

GLP-1 AGONISTS, COMPOSITIONS, METHODS AND USES

[1] BACKGROUND OF THE INVENTION

[2] FIELD OF THE INVENTION

5 [3] The present invention relates to mammalian glucagons like peptide-1 (GLP-1) agonists, such as GLP-1 mimetibodies, specified portions and variants specific for biologically active proteins, fragment or ligands, GLP-1 agonist encoding and complementary nucleic acids, host cells, and methods of making and using thereof, including diabetes related therapeutic formulations, administration and devices

10 [4] RELATED ART

[5] Recombinant proteins are an emerging class of therapeutic agents. The use of recombinant proteins as potential therapeutics have provided an opportunity for advances in therapeutic protein formulations, also including the use of chemical modifications. Such modifications can potentially enhance the therapeutic utility of
15 therapeutic proteins, potentially such as by increasing serum half lives (e.g., by blocking their exposure to proteolytic enzymes), enhancing biological activity, and/or reducing unwanted side effects. One such modification is the use of immunoglobulin fragments fused to receptor proteins, such as entercept. Fusion proteins have also been constructed using the antibody Fc domain in an attempt to provide a longer half-life or
20 to incorporate functions such as Fc receptor binding, protein A binding, and complement fixation.

[6] Diabetes is a growing epidemic that is estimated to affect over 300 million people by the year 2025 pending an effective pharmaceutical cure. Most cases of diabetes fall into two clinical types. Type 1, also known as juvenile-onset diabetes or insulin dependent
25 diabetes mellitus (IDDM), and Type 2, also known as adult-onset diabetes, that accounts for 90-95% of all cases.

[7] IDDM is characterized by a partial or complete inability to produce insulin. It is generally recognized that Type 1 diabetes results from a progressive autoimmune response, which selectively destroys the insulin-producing β cells of the islets of
30 Langerhans in individuals who are genetically predisposed.

- [8] The pancreas of a patient diagnosed with Type 2 diabetes is still able to synthesize and secrete insulin. However, the sensitivity of the tissues on which insulin exerts its effects is impaired. Additionally, a number of Type 2 diabetics are insulin dependent at the later stage and require insulin injections to improve their insulin resistance.
- 5 [9] Complications resulting from sustained elevated plasma glucose levels include cardiovascular disease, nephropathy, neuropathy, and retinopathy. Insulin replacement therapy through insulin injections or an insulin pump is the standard treatment for IDDM. However, the delivery of such insulin is not regulated and often results in fluctuations of the patient's blood glucose both above and below the normal range. A
- 10 potential alternative method under study for treating insulin-requiring diabetes is the transplantation of pancreas or islets from a normal donor to a diabetic patient. If the problems of transplant rejection can be ameliorated to some extent using immunosuppression therapy or other means, pancreas or islet cell transplant can potentially normalize blood glucose levels and restore the physiological manner in
- 15 which islets secrete insulin in response to glucose. However, aside from the complications attendant with the required immunosuppression regimen to prevent the rejection of the transplant, a major factor limiting this approach is the shortage of human donor tissue. In addition, the living donor strategy for transplantation would likely result in the donor becoming diabetic.
- 20 [10] For most Type 2 diabetic patients, oral medications are used to attempt to lower blood glucose by partially increasing the insulin sensitivity, reduce insulin resistance, and/or increase insulin production. However, such therapies are not ultimately effective, e.g., in many cases, the oral agents are not effective or become ineffective over time and insulin injections are required at some stage to maintain appropriate
- 25 glucose levels.
- [11] Most current treatments for diabetes are associated with a variety of deleterious side effects including hypoglycemia and weight gain. In addition, despite all interventions available, there are still a significant number of patients that are unable to maintain their blood glucose in an acceptable range. Therefore, there is a need to provide alternative
- 30 method of treating Type 1 and/or Type 2 diabetes as well as complications resulted therefrom.

[12] Glucagon like peptide-1 (GLP-1) is a 37-amino acid peptide secreted from the L-cells of the intestine following an oral glucose challenge. A subsequent endogenous cleavage between the 6th and 7th position produces the biologically active GLP-1 (7-37) peptide. The GLP-1 (7-37) peptide sequence can be divided into 2 structural domains.

5 The amino terminal domain of the peptide may be involved in signaling while the remainder of the peptide appears to bind to the extracellular loops of the GLP-1 receptor in a helical conformation. In response to glucose, the active GLP-1 appears to bind to the GLP-1 receptor on the pancreas and causes an increase in insulin secretion (insulinotropic action). In addition, it has been shown that GLP-1 reduces gastric
10 emptying which decreases the bolus of glucose that is released into the circulation and may reduce food intake. GLP-1 has also been shown to inhibit glucagon secretion, thereby reducing the endogenous release of stored glucose in the liver. These actions, singly, or in combination lower blood glucose levels. GLP-1 has also been shown in in vitro and in vivo studies to inhibit apoptosis and increase proliferation of the β -cells in
15 the pancreas. In addition, GLP-1 activity has also been shown to be controlled by blood glucose levels. When blood glucose levels drop to a certain threshold level, GLP-1 is not active. Therefore, there appears to be little risk of hypoglycemia associated with the administration of GLP-1 to animals.

[13] However, GLP-1 is rapidly inactivated *in vivo* by the protease dipeptidyl-peptidase IV (DPP-IV). Therefore, the potential usefulness of therapy involving GLP-1
20 peptides has been limited by their fast clearance and short half-lives. For example, GLP-1 (7-37) has a serum half-life of only 3 to 5 minutes. GLP-1 (7-36) amide has a time action of about 50 minutes when administered subcutaneously. Even analogs and derivatives that are resistant to endogenous protease cleavage, do not have half-lives
25 long enough to avoid repeated administrations over a 24 hour period. For example, exenatide is resistant to DPP-IV, yet it still requires twice daily preprandial dosing because of the short half-life and significant variability in *in vivo* pharmacokinetics. NN2211, another compound currently in clinical trials, is a lipidated GLP-1 analogue. It is expected to be dosed once daily.

30 [14] Fast clearance of a therapeutic agent is inconvenient in cases where it is desired to maintain a high blood level of the agent over a prolonged period of time since repeated administrations will then be necessary. Furthermore, a long-acting compound

is particularly important for diabetic patients whose past treatment regimen has involved taking only oral medication. These patients often have an extremely difficult time transitioning to a regimen that involves multiple injections of medication. A GLP-1 therapy that has an increased half-life would have a significant advantage over other GLP-1 peptides and compounds in development.

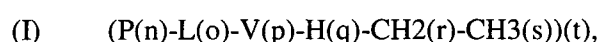
[15] Accordingly, there is a need to provide improved and/or modified versions of GLP-1 therapeutic proteins, which overcome one or more of these and other problems known in the art

[16] SUMMARY OF THE INVENTION

10 [17] The present invention provides human GLP-1 agonists, including GLP-1 mimetibodies, including modified proteins, peptides, immunoglobulins, cleavage products and other specified portions and variants thereof, as well as GLP-1 agonist or mimetibody compositions, encoding or complementary nucleic acids, vectors, host cells, compositions, formulations, devices, transgenic animals, transgenic plants, and methods
15 of making and using thereof, as described and/or enabled herein, in combination with what is known in the art

[18] The present invention also provides at least one isolated GLP-1 mimetibody or specified portion or variant as described herein and/or as known in the art. The GLP-1 mimetibody can optionally comprise at least one CH3 region directly linked with at least one CH2 region directly linked with at least one portion of at least one hinge region or
20 fragment thereof (H), directly linked with at least one partial variable region (V), directly linked with an optional linker sequence (L), directly linked to at least one GLP-1 therapeutic peptide (P)

[19] In a preferred embodiment a pair of a CH3-CH2-hinge-partial V region sequence-linker-therapeutic peptide sequence, the pair optionally linked by association or covalent linkage, such as, but not limited to, at least one Cys-Cys disulfide bond or at least one CH4 or other immunoglobulin sequence. In one embodiment, a GLP-1 mimetibody comprises formula (I)



30 [20] wherein P is at least one bioactive GLP-1 peptide, variant or derivative, L is at least one linker sequence, which can be a polypeptide that provides structural flexibility by allowing

the mimetibody to have alternative orientations and binding properties, V is at least one portion of a C-terminus of an immunoglobulin variable region, H is at least one portion of an immunoglobulin variable hinge region, CH2 is at least a portion of an immunoglobulin CH2 constant region, CH3 is at least a portion of an immunoglobulin CH3 constant region, n is an integer from 1 to 10, and o, p, q, r, s, and t can be independently an integer from 0 to 10, mimicing different types of immunoglobulin molecules, e g , but not limited to IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgM, IgD, IgE, or any subclass thereof, and the like, or any combination thereof

[21] The variable region of the antibody sequence can be, but not limited to, at least one portion of at least one of SEQ ID NOS 47-55, or fragment thereof, further optionally comprising at least one substitution, insertion or deletion The CH2, CH3 and hinge region can be, but not limited to, at least one portion of at least one of SEQ ID NOS 56-64, or fragment thereof, further optionally comprising at least one substitution, insertion or deletion (Kevin, these sequences are included in the sequence listing, but if you want to incorporate by reference, then we don't need them)

[22] Thus, a GLP-1 mimetibody of the present invention mimics at least a portion of an antibody or immnuoglobulin structure or function with its inherent properties and functions, while providing a GLP-1 therapeutic peptide and its inherent or acquired *in vitro*, *in vivo* or *in situ* properties or activities. The various portions of the antibody and therapeutic peptide portions of GLP-1 mimetibody of the present invention can vary as described herein in combination with what is known in the art

