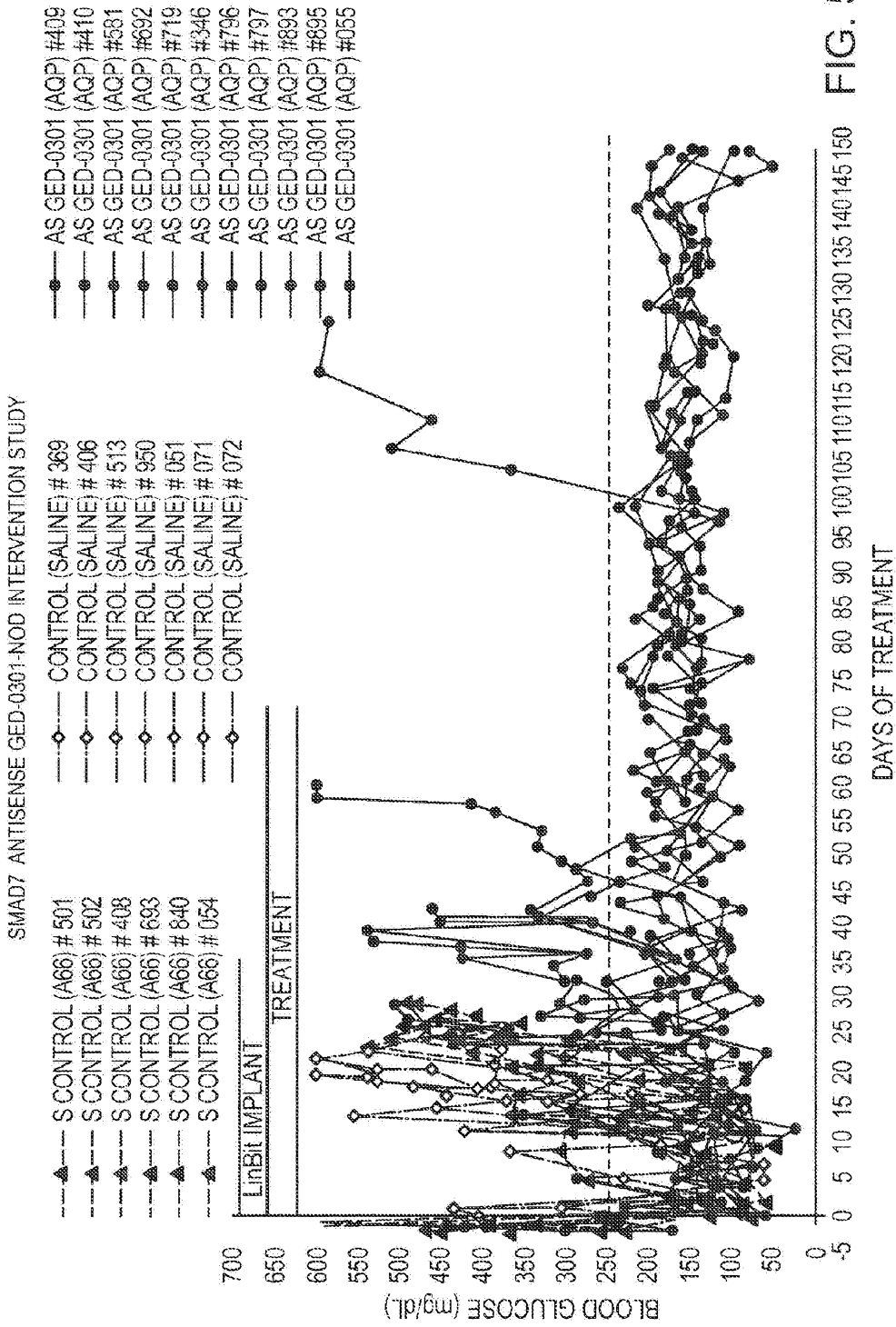
FIG. 5A

GED-0301 INTERVENTION STUDY IN HYPERGLYCEMIC NOD MICE,