[23] The present invention also provides at least one isolated GLP-1 agonist or mimetibody or specified portion or variant that has at least one activity, such as, but not limited to known biological activities of at least one bioactive GLP-1 peptide or polypeptide corresponding to the P portion of formula (I), as described herein or known in the art

[24] In one aspect, the present invention provides at least one isolated human GLP-1 agonist or mimetibody comprising at least one polypeptide sequence of SEQ ID NO·1, or optionally with one or more substitutions, deletions or insertions as described herein or as known in the art. In another aspect, at least one GLP-1 agonist or mimetibody or specified portion or variant of the invention mimics the binding of at least one GLP-1 peptide or polypeptide corresponding to the P portion of the mimetibody in formula (I), to at least one epitope comprising at least 1-3, to the entire amino acid sequence of at

least one ligand, e.g., but not limited to, a GLP-1 receptor, or fragment thereof, wherein the ligand binds to at least a portion of SEQ ID NO 1, or optionally with one or more substitutions, deletions or insertions as described herein or as known in the art. The at least one GLP-1 agonist or mimetibody can optionally bind GLP-1 receptor with an

5 affinity of at least 10^{-9} M, at least 10^{-10} M, at least 10^{-11} M, or at least 10^{-12} M. A GLP-1 agonist or mimetibody can thus be screened for a corresponding activity according to known methods, such as, but not limited to the binding activity towards a receptor or fragment thereof.

[25] The present invention further provides at least one anti-idiotypic antibody to at

10 least one GLP-1 agonist or mimetibody of the present invention. The anti-idiotypic antibody or fragment specifically binds at least one GLP-1 agonist or mimetibody of the present invention. The anti-idiotypic antibody includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule, such as but not limited to at least one complementarity determining region (CDR) of a heavy or light

15 chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework region, or any portion thereof, that competitively binds a GLP-1 ligand binding region of at least one GLP-1 agonist or mimetibody of the present invention. Such idiotypic antibodies of the invention can include or be derived from any mammal, such as but not limited to a human, a mouse, a

20 rabbit, a rat, a rodent, a primate, and the like.

[26] The present invention provides, in one aspect, isolated nucleic acid molecules comprising, complementary, having significant identity or hybridizing to, a polynucleotide encoding at least one GLP-1 agonist or mimetibody or GLP-1 agonist or mimetibody anti-idiotypic antibody, or specified portions or variants thereof, comprising

25 at least one specified sequence, domain, portion or variant thereof. The present invention further provides recombinant vectors comprising at least one of said isolated GLP-1 agonist or mimetibody or GLP-1 agonist or mimetibody anti-idiotypic antibody encoding nucleic acid molecules, host cells containing such nucleic acids and/or recombinant vectors, as well as methods of making and/or using such GLP-1 agonist or

30 mimetibody or GLP-1 agonist or mimetibody anti-idiotypic antibody nucleic acids, vectors and/or host cells.

[27] Also provided is an isolated nucleic acid encoding at least one isolated mammalian GLP-1 agonist or mimetibody or GLP-1 agonist or mimetibody anti-idiotypic antibody, an isolated nucleic acid vector comprising the isolated nucleic acid, and/or a prokaryotic or eukaryotic host cell comprising the isolated nucleic acid. The host cell can optionally be at least one selected from COS-1, COS-7, HEK293, BHK21, CHO, BSC-1, Hep G2, 653, SP2/0, 293, HeLa, myeloma, or lymphoma cells, or any derivative, immortalized or transformed cell thereof.

[28] The present invention also provides at least one method for expressing at least one GLP-1 agonist or mimetibody or GLP-1 agonist or mimetibody anti-idiotypic antibody, or specified portion or variant in a host cell, comprising culturing a host cell as described herein and/or as known in the art under conditions wherein at least one GLP-1 agonist or mimetibody or GLP-1 agonist or mimetibody anti-idiotypic antibody, or specified portion or variant is expressed in detectable and/or recoverable amounts. Also provided is a method for producing at least one GLP-1 agonist or mimetibody or GLP-1 agonist or mimetibody anti-idiotypic antibody, comprising translating the GLP-1 agonist or mimetibody or GLP-1 agonist or mimetibody anti-idiotypic antibody encoding nucleic acid under conditions *in vitro*, *in vivo* or *in situ*, such that the GLP-1 agonist or mimetibody or GLP-1 agonist or mimetibody anti-idiotypic antibody is expressed in detectable or recoverable amounts.

[29] Also provided is a method for producing at least one isolated human GLP-1 agonist or mimetibody or GLP-1 anti-idiotypic antibody of the present invention, comprising providing a host cell or transgenic animal or transgenic plant capable of expressing in recoverable amounts the GLP-1 agonist or mimetibody or GLP-1 anti-idiotypic antibody.

[30] Further provided in the present invention is at least one GLP-1 agonist or mimetibody produced by the above methods.

[31] The present invention also provides at least one composition comprising (a) an isolated GLP-1 agonist or mimetibody or specified portion or variant encoding nucleic acid and/or GLP-1 agonist or mimetibody as described herein, and (b) a suitable carrier or diluent. The carrier or diluent can optionally be pharmaceutically acceptable,

according to known methods. The composition can optionally further comprise at least one further compound, protein or composition.

[32] Also provided is a composition comprising at least one isolated human GLP-1 agonist or mimetibody and at least one pharmaceutically acceptable carrier or diluent.

5 The composition can optionally further comprise an effective amount of at least one compound or protein selected from at least one of a diabetes drug, an insulin metabolism related drug, a glucose metabolism related drug, a detectable label or reporter, an anti-infective drug, a cardiovascular (CV) system drug, a central nervous system (CNS) drug, an autonomic nervous system (ANS) drug, a respiratory tract drug, a
10 gastrointestinal (GI) tract drug, a hormonal drug, a drug for fluid or electrolyte balance, a hematologic drug, an antineoplastic, an immunomodulation drug, an ophthalmic, otic or nasal drug, a topical drug, a nutritional drug, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial,
15 an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, or a cytokine antagonist.

20 [33] The present invention also provides at least one composition, device and/or method of delivery of a therapeutically or prophylactically effective amount of at least one GLP-1 agonist or mimetibody or specified portion or variant, according to the present invention.

[34] The present invention further provides at least one GLP-1 agonist or
25 mimetibody method or composition, for administering a therapeutically effective amount to modulate or treat at least one GLP-1 related condition in a cell, tissue, organ, animal or patient and/or, prior to, subsequent to, or during a related condition, as known in the art and/or as described herein.

[35] The present invention further provides at least one GLP-1 agonist or
30 mimetibody, specified portion or variant in a method or composition, when administered in a therapeutically effective amount, for modulation, for treating or reducing the

symptoms of, at least one metabolic, immune, cardiovascular, infectious, malignant, and/or neurologic disease in a cell, tissue, organ, animal or patient and/or, as needed in many different conditions, such as but not limited to, prior to, subsequent to, or during a related disease or treatment condition, as known in the art

5 [36] The present invention further provides at least one GLP-1 agonist or mimetibody, specified portion or variant in a method or composition, when administered in a therapeutically effective amount, for modulation, for treating or reducing the symptoms of at least one of a diabetes or an insulin metabolism related disorder, a glucose metabolism related disorder, a bone and joint disorder, cardiovascular disorder, a
10 dental or oral disorder, a dermatologic disorder, an ear, nose or throat disorder, an endocrine or metabolic disorder, a gastrointestinal disorder, a gynecologic disorder, a hepatic or biliary disorder, a an obstetric disorder, a hematologic disorder, an immunologic or allergic disorder, an infectious disease, a musculoskeletal disorder, a oncologic disorder, a neurologic disorder, a nutritrional disorder, an ophthalmologic
15 disorder, a pediatric disorder, a poisoning disorder, a psychiatric disorder, a renal disorder, a pulmonary disorder, or any other known disorder, (See, e g , The Merck Manual, 17th ed , Merck Research Laboratories, Merck and Co , Whitehouse Station, NJ (1999), entirely incoporated herein by reference), as needed in many different conditions, such as but not limited to, prior to, subsequent to, or during a related disease
20 or treatment condition, as known in the art

[37] The present invention also provides at least one composition, device and/or method of delivery, for diagnosing GLP-1 related conditions, of at least one GLP-1 agonist or mimetibody, according to the present invention

[38] The present invention further provides at least one GLP-1 agonist or
25 mimetibody method or composition, for diagnosing at least one GLP-1 related condition in a cell, tissue, organ, animal or patient and/or, prior to, subsequent to, or during a related condition, as known in the art and/or as described herein

[39] Also provided is a method for diagnosing or treating a disease condition in a cell, tissue, organ or animal, comprising (a) contacting or administering a composition
30 comprising an effective amount of at least one isolated human GLP-1 agonist or mimetibody of the invention with, or to, the cell, tissue, organ or animal The method

can optionally further comprise using an effective amount of 0.001-50 mg/kilogram, or equivalent concentration or molarity if done in vitro or in situ, of the cells, tissue, organ or animal per 0-24 hours, 1-7 days, 1-52 weeks, 1-24 months, 1-50 years or any range or value therein. The method can optionally further comprise using the contacting or the administering by at least one mode selected from in vitro, parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelical, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal. The method can optionally further comprise administering, prior, concurrently or after the (a) contacting or administering, at least one composition comprising an effective amount of at least one compound or protein selected from at least one of a diabetes drug, an insulin metabolism related drug, a glucose metabolism related drug, a detectable label or reporter, an anti-infective drug, a cardiovascular (CV) system drug, a central nervous system (CNS) drug, an autonomic nervous system (ANS) drug, a respiratory tract drug, a gastrointestinal (GI) tract drug, a hormonal drug, a drug for fluid or electrolyte balance, a hematologic drug, an antineoplastic, an immunomodulation drug, an ophthalmic, otic or nasal drug, a topical drug, a nutritional drug, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, a cytokine antagonist, vitamins, growth factors, or antioxidants.

[40] Also provided is a medical device, comprising at least one isolated human GLP-1 agonist or mimetibody of the invention, wherein the device is suitable to contacting or administering the at least one GLP-1 agonist or mimetibody by at least one mode selected from in vitro, parenteral, subcutaneous, intramuscular, intravenous,

intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal

[41] Also provided is an article of manufacture for human pharmaceutical or diagnostic use, comprising packaging material and a container comprising a solution or a lyophilized form of at least one isolated human GLP-1 agonist or mimetibody of the present invention. The article of manufacture can optionally comprise having the container as a component of an in vitro, a parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal delivery device or system.

[42] The present invention further provides any invention described herein

[43] DESCRIPTION OF THE FIGURES

[44] Figure 1 illustrates the nucleotide and peptide sequences of GLP-1 mimetibody in IgG1 scaffold showing important functional domains

[45] Figures 2A-2C illustrate FACS binding assays of GLP-1 mimetibody.

[46] Figure 2A shows that GLP-1 mimetibody binds to HEK293 cells over-expressing the GLP-1R. Grey: GLP-1 mimetibody but no secondary; black: secondary only; red, negative control mimetibody and secondary; blue: GLP-1 mimetibody and secondary

[47] Figure 2B shows that the GLP-1 mimetibody does not bind to the control HEK293 cells. Grey: GLP-1 mimetibody but no secondary, black: secondary only, blue: GLP-1 mimetibody and secondary.

[48] Figure 2C shows that a GLP-1 peptide analogue (A2S) is able to compete with GLP-1 mimetibody for binding to HEK293 cells over-expressing the GLP-1R. Grey: GLP-1 mimetibody but no secondary; black: GLP-1 mimetibody and secondary, orange: GLP-1 mimetibody, 0.2 nM competitor, secondary, blue: GLP-1 mimetibody, 20 nM competitor, secondary, red: GLP-1 mimetibody, 100 nM competitor, secondary).

[49] Figures 3A-3E illustrate cAMP assays of GLP-1 mimetibody. Figure 3A: wt GLP-1 mimetibody in IgG1 scaffold, Figure 3B: GLP-1 peptide, Figure 3C: GLP-1 (A2G) mimetibody in IgG4 (Ala/Ala, Ser -> Pro) scaffold, Figure 3D: GLP-1 (A2S) mimetibody in IgG4 (Ala/Ala, Ser -> Pro) scaffold, Figure 3E: wt GLP-1 mimetibody in IgG4 (Ala/Ala, Ser -> Pro) scaffold.

[50] Figure 4 illustrates the resistance of GLP-1 mimetibody to DPP-IV cleavage.

[51] Figure 5 shows the improved stability of GLP-1 mimetibody as compared to GLP-1 peptide in human serum.

[52] Figure 6 demonstrates that GLP-1 mimetibodies cause insulin secretion in RINm cells. Figure 6A shows that GLP-1 (7-36) peptide and exendin-4 peptide stimulates insulin release in RINm cells. Figure 6B shows that GLP-1 (A2S) mimetibody in either IgG1 or IgG4 (Ala/Ala, Ser -> Pro) scaffold, or GLP-1 (A2G) mimetibody in IgG4 (Ala/Ala, Ser -> Pro) scaffold are active in stimulating insulin secretion in RINm cells.

[53] Figure 7 shows the effect of GLP-1 mimetibody on fasting blood glucose in diabetic mice. Figure 7A demonstrates that GLP-1 mimetibody lowers fasting blood glucose. Figure 7B demonstrates that the effect is dose-dependent.

[54] Figure 8A shows the effects of GLP-1 mimetibody on the proliferation of isolated rat islets *in vitro*. Figure 8B shows the effects of GLP-1 mimetibody dose on rat islet proliferation.

[55] Figure 9 shows the effects of GLP-1 mimetibody on the proliferation of isolated non-human primate (NHP) islets *in vitro*.

- [56] Figure 10A shows the effects of GLP-1 mimetibody on the proliferation of isolated human islets *in vitro* Figure 10B shows the effects of GLP-1 mimetibody dose on human islet proliferation
- [57] Figure 11 demonstrates the effects of GLP-1 mimetibody on the insulin
5 secretion from isolated rat islets *in vitro*
- [58] Figure 12 shows the pharmacokinetic profile of GLP-1 peptide (Figure 12A) compared to GLP-1 (A2S) mimetibody (Figure 12B) in mice
- [59] Figure 13 shows the stability of GLP-1 mimetibody in blood from mouse (Figure 13A), rat (Figure 13B), cynomolgus monkey (Figure 13C) and human (Figure
10 13D)
- [60] Figure 14 shows *in vivo* stability of GLP-1 mimetibody in cynomolgus monkey
- [61] Figure 15 demonstrates the effect of GLP-1 mimetibody treatment on blood glucose following an oral glucose challenge in db/db mice (Figure 15A) and DIO mice
15 (Figure 15B)
- [62] Figure 16 demonstrates that GLP-1 mimetibody inhibits cytokine-induced apoptosis in a dose-dependent manner The percent of apoptosis relative to the untreated control is plotted versus the concentration of GLP-1 MMB
- [63] Figure 17 demonstrates that GLP-1 mimetibody increases glucose-dependent
20 insulin secretion in INS-1E cells **A.** The bar graph shows the amount of insulin secreted at each concentration of GLP-1 MMB in the presence of 5.5 mM glucose In addition, the insulin secreted at 3 and 7.5 mM glucose only is plotted **B.** The amount of insulin secreted is plotted vs. the concentration of GLP-1 MMB The data were fit to a Hill equation, providing an EC_{50} of 0.07 nM
- 25 [64] Figure 18 demonstrates that GLP-1 mimetibody increases glucose-dependent insulin secretion in rat (Figure 18A) and human islets (Figure 18B)
- [65] Figure 19 shows the effects of GLP-1 MMB treatment on the blood glucose levels of normal C57/BLK6 mice
- [66] Figure 20 shows the effects of GLP-1 MMB treatment on the blood glucose
30 levels (Figure 20A) and glucose tolerance (Figure 20B) in STZ treated diabetic nude

mice transplanted with a marginal Mass of human islets In Figure 20A, blood glucose was plotted against time (days) for control mice treated with PBS (X) and GLP-1 MMB treated mice (■) receiving daily IP injections. A marginal mass of human islets (50 IEQ/g) was transplanted on day 0 and blood glucose in the animals was monitored thereafter The arrow indicates when the transplanted islets were removed, resulting in the prompt return to the diabetic state In Figure 20B, blood glucose plotted against time (minutes) for control mice treated with PBS (X) and GLP-1 MMB (0.5 mg/kg) (■) The IVGTT was done after the mice received daily IP injections for >30 days Animals were fasted overnight followed by an IV glucose injection (1.5 g/kg) The blood glucose of the mice was monitored and plotted against time post injection. The insert represents the area under the curve for the control and GLP-1 MMB treated animals

[67] Figure 21 shows the effects of GLP-1 MMB on glucose-induced insulin secretion in non-human primate (NHP) islets

[68] Figure 22 shows the effects of GLP-1 MMB on the exogenous insulin requirements (Figure 22A) and the Hemoglobin A1c (HgbA1c) (Figure 22B) of STZ diabetic NHPs transplanted with a marginal mass

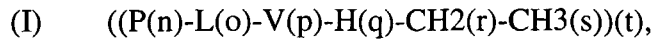
[69] DESCRIPTION OF THE INVENTION

[70] The present invention provides isolated, recombinant and/or synthetic mimetibodies or specified portions or variants, as well as compositions and encoding nucleic acid molecules comprising at least one polynucleotide encoding at least one GLP-1 agonist or mimetibody Such mimetibodies or specified portions or variants of the present invention comprise specific GLP-1 agonist or mimetibody sequences, domains, fragments and specified variants thereof, and methods of making and using said nucleic acids and mimetibodies or specified portions or variants, including therapeutic compositions, methods and devices.

[71] The present invention also provides at least one isolated GLP-1 agonist or mimetibody or specified portion or variant as described herein and/or as known in the art The GLP-1 mimetibody can optionally comprise at least one CH3 region directly linked with at least one CH2 region directly linked with at least one hinge region or fragment thereof (H), directly linked with at least one partial variable region (V),

directly linked with an optional linker sequence (L), directly linked to at least one GLP-1 therapeutic peptide (P)

[72] In a preferred embodiment a GLP-1 mimetibody comprises formula (I):



5 [73] where P is at least one bioactive GLP-1 polypeptide, L is at least one linker sequence, which can be a polypeptide that provides structural flexibility by allowing the mimetibody to have alternative orientations and binding properties, V is at least one portion of a C-terminus of an immunoglobulin variable region, H is at least one portion of an immunoglobulin variable hinge region, CH₂ is at least a portion of an
 10 immunoglobulin CH₂ constant region, CH₃ is at least a portion of an immunoglobulin CH₃ constant region, m, n, o, p, q, r, s and t can be independently an integer between and including 0 and 10, mimicing different types of immunoglobulin molecules, e g , but not limited to IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgM, IgD, IgE, or any subclass thereof, and the like, or any combination thereof

15 [74] Thus, a GLP-1 mimetibody of the present invention mimics an antibody structure with its inherent properties and functions, while providing a therapeutic peptide and its inherent or acquired *in vitro*, *in vivo* or *in situ* properties or activities. In a preferred embodiment where t=1, the monomer CH₃-CH₂-hinge-partial J (J not previously described) sequence-linker-therapeutic peptide can be linked to other
 20 monomers by association or covalent linkage, such as, but not limited to, a Cys-Cys disulfide bond The various portions of the antibody and the GLP-1 therapeutic peptide portions of at least one GLP-1 mimetibody of the present invention can vary as described herein in combination with what is known in the art.