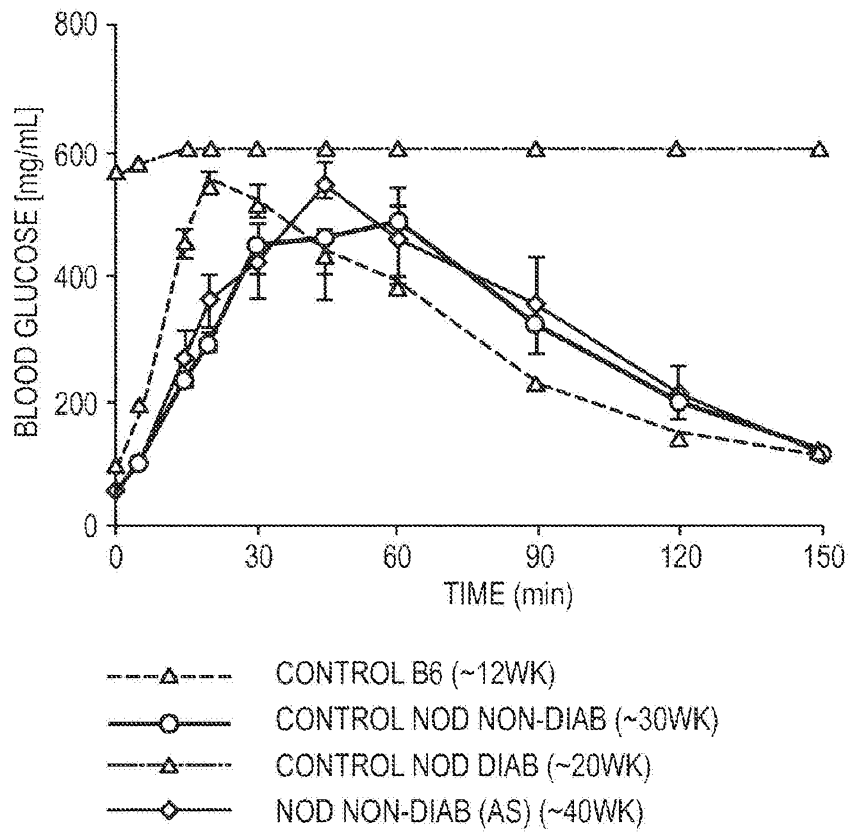KAPLAN-MEIER SURVIVAL PLOT
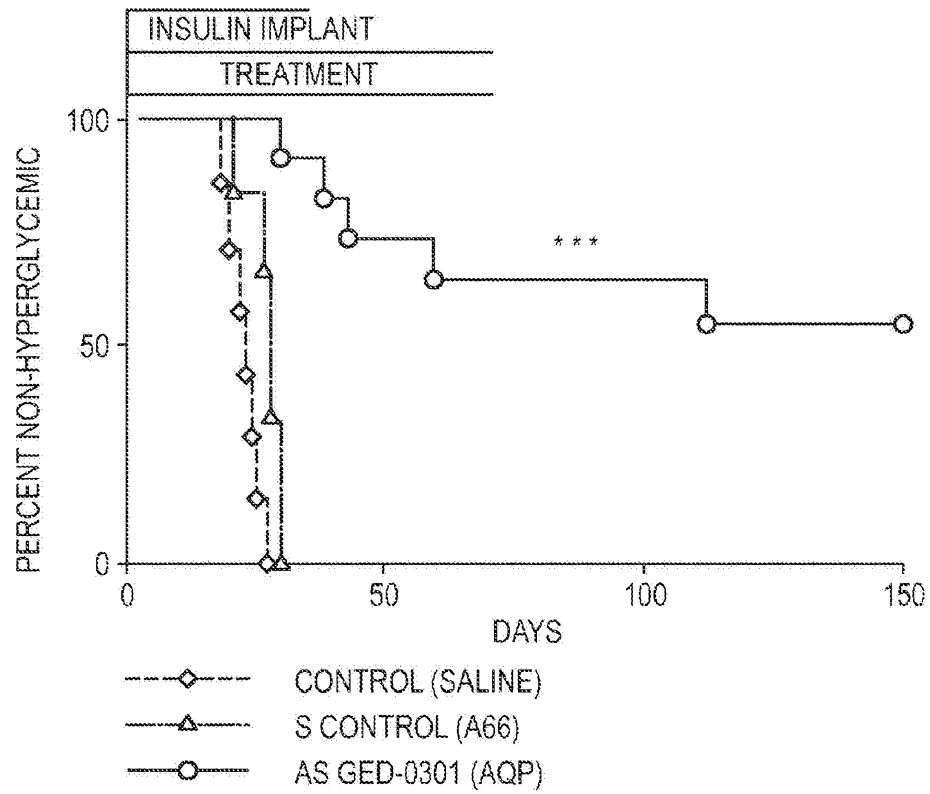
FIG. 5B