[75] The portion of CH₃-CH₂-hinge may be extensively modified to form a variant in
 25 accordance with this invention, provided binding to the salvage receptor is maintained In such variants, one may remove one or more native sites that provide structural features or functional activity not required by the fusion molecules of this invention One may remove these sites by, for example, substituting or deleting residues, inserting residues into the site, or truncating portions containing the site The inserted or
 30 substituted residues may also be altered amino acids, such as peptidomimetics or D-amino acids. A variant of CH₃-CH₂-hinge may lack one or more native sites or residues

that affect or are involved in (1) disulfide bond formation, (2) incompatibility with a selected host cell, (3) heterogeneity upon expression in a selected host cell, (4) glycosylation, (5) interaction with complement, (6) binding to an Fc receptor other than a salvage receptor, or (7) antibody-dependent cellular cytotoxicity (ADCC) Exemplary

5 CH3-CH2-hinge variants include molecules and sequences in which 1 Sites involved in disulfide bond formation are removed Such removal may avoid reaction with other cysteine-containing proteins present in the host cell used to produce the molecules of the invention. For this purpose, the cysteine residues may be deleted or substituted with other amino acids (e.g., alanyl, seryl) Even when cysteine residues are removed, the

10 single chain CH3-CH2-hinge domains can still form a dimeric CH3-CH2-hinge domain that is held together non-covalently, 2. The CH3-CH2-hinge region is modified to make it more compatible with a selected host cell For example, when the molecule is expressed recombinantly in a bacterial cell such as *E. coli*, one may remove the PA sequence in the hinge, which may be recognized by a digestive enzyme in *E. coli* such as proline

15 iminopeptidase, 3 A portion of the hinge region is deleted or substituted with other amino acids to prevent heterogeneity when expressed in a selected host cell; 4 One or more glycosylation sites are removed Residues that are typically glycosylated (e.g., asparagine) may confer cytolytic response. Such residues may be deleted or substituted with unglycosylated residues (e.g., alanine); 5 Sites involved in interaction with

20 complement, such as the C1q binding site, are removed Complement recruitment may not be advantageous for the molecules of this invention and so may be avoided with such a variant, 6 Sites are removed that affect binding to Fc receptors other than a salvage receptor. The CH3-CH2-hinge region may have sites for interaction with certain white blood cells that are not required for the fusion molecules of the present invention

25 and so may be removed, 7 The ADCC site is removed ADCC sites are known in the art, see, for example, *Molec. Immunol.* 29 (5) 633-9 (1992) with regard to ADCC sites in IgG1 These sites, as well, are not required for the fusion molecules of the present invention and so may be removed.

[76] Linker polypeptide provides structural flexibility by allowing the agonist or

30 mimetibody to have alternative orientations and binding properties When present, its chemical structure is not critical The linker is preferably made up of amino acids linked together by peptide bonds Thus, in preferred embodiments, the linker is made up of

from 1 to 20 amino acids linked by peptide bonds, wherein the amino acids are selected from the 20 naturally occurring amino acids. Some of these amino acids may be glycosylated, as is well understood by those in the art. In a more preferred embodiment, the 1 to 20 amino acids are selected from glycine, alanine, serine, proline, asparagine, glutamine, and lysine. Even more preferably, a linker is made up of a majority of amino acids that are sterically unhindered, such as glycine and alanine. Thus, preferred linkers are poly(Gly-Ser), polyglycines (particularly (Gly)₄, (Gly)₅), poly(Gly-Ala), and polyalanines. Other specific examples of linkers are (Gly)₃Lys(Gly)₄ (SEQ ID NO:65), (Gly)₃AsnGlySer(Gly)₂ (SEQ ID NO 66), (Gly)₃Cys(Gly)₄ (SEQ ID NO 67), and GlyProAsnGlyGly (SEQ ID NO 68).

[77] To explain the above nomenclature, for example, (Gly)₃Lys(Gly)₄ means Gly-Gly-Gly-Lys-Gly-Gly-Gly-Gly. Combinations of Gly and Ala are also preferred. The linkers shown here are exemplary, linkers within the scope of this invention may be much longer and may include other residues.

[78] Non-peptide linkers are also possible. For example, alkyl linkers such as -NH-(CH₂)_s-C(O)-, wherein s=2-20 could be used. These alkyl linkers may further be substituted by any non-sterically hindering group such as lower alkyl (e.g., C₁ - C₆), lower acyl, halogen (e.g., Cl, Br), CN, NH₂, phenyl, etc. An exemplary non-peptide linker is a PEG linker which has a molecular weight of 100 to 5000 kD, preferably 100 to 500 kD. The peptide linkers may be altered to form derivatives in the same manner as described above.

As used herein, a "GLP-1 peptide," or "GLP-1 peptide, variant, or derivative" can be at least one GLP-1 peptide, GLP-1 fragment, GLP-1 homolog, GLP-1 analog, or GLP-1 derivative. A GLP-1 peptide has from about twenty-five to about forty-five naturally occurring or non-naturally occurring amino acids that have sufficient homology to native GLP-1 (7-37) such that they exhibit insulinotropic activity by binding to the GLP-1 receptor on β -cells in the pancreas. GLP-1 (7-37) has the amino acid sequence of SEQ ID NO 15: His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly.

[79] A GLP-1 fragment is a polypeptide obtained after truncation of one or more amino acids from the N-terminus and/or C-terminus of GLP-1 (7-37) or an analog or

derivative thereof. A GLP-1 homolog is a peptide in which one or more amino acids have been added to the N-terminus and/or C-terminus of GLP-1 (7-37), or fragments or analogs thereof. A GLP-1 analog is a peptide in which one or more amino acids of GLP-1 (7-37) have been modified and/or substituted. A GLP-1 analog has sufficient
5 homology to GLP-1 (7-37) or a fragment of GLP-1 (7-37) such that the analog has insulinotropic activity. A GLP-1 derivative is defined as a molecule having the amino acid sequence of a GLP-1 peptide, a GLP-1 homolog or a GLP-1 analog, but additionally having chemical modification of one or more of its amino acid side groups, α -carbon atoms, terminal amino group, or terminal carboxylic acid group.

10 [80] Numerous active GLP-1 fragments, analogs and derivatives are known in the art and any of these analogs and derivatives can also be part of the GLP-1 agonist or mimetibody of the present invention. Some GLP-1 analogs and GLP-1 fragments known in the art are disclosed in U.S. Pat. Nos. 5,118,666, 5,977,071, and 5,545,618, and Adelhorst, et al., *J. Biol. Chem.* 269:6275 (1994). Examples include, but not limited
15 to, GLP-1 (7-34), GLP-1 (7-35), GLP-1 (7-36), Gln9-GLP-1(7-37), D-Gln9-GLP-1(7-37), Thr16-Lys18-GLP-1 (7-37), and Lys18-GLP-1 (7-37).

[81] A "GLP-1 agonist or mimetibody," "GLP-1 agonist or mimetibody portion," or "GLP-1 agonist or mimetibody fragment" and/or "GLP-1 agonist or mimetibody variant" and the like has, mimics or simulates at least one biological activity, such as but
20 not limited to ligand binding, *in vitro*, *in situ* and/or preferably *in vivo*, of at least one GLP-1 peptide, variant or derivative, such as but not limited to at least one of SEQ ID NO 1. For example, a suitable GLP-1 agonist or mimetibody, specified portion, or variant can also modulate, increase, modify, activate, at least one GLP-1 receptor signaling or other measurable or detectable activity.

25 [82] GLP-1 mimetibodies useful in the methods and compositions of the present invention are characterized by suitable affinity binding to protein ligands, for example, GLP-1 receptors, and optionally and preferably having low toxicity. In particular, a GLP-1 mimetibody, where the individual components, such as the portion of variable region, constant region (without a CH1 portion) and framework, or any portion thereof
30 (e.g., a portion of the J, D or V regions of the variable heavy or light chain, at least a portion of at least one hinge region, the constant heavy chain or light chain, and the like) individually and/or collectively optionally and preferably possess low immunogenicity,

is useful in the present invention. The mimetibodies that can be used in the invention are optionally characterized by their ability to treat patients for extended periods with good to excellent alleviation of symptoms and low toxicity. Low immunogenicity and/or high affinity, as well as other undefined properties, may contribute to the therapeutic results achieved. "Low immunogenicity" is defined herein as raising significant HAMA, HACA or HAHA responses in less than about 75%, or preferably less than about 50, 45, 40, 35, 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, and/or 1% of the patients treated and/or raising low titres in the patient treated (less than about 300, preferably less than about 100 measured with a double antigen enzyme immunoassay) (see, e.g., Elliott *et al*, *Lancet* 344 1125-1127 (1994))

[83] *Utility.* The isolated nucleic acids of the present invention can be used for production of at least one GLP-1 agonist or mimetibody, fragment or specified variant thereof, which can be used to effect a cell, tissue, organ or animal (including mammals and humans), to modulate, treat, alleviate, help prevent the incidence of, or reduce the symptoms of, at least one protein related condition, selected from, but not limited to, at least one of a diabetes related disorder, an insulin metabolism related disorder, a glucose metabolism related disorder, an immune disorder or disease, a cardiovascular disorder or disease, an infectious, malignant, and/or neurologic disorder or disease, as well as other known or specified protein related conditions

[84] Such a method can comprise administering an effective amount of a composition or a pharmaceutical composition comprising at least one GLP-1 agonist or mimetibody or specified portion or variant to a cell, tissue, organ, animal or patient in need of such modulation, treatment, alleviation, prevention, or reduction in symptoms, effects or mechanisms. The effective amount can comprise an amount of about 0.0001 to 500 mg/kg per single or multiple administration, or to achieve a serum concentration of 0.01-5000 µg/ml serum concentration per single or multiple administration, (need to include in vitro concentrations) or any effective range or value therein, as done and determined using known methods, as described herein or known in the relevant arts

[85] *Citations.* All publications or patents cited herein are entirely incorporated herein by reference as they show the state of the art at the time of the present invention and/or to provide description and enablement of the present invention. Publications refer to any scientific or patent publications, or any other information available in any media

format, including all recorded, electronic or printed formats. The following references are entirely incorporated herein by reference: Ausubel, et al., ed., Current Protocols in Molecular Biology, John Wiley & Sons, Inc., NY, NY (1987-2005); Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor, NY

- 5 (1989), Harlow and Lane, Antibodies, a Laboratory Manual, Cold Spring Harbor, NY (1989); Colligan, et al., eds., Current Protocols in Immunology, John Wiley & Sons, Inc., NY (1994-2005), Colligan et al., Current Protocols in Protein Science, John Wiley & Sons, NY, NY, (1997-2005).

- [86] **Mimetibodies of the Present Invention.** The GLP-1 mimetibody can
- 10 optionally comprise at least one CH3 region directly linked with at least one CH2 region directly linked with at least one portion of at least one hinge region fragment (H), such as comprising at least one core hinge region, directly linked with at least one partial variable region (V), directly linked with an optional linker sequence (L), directly linked to at least one GLP-1 therapeutic peptide (P). In a preferred embodiment, a pair of a
- 15 CH3-CH2-H-V-L-P can be linked by association or covalent linkage, such as, but not limited to, a Cys-Cys disulfide bond. Thus, a GLP-1 mimetibody of the present invention mimics an antibody structure with its inherent properties and functions, while providing a therapeutic peptide and its inherent or acquired *in vitro*, *in vivo* or *in situ* properties or activities. The various portions of the antibody and therapeutic peptide
- 20 portions of at least one GLP-1 mimetibody of the present invention can vary as described herein in combination with what is known in the art.

- [87] Mimetibodies of the present invention thus provide at least one suitable property as compared to known proteins, such as, but not limited to, at least one of increased half-life, increased activity, more specific activity, increased avidity, increased
- 25 or decrease off rate, a selected or more suitable subset of activities, less immunogenicity, increased quality or duration of at least one desired therapeutic effect, less side effects, and the like.

- [88] Fragments of mimetibodies according to Formula (I) can be produced by enzymatic cleavage, synthetic or recombinant techniques, as known in the art and/or as
- 30 described herein. Mimetibodies can also be produced in a variety of truncated forms using antibody genes in which one or more stop codons have been introduced upstream of the natural stop site. The various portions of mimetibodies can be joined together

chemically by conventional techniques, or can be prepared as a contiguous protein using genetic engineering techniques. For example, a nucleic acid encoding at least one of the constant regions of a human antibody chain can be expressed to produce a contiguous protein for use in mimetibodies of the present invention. See, e.g., Ladner *et al.*, U.S. Patent No. 4,946,778 and Bird, R.E. *et al.*, *Science*, 242: 423-426 (1988), regarding single chain antibodies.

[89] As used herein, the term "human mimetibody" refers to an antibody in which substantially every part of the protein (e.g., GLP-1 peptide, C_H domains (e.g., C_H2, C_H3), hinge, V) is expected to be substantially non-immunogenic in humans with only minor sequence changes or variations. Such changes or variations optionally and preferably retain or reduce the immunogenicity in humans relative to non-modified human antibodies, or mimetibodies of the present invention. Thus, a human antibody and corresponding GLP-1 mimetibody of the present invention is distinct from a chimeric or humanized antibody. It is pointed out that the GLP-1 mimetibody can be produced by a non-human animal or cell that is capable of expressing human immunoglobulins (e.g., heavy chain and/or light chain) genes.

[90] Human mimetibodies that are specific for at least one protein ligand thereof can be designed against an appropriate ligand, such as an isolated GLP-1 receptor, or a portion thereof (including synthetic molecules, such as synthetic peptides). Preparation of such mimetibodies are performed using known techniques to identify and characterize ligand binding regions or sequences of at least one protein or portion thereof.

[91] In a preferred embodiment, at least one GLP-1 agonist or mimetibody or specified portion or variant of the present invention is produced by at least one cell line, mixed cell line, immortalized cell or clonal population of immortalized and/or cultured cells. Immortalized protein producing cells can be produced using suitable methods. Preferably, the at least one GLP-1 agonist or mimetibody or specified portion or variant is generated by providing nucleic acid or vectors comprising DNA derived or having a substantially similar sequence to, at least one human immunoglobulin locus that is functionally rearranged, or which can undergo functional rearrangement, and which further comprises a agonist or mimetibody structure as described herein, e.g., but not

limited to Formula (I), wherein portions of C- terminal variable regions can be used for V, hinge regions for H, CH2 for CH2 and CH3 for CH3, as known in the art

[92] The term “functionally rearranged,” as used herein refers to a segment of nucleic acid from an immunoglobulin locus that has undergone V(D)J recombination, thereby producing an immunoglobulin gene that encodes an immunoglobulin chain (e.g., heavy chain), or any portion thereof. A functionally rearranged immunoglobulin gene can be directly or indirectly identified using suitable methods, such as, for example, nucleotide sequencing, hybridization (e.g., Southern blotting, Northern blotting) using probes that can anneal to coding joints between gene segments or enzymatic amplification of immunoglobulin genes (e.g., polymerase chain reaction) with primers that can anneal to coding joints between gene segments. Whether a cell produces a GLP-1 agonist or mimetibody or portion or variant comprising a particular variable region or a variable region comprising a particular sequence (e.g., at least one P sequence) can also be determined using suitable methods

[93] Mimetibodies, specified portions and variants of the present invention can also be prepared using at least one GLP-1 agonist or mimetibody or specified portion or variant encoding nucleic acid to provide transgenic animals or mammals, such as goats, cows, horses, sheep, and the like, that produce such mimetibodies or specified portions or variants in their milk. Such animals can be provided using known methods as applied for antibody encoding sequences. See, e.g., but not limited to, US patent nos 5,827,690, 5,849,992, 4,873,316, 5,849,992; 5,994,616, 5,565,362; 5,304,489, and the like, each of which is entirely incorporated herein by reference

[94] Mimetibodies, specified portions and variants of the present invention can additionally be prepared using at least one GLP-1 agonist or mimetibody or specified portion or variant encoding nucleic acid to provide transgenic plants and cultured plant cells (e.g., but not limited to tobacco and maize) that produce such mimetibodies, specified portions or variants in the plant parts or in cells cultured therefrom. As a non-limiting example, transgenic tobacco leaves expressing recombinant proteins have been successfully used to provide large amounts of recombinant proteins, e.g., using an inducible promoter. See, e.g., Cramer et al., *Curr Top Microbiol Immunol* 240 95-118 (1999) and references cited therein. Also, transgenic maize or corn have been used to express mammalian proteins at commercial production levels, with biological activities

equivalent to those produced in other recombinant systems or purified from natural sources. See, e.g., Hood et al., *Adv. Exp. Med. Biol.* 464:127-147 (1999) and references cited therein. Antibodies have also been produced in large amounts from transgenic plant seeds including antibody fragments, such as single chain mimetibodies (scFv's),
 5 including tobacco seeds and potato tubers. See, e.g., Conrad et al., *Plant Mol. Biol.* 38:101-109 (1998) and references cited therein. Thus, mimetibodies, specified portions and variants of the present invention can also be produced using transgenic plants, according to known methods. See also, e.g., Fischer et al., *Biotechnol. Appl. Biochem.* 30:99-108 (Oct., 1999), Ma et al., *Trends Biotechnol.* 13:522-7 (1995); Ma et al., *Plant*
 10 *Physiol.* 109:341-6 (1995); Whitlam et al., *Biochem. Soc. Trans.* 22:940-944 (1994), and references cited therein. The above references are entirely incorporated herein by reference.

[95] The mimetibodies of the invention can bind human protein ligands with a wide range of affinities (K_D). In a preferred embodiment, at least one human GLP-1 agonist
 15 or mimetibody of the present invention can optionally bind at least one protein ligand with high affinity. For example, at least one GLP-1 agonist or mimetibody of the present invention can bind at least one protein ligand with a K_D equal to or less than about 10^{-7} M or, more preferably, with a K_D equal to or less than about 0.1-9.9 (or any range or value therein) $\times 10^{-7}$, 10^{-8} , 10^{-9} , 10^{-10} , 10^{-11} , 10^{-12} , or 10^{-13} M, or any range or
 20 value therein.