GED-0301 INTERVENTION STUDY IN HYPERGLYCEMIC NOD MICE,
ORAL GLUCOSE TOLERANCE TEST (OGTT)

FIG. 6

GED-0301 INTERVENTION STUDY IN HYPERGLYCEMIC NOD MICE,
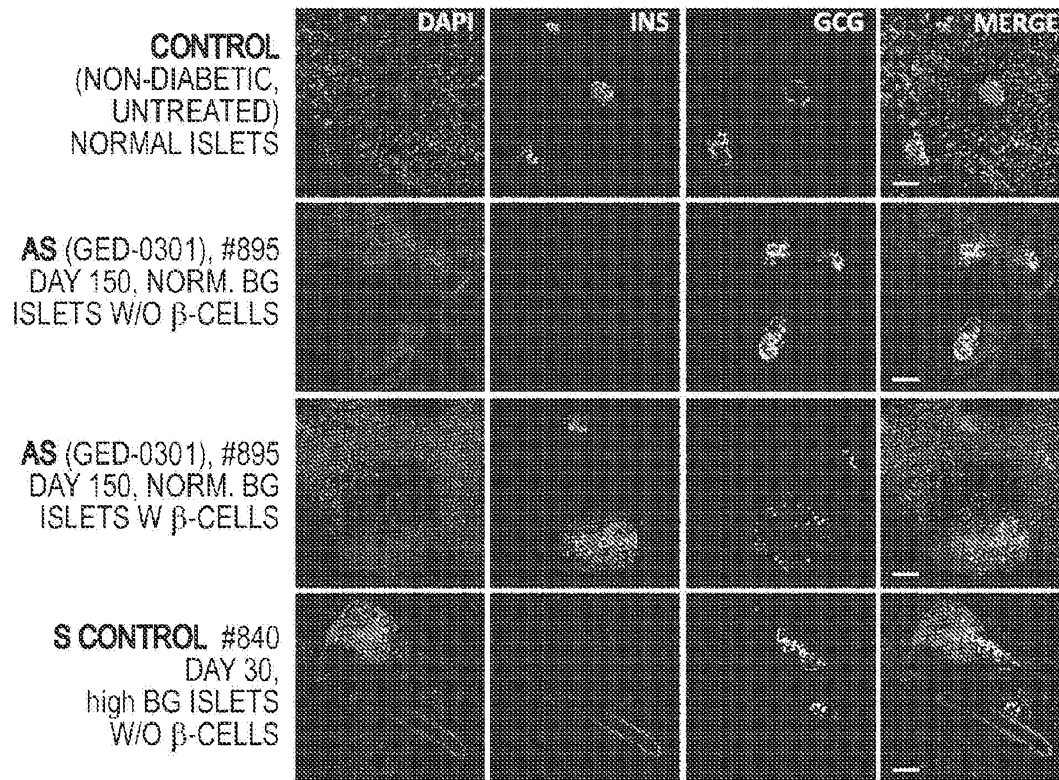REPRESENTATIVE PANCREAS SECTIONS STAINED FOR INSULIN AND GLUCAGON

FIG. 7