[96] The affinity or avidity of a GLP-1 agonist or mimetibody for at least one protein ligand can be determined experimentally using any suitable method, e.g., as used for determining antibody-antigen binding affinity or avidity. (See, for example, Berzofsky, *et al.*, "Antibody-Antigen Interactions," In *Fundamental Immunology*, Paul, W. E., Ed.,
 25 Raven Press: New York, NY (1984), Kubly, Janis *Immunology*, W. H. Freeman and Company: New York, NY (1992), and methods described herein). The measured affinity of a particular GLP-1 agonist or mimetibody-ligand interaction can vary if measured under different conditions (e.g., salt concentration, pH). Thus, measurements of affinity and other ligand-binding parameters (e.g., K_D , K_a , K_d) are preferably made
 30 with standardized solutions of GLP-1 agonist or mimetibody and ligand, and a standardized buffer, such as the buffer described herein or known in the art.

- [97] **Nucleic Acid Molecules.** Using the information provided herein, such as the nucleotide sequences encoding at least 90-100% of the contiguous amino acids of at least one of SEQ ID NOS 1 and 6, as well as at least one portion of an antibody, wherein the above sequences are inserted as the P sequence of Formula (I) to provide a GLP-1 agonist or mimetibody of the present invention, further comprising specified fragments, variants or consensus sequences thereof, or a deposited vector comprising at least one of these sequences, a nucleic acid molecule of the present invention encoding at least one GLP-1 agonist or mimetibody or specified portion or variant can be obtained using methods described herein or as known in the art.
- 10 [98] Nucleic acid molecules of the present invention can be in the form of RNA, such as mRNA, hnRNA, tRNA or any other form, or in the form of DNA, including, but not limited to, cDNA and genomic DNA obtained by cloning or produced synthetically, or any combination thereof. The DNA can be triple-stranded, double-stranded or single-stranded, or any combination thereof. Any portion of at least one strand of the DNA or
- 15 RNA can be the coding strand, also known as the sense strand, or it can be the non-coding strand, also referred to as the anti-sense strand.
- [99] Isolated nucleic acid molecules of the present invention can include nucleic acid molecules comprising an open reading frame (ORF), optionally with one or more introns, nucleic acid molecules comprising the coding sequence for a GLP-1 agonist or mimetibody or specified portion or variant, and nucleic acid molecules which comprise a nucleotide sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode at least one GLP-1 agonist or mimetibody as described herein and/or as known in the art. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate
- 20 such degenerate nucleic acid variants that code for specific GLP-1 agonist or mimetibody or specified portion or variants of the present invention. See, e.g., Ausubel, et al., *supra*, and such nucleic acid variants are included in the present invention.
- [100] As indicated herein, nucleic acid molecules of the present invention which comprise a nucleic acid encoding a GLP-1 agonist or mimetibody or specified portion or variant can include, but are not limited to, those encoding the amino acid sequence of a GLP-1 agonist or mimetibody fragment, by itself, the coding sequence for the entire GLP-1 agonist or mimetibody or a portion thereof; the coding sequence for a GLP-1
- 30

agonist or mimetibody, fragment or portion, as well as additional sequences, such as the coding sequence of at least one signal leader or fusion peptide, with or without the aforementioned additional coding sequences, such as at least one intron, together with additional, non-coding sequences, including but not limited to, non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals (for example - ribosome binding and stability of mRNA), an additional coding sequence that codes for additional amino acids, such as those that provide additional functionalities. Thus, the sequence encoding a GLP-1 agonist or mimetibody or specified portion or variant can be fused to a marker sequence, such as a sequence encoding a peptide that facilitates purification of the fused GLP-1 agonist or mimetibody or specified portion or variant comprising a GLP-1 agonist or mimetibody fragment or portion.

[101] **Polynucleotides Which Selectively Hybridize to a Polynucleotide as Described Herein.** The present invention provides isolated nucleic acids that hybridize under selective hybridization conditions to a polynucleotide disclosed herein, or others disclosed herein, including specified variants or portions thereof. Thus, the polynucleotides of this embodiment can be used for isolating, detecting, and/or quantifying nucleic acids comprising such polynucleotides.

[102] Low or moderate stringency hybridization conditions are typically, but not exclusively, employed with sequences having a reduced sequence identity relative to complementary sequences. Moderate and high stringency conditions can optionally be employed for sequences of greater identity. Low stringency conditions allow selective hybridization of sequences having about 40-99% sequence identity and can be employed to identify orthologous or paralogous sequences.

[103] Optionally, polynucleotides of this invention will encode at least a portion of a GLP-1 agonist or mimetibody or specified portion or variant encoded by the polynucleotides described herein. The polynucleotides of this invention embrace nucleic acid sequences that can be employed for selective hybridization to a polynucleotide encoding a GLP-1 agonist or mimetibody or specified portion or variant of the present invention. See, e.g., Ausubel, *supra*; Colligan, *supra*, each entirely incorporated herein by reference.

[104] **Construction of Nucleic Acids.** The isolated nucleic acids of the present invention can be made using (a) recombinant methods, (b) synthetic techniques, (c) purification techniques, or combinations thereof, as well-known in the art.

[105] The nucleic acids can conveniently comprise sequences in addition to a polynucleotide of the present invention. For example, a multi-cloning site comprising one or more endonuclease restriction sites can be inserted into the nucleic acid to aid in isolation of the polynucleotide. Also, translatable sequences can be inserted to aid in the isolation of the translated polynucleotide of the present invention. For example, a hexahistidine marker sequence provides a convenient means to purify the proteins of the present invention. The nucleic acid of the present invention - excluding the coding sequence - is optionally a vector, adapter, or linker for cloning and/or expression of a polynucleotide of the present invention

[106] Additional sequences can be added to such cloning and/or expression sequences to optimize their function in cloning and/or expression, to aid in isolation of the polynucleotide, or to improve the introduction of the polynucleotide into a cell. Use of cloning vectors, expression vectors, adapters, and linkers is well known in the art. See, e.g., Ausubel, *supra*, or Sambrook, *supra*

[107] **Recombinant Methods for Constructing Nucleic Acids.** The isolated nucleic acid compositions of this invention, such as RNA, cDNA, genomic DNA, or any combination thereof, can be obtained from biological sources using any number of cloning methodologies known to those of skill in the art. In some embodiments, oligonucleotide probes that selectively hybridize, under suitable stringency conditions, to the polynucleotides of the present invention are used to identify the desired sequence in a cDNA or genomic DNA library. The isolation of RNA, and construction of cDNA and genomic libraries, is well known to those of ordinary skill in the art. (See, e.g., Ausubel, *supra*, or Sambrook, *supra*)

[108] **Synthetic Methods for Constructing Nucleic Acids.** The isolated nucleic acids of the present invention can also be prepared by direct chemical synthesis by known methods (see, e.g., Ausubel, et al., *supra*). Chemical synthesis generally produces a single-stranded oligonucleotide, which can be converted into double-stranded DNA by hybridization with a complementary sequence, or by polymerization

with a DNA polymerase using the single strand as a template. One of skill in the art will recognize that while chemical synthesis of DNA can be limited to sequences of about 100 or more bases, longer sequences can be obtained by the ligation of shorter sequences

5 [109] **Recombinant Expression Cassettes.** The present invention further provides recombinant expression cassettes comprising a nucleic acid of the present invention. A nucleic acid sequence of the present invention, for example a cDNA or a genomic sequence encoding a GLP-1 agonist or mimetibody or specified portion or variant of the present invention, can be used to construct a recombinant expression cassette that can be
10 introduced into at least one desired host cell. A recombinant expression cassette will typically comprise a polynucleotide of the present invention operably linked to transcriptional initiation regulatory sequences that will direct the transcription of the polynucleotide in the intended host cell. Both heterologous and non-heterologous (i.e., endogenous) promoters can be employed to direct expression of the nucleic acids of the
15 present invention.

[110] In some embodiments, isolated nucleic acids that serve as promoter, enhancer, or other elements can be introduced in the appropriate position (upstream, downstream or in intron) of a non-heterologous form of a polynucleotide of the present invention so as to up or down regulate expression of a polynucleotide of the present invention. For
20 example, endogenous promoters can be altered *in vivo* or *in vitro* by mutation, deletion and/or substitution, as known in the art. A polynucleotide of the present invention can be expressed in either sense or anti-sense orientation as desired. It will be appreciated that control of gene expression in either sense or anti-sense orientation can have a direct impact on the observable characteristics. Another method of suppression is sense
25 suppression. Introduction of nucleic acid configured in the sense orientation has been shown to be an effective means by which to block the transcription of target genes.

[111] **Vectors And Host Cells.** The present invention also relates to vectors that include isolated nucleic acid molecules of the present invention, host cells that are genetically engineered with the recombinant vectors, and the production of at least one
30 GLP-1 agonist or mimetibody or specified portion or variant by recombinant techniques, as is well known in the art. See, e.g., Sambrook, et al., *supra*; Ausubel, et al., *supra*, each entirely incorporated herein by reference.

[112] The polynucleotides can optionally be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced into a cell using suitable known methods, such as electroporation and the like, other known methods include the use of the vector as a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it can be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

[113] The DNA insert should be operatively linked to an appropriate promoter. The expression constructs will further contain sites optionally for at least one of transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will preferably include a translation initiating at the beginning and a termination codon (e.g., UAA, UGA or UAG) appropriately positioned at the end of the mRNA to be translated, with UAA and UAG preferred for mammalian or eukaryotic cell expression.

[114] Expression vectors will preferably but optionally include at least one selectable marker. Such markers include, e.g., but not limited to, methotrexate (MTX), dihydrofolate reductase (DHFR, US Pat Nos. 4,399,216, 4,634,665, 4,656,134, 4,956,288, 5,149,636, 5,179,017, ampicillin, neomycin (G418), mycophenolic acid, or glutamine synthetase (GS, US Pat Nos. 5,122,464, 5,770,359, 5,827,739) resistance for eukaryotic cell culture, and tetracycline or ampicillin resistance genes for culturing in *E. coli* and other bacteria or prokaryotics (the above patents are entirely incorporated hereby by reference). Appropriate culture mediums and conditions for the above-described host cells are known in the art. Suitable vectors will be readily apparent to the skilled artisan. Introduction of a vector construct into a host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other known methods. Such methods are described in the art, such as Sambrook, *supra*, Chapters 1-4 and 16-18, Ausubel, *supra*, Chapters 1, 9, 13, 15, 16.

[115] At least one GLP-1 agonist or mimetibody or specified portion or variant of the present invention can be expressed in a modified form, such as a fusion protein, and can include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, can

be added to the N-terminus of a GLP-1 agonist or mimetibody or specified portion or variant to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties can be added to a GLP-1 agonist or mimetibody or specified portion or variant of the present invention to
 5 facilitate purification. Such regions can be removed prior to final preparation of a GLP-1 agonist or mimetibody or at least one fragment thereof. Such methods are described in many standard laboratory manuals, such as Sambrook, *supra*, Chapters 17-17.42 and 18.1-18.74; Ausubel, *supra*, Chapters 16, 17 and 18.

[116] Those of ordinary skill in the art are knowledgeable in the numerous expression
 10 systems available for expression of a nucleic acid encoding a protein of the present invention.

Illustrative of cell cultures useful for the production of the mimetibodies, specified portions or variants thereof, are mammalian cells. Mammalian cell systems often will be in the form of monolayers of cells although mammalian cell suspensions or
 15 bioreactors can also be used. A number of suitable host cell lines capable of expressing intact glycosylated proteins have been developed in the art, and include the COS-1 (e.g., ATCC CRL 1650), COS-7 (e.g., ATCC CRL-1651), HEK293, BHK21 (e.g., ATCC CRL-10), CHO (e.g., ATCC CRL 1610, DG-44) and BSC-1 (e.g., ATCC CRL-26) cell lines, hepG2 cells, P3X63Ag8.653, SP2/0-Ag14, 293 cells, HeLa cells and the
 20 like, which are readily available from, for example, American Type Culture Collection, Manassas, Va. Preferred host cells include cells of lymphoid origin such as myeloma and lymphoma cells. Particularly preferred host cells are P3X63Ag8.653 cells (ATCC Accession Number CRL-1580) and SP2/0-Ag14 cells (ATCC Accession Number CRL-1851).

[117] Expression vectors for these cells can include one or more of the following expression control sequences, such as, but not limited to an origin of replication, a promoter (e.g., late or early SV40 promoters, the CMV promoter (e.g., US Pat Nos 5,168,062; 5,385,839), an HSV tk promoter, a pgk (phosphoglycerate kinase) promoter, an EF-1 alpha promoter (e.g., US Pat No. 5,266,491), at least one human
 25 immunoglobulin promoter, an enhancer, and/or processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites (e.g., an SV40 large T Ag poly A addition site), and transcriptional terminator sequences. See, e.g., Ausubel et

al., *supra*; Sambrook, et al , *supra* Other cells useful for production of nucleic acids or proteins of the present invention are known and/or available, for instance, from the American Type Culture Collection Catalogue of Cell Lines and Hybridomas (www.atcc.org) or other known or commercial sources.

- 5 [118] When eukaryotic host cells are employed, polyadenylation or transcription terminator sequences are typically incorporated into the vector An example of a terminator sequence is the polyadenylation sequence from the bovine growth hormone gene. Sequences for accurate splicing of the transcript can also be included An example of a splicing sequence is the VP1 intron from SV40 (Sprague, et al , J Virol
10 45 773-781 (1983)) Additionally, gene sequences to control replication in the host cell can be incorporated into the vector, as known in the art

- [119] **Purification of a GLP-1 agonist or mimetibody or specified portion or variant thereof.** A GLP-1 agonist or mimetibody or specified portion or variant can be recovered and purified from recombinant cell cultures by well-known methods
15 including, but not limited to, protein A purification, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography High performance liquid chromatography ("HPLC") can also be employed for purification
20 See, e g , Colligan, Current Protocols in Immunology, or Current Protocols in Protein Science, John Wiley & Sons, NY, NY, (1997-2005), e g , Chapters 1, 4, 6, 8, 9, 10, each entirely incorporated herein by reference

- [120] Mimetibodies or specified portions or variants of the present invention include naturally purified products, products of chemical synthetic procedures, and products
25 produced by recombinant techniques from a eukaryotic host, including, for example, yeast, higher plant, insect and mammalian cells Depending upon the host employed in a recombinant production procedure, the GLP-1 agonist or mimetibody or specified portion or variant of the present invention can be glycosylated or can be non-glycosylated, with glycosylated preferred Such methods are described in many standard
30 laboratory manuals, such as Sambrook, *supra*, Sections 17.37-17.42, Ausubel, *supra*, Chapters 10, 12, 13, 16, 18 and 20, Colligan, Protein Science, *supra*, Chapters 12-14, all entirely incorporated herein by reference

[121] MIMETIBODIES, SPECIFIED FRAGMENTS AND/OR VARIANTS.

The isolated mimetibodies of the present invention comprise a GLP-1 agonist or mimetibody or specified portion or variant encoded by any one of the polynucleotides of the present invention as discussed more fully herein, or any isolated or prepared GLP-1

5 agonist or mimetibody or specified portion or variant thereof

[122] Preferably, the GLP-1 agonist or mimetibody or ligand-binding portion or variant binds at least one GLP-1 protein ligand and thereby provides at least one GLP-1 biological activity of the corresponding protein or a fragment thereof. Different therapeutically or diagnostically significant proteins are well known in the art and

10 suitable assays or biological activities of such proteins are also well known in the art

[123] Non-limiting examples of suitable GLP-1 peptides, variants and derivatives for this invention appear as SEQ ID NO 1: His-Xaa2-Xaa3-Gly-Xaa5-Xaa6-Xaa7-Xaa8-Xaa9-Xaa10-Xaa11-Xaa12-Xaa13-Xaa14-Xaa15-Xaa16-Xaa17-Xaa18-Xaa19-Xaa20-Xaa21-Phe-Xaa23-Xaa24-Xaa25-Xaa26-Xaa27-Xaa28-Xaa29-Xaa30-Xaa31, wherein:

15 Xaa2 is Ala, Gly, Ser, Thr, Leu, Ile, Val, Glu, Asp, or Lys, Xaa3 is Glu, Asp, or Lys, Xaa5 is Thr, Ala, Gly, Ser, Leu, Ile, Val, Arg, His, Glu, Asp, or Lys, Xaa6 is Phe, His, Trp, or Tyr, Xaa7 is Thr or Asn, Xaa8 is Ser, Ala, Gly, Thr, Leu, Ile, Val, Glu, Asp, or Lys, Xaa9 is Asp or Glu, Xaa10 is Val, Ala, Gly, Ser, Thr, Leu, Ile, Met, Tyr, Trp, His, Phe, Glu, Asp, or Lys; Xaa11 is Ser, Val, Ala, Gly, Thr, Leu, Ile, Glu, Asp, or Lys,

20 Xaa12 is Ser, Val, Ala, Gly, Thr, Leu, Ile, Glu, Asp or Lys, Xaa13 is Tyr, Phe, Trp, Glu, Asp or Lys; Xaa14 is Leu, Ala, Met, Gly, Ser, Thr, Leu, Ile, Val, Glu, Asp or Lys, Xaa15 is Glu, Ala, Thr, Ser, Gly, Gln, Asp or Lys, Xaa16 is Gly, Ala, Ser, Thr, Leu, Ile, Val, Gln, Asn, Arg, Cys, Glu, Asp or Lys, Xaa17 is Gln, Asn, Arg, His, Glu, Asp or Lys, Xaa18 is Ala, Gly, Ser, Thr, Leu, Ile, Val, Arg, Glu, Asp or Lys, Xaa19 is Ala,

25 Gly, Ser, Thr, Leu, Ile, Val, Met, Glu, Asp or Lys, Xaa20 is Lys, Arg, His, Gln, Trp, Tyr, Phe, Glu or Asp; Xaa21 is Glu, Leu, Ala, His, Phe, Tyr, Trp, Arg, Gln, Thr, Ser, Gly, Asp or Lys, Xaa23 is Ile, Ala, Val, Leu or Glu; Xaa24 is Ala, Gly, Ser, Thr, Leu, Ile, Val, His, Glu, Asp or Lys, Xaa25 is Trp, Phe, Tyr, Glu, Asp or Lys, Xaa26 is Leu, Gly, Ala, Ser, Thr, Ile, Val, Glu, Asp or Lys, Xaa27 is Val, Leu, Gly, Ala, Ser, Thr, Ile,

30 Arg, Glu, Asp or Lys, Xaa28 is Lys, Asn, Arg, His, Glu or Asp, Xaa29 is Gly, Ala, Ser, Thr, Leu, Ile, Val, Arg, Trp, Tyr, Phe, Pro, His, Glu, Asp or Lys, Xaa30 is Arg, His,

Thr, Ser, Trp, Tyr, Phe, Glu, Asp or Lys; and Xaa31 is Gly, Ala, Ser, Thr, Leu, Ile, Val, Arg, Trp, Tyr, Phe, His, Glu, Asp, Lys

[124] Another preferred group of GLP-1 peptides, variants or derivatives are exemplified in SEQ ID NO 6 His-Xaa2-Xaa3-Gly-Thr-Xaa6- Xaa7-Xaa8-Xaa9-Xaa10-
 5 Ser-Xaa12-Tyr-Xaa14-Glu-Xaa16-Xaa17-Xaa18-Xaa19-Lys-Xaa21-Phe-Xaa23-Ala-Trp-Leu-Xaa27-Xaa28-Gly-Xaa30, wherein Xaa2 is Ala, Gly, or Ser, Xaa3 is Glu or Asp, Xaa6 is Phe or Tyr; Xaa7 is Thr or Asn; Xaa8 is Ser, Thr or Ala, Xaa9 is Asp or Glu, Xaa10 is Val, Leu, Met or Ile; Xaa12 is Ser or Lys, Xaa14 is Leu, Ala or Met, Xaa16 is Gly, Ala, Glu or Asp, Xaa17 is Gln or Glu, Xaa18 is Ala or Lys, Xaa19 is Ala,
 10 Val, Ile, Leu or Met, Xaa21 is Glu or Leu; Xaa23 is Ile, Ala, Val, Leu or Glu, Xaa27 is Val or Lys; Xaa28 is Lys or Asn; and Xaa30 is Arg or Glu

[125] These peptides can be prepared by methods disclosed and/or known in the art The Xaas in the sequence (and throughout this specification, unless specified otherwise in a particular instance) include specified amino acid residues, derivatives or modified
 15 amino acids thereof Because the enzyme, dipeptidyl-peptidase IV (DPP-IV), may be responsible for the observed rapid in vivo inactivation of administered GLP-1, GLP-1 peptides, homologs, analogs and derivatives that are protected from the activity of DPP-IV in the context of agonist or mimetibody are preferred

[126] A GLP-1 agonist or mimetibody, or specified portion or variant thereof, that
 20 partially or preferably substantially provides at least one GLP-1 biological activity, can bind the GLP-1 ligand and thereby provide at least one activity that is otherwise mediated through the binding of GLP-1 to at least one ligand, such as a GLP-1 receptor, or through other protein-dependent or mediated mechanisms As used herein, the term "GLP-1 agonist or mimetibody activity" refers to a GLP-1 agonist or mimetibody that
 25 can modulate or cause at least one GLP-1 dependent activity by about 20-10,000%, preferably by at least about 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, 450, 500, 550, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000 % or more, depending on the assay

30 [127] The capacity of a GLP-1 agonist or mimetibody or specified portion or variant to provide at least one protein-dependent activity is preferably assessed by at least one

suitable protein biological assay, as described herein and/or as known in the art. A human GLP-1 agonist or mimetibody or specified portion or variant of the invention can be similar to any class (IgG, IgA, IgM, etc) or isotype and can comprise at least a portion of a kappa or lambda light chain. In one embodiment, the human GLP-1 agonist or mimetibody or specified portion or variant comprises IgG heavy chain variable fragments, hinge region, CH2 and CH3 of, at least one of isotypes, e g , IgG1, IgG2, IgG3 or IgG4.

[128] At least one GLP-1 agonist or mimetibody or specified portion or variant of the invention binds at least one ligand, subunit, fragment, portion or any combination thereof. The at least one GLP-1 peptide, variant or derivative of at least one GLP-1 agonist or mimetibody, specified portion or variant of the present invention can optionally bind at least one specified epitope of the ligand. The binding epitope can comprise any combination of at least one amino acid sequence of at least 1-3 amino acids to the entire specified portion of contiguous amino acids of the sequences of a protein ligand, such as a GLP-1 receptor or portion thereof.

[129] Such mimetibodies can be prepared by joining together the various portions of Formula (I) of the GLP-1 agonist or mimetibody using known techniques, by preparing and expressing at least one nucleic acid molecules that encode the GLP-1 agonist or mimetibody, using known techniques of recombinant DNA technology or by using any other suitable method, such as chemical synthesis.

[130] Mimetibodies that bind to human GLP-1 ligands, such as receptors, and that comprise a defined heavy or light chain variable region or portion thereof, can be prepared using suitable methods, such as phage display (Katsube, Y , *et al* , *Int J Mol Med*, 1(5) 863-868 (1998)) or methods that employ transgenic animals, as known in the art. The GLP-1 agonist or mimetibody, specified portion or variant can be expressed using the encoding nucleic acid or portion thereof in a suitable host cell.

[131] The invention also relates to mimetibodies, ligand-binding fragments and immunoglobulin chains comprising amino acids in a sequence that is substantially the same as an amino acid sequence described herein. Preferably, such mimetibodies or ligand-binding fragments thereof can bind human GLP-1 ligands, such as receptors, with high affinity (e g , K_D less than or equal to about 10^{-7} M). Amino acid sequences

that are substantially the same as the sequences described herein include sequences comprising conservative amino acid substitutions, as well as amino acid deletions and/or insertions. A conservative amino acid substitution refers to the replacement of a first amino acid by a second amino acid that has chemical and/or physical properties (e.g., charge, structure, polarity, hydrophobicity/ hydrophilicity) that are similar to those of the first amino acid. Conservative substitutions include replacement of one amino acid by another within the following groups: lysine (K), arginine (R) and histidine (H), aspartate (D) and glutamate (E), asparagine (N), glutamine (Q), serine (S), threonine (T), tyrosine (Y), K, R, H, D and E; alanine (A), valine (V), leucine (L), isoleucine (I), proline (P), phenylalanine (F), tryptophan (W), methionine (M), cysteine (C) and glycine (G), F, W and Y; C, S and T

[132] Amino Acid Codes. The amino acids that make up mimetibodies or specified portions or variants of the present invention are often abbreviated. The amino acid designations can be indicated by designating the amino acid by its single letter code, its three letter code, name, or three nucleotide codon(s) as is well understood in the art (see Alberts, B, et al, Molecular Biology of The Cell, Third Ed, Garland Publishing, Inc, New York, 1994).

[133] A GLP-1 agonist or mimetibody or specified portion or variant of the present invention can include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation, as specified herein. Such or other sequences that can be used in the present invention, include, but are not limited to the following sequences presented in SEQ ID NOS 47-64

[134] Of course, the number of amino acid substitutions a skilled artisan would make depends on many factors, including those described above. Generally speaking, the number of amino acid substitutions, insertions or deletions for at least one of a GLP-1 agonist or mimetibody will not be more than 40, 30, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 amino acids, such as 1-30 or any range or value therein, as specified herein

[135] In formula I of the present invention ((P(n)-L(o)-V(p)-H(q)-CH₂(r)-CH₃(s))(t), the V, H, CH₂, CH₃ portions according to Formula I can be any suitable human or human compatible sequence, e.g., as presented in Figures 1-18 and Table 1, or as

known in the art, or any combination or consensus sequence thereof, or any fusion protein thereof, preferably of human origin or engineered to minimize immunogenicity when administered to humans

[136] The P portion can comprise at least one GLP-1 therapeutic peptide known in the art or described herein, such as, but not limited to those presented in SEQ ID NO 1, or any combination or consensus sequence thereof, or any fusion protein thereof. In a preferred embodiment, the P portion can comprise at least one GLP-1 peptide having the sequence of at least one of SEQ ID NO 6, or any combination or consensus sequence thereof, or any fusion protein thereof

[137] The optional linker sequence can be any suitable peptide linker as known in the art. Preferred sequences include any combination of G and S, e.g., X1-X2-X3-X4-...-Xn, where X can be G or S, and n can be 5-30. Non-limiting examples include GS, GGS, GGGS (SEQ ID NO 16), GSGGGS (SEQ ID NO 17), GGS GGGS (SEQ ID NO 18), GGS GGGS GG (SEQ ID NO 19) and GGS GGGS GG (SEQ ID NO 20), and the like.

[138] Amino acids in a GLP-1 agonist or mimetibody or specified portion or variant of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (e.g., Ausubel, supra, Chapters 8, 15; Cunningham and Wells, Science 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity, such as, but not limited to at least one protein related activity, as specified herein or as known in the art. Sites that are critical for GLP-1 agonist or mimetibody or specified portion or variant binding can also be identified by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith, et al., J. Mol. Biol. 224:899-904 (1992) and de Vos, et al., Science 255:306-312 (1992)).

[139] Mimetibodies or specified portions or variants of the present invention can comprise as the P portion of Formula (I), e.g., but not limited to, at least one portion of at least one of SEQ ID NOS 1 and 6. A GLP-1 agonist or mimetibody or specified portion or variant can further optionally comprise at least one functional portion of at least one polypeptide as P portion of Formula (I), at least 90-100% of at least one of SEQ ID

NOS.1 and 6 Non-limiting variants that can enhance or maintain at least one of the listed activities above include, but are not limited to, any of the above polypeptides, further comprising at least one mutation corresponding to at least one substitution, insertion or deletion that does not significantly affect the suitable biological activities or
5 functions of said GLP-1 agonist or mimetibody.

[140] In one embodiment, the P amino acid sequence, or portion thereof, has about 90-100% identity (i.e., 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or any range or value therein) to the corresponding amino acid sequence of the corresponding portion of at least one of SEQ ID NOS 1 and 6 Preferably, 90-100% amino acid identity (i.e , 90,
10 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or any range or value therein) is determined using a suitable computer algorithm, as known in the art

[141] Mimetibodies or specified portions or variants of the present invention can comprise any number of contiguous amino acid residues from a GLP-1 agonist or mimetibody or specified portion or variant of the present invention, wherein that number
15 is selected from the group of integers consisting of from 10-100% of the number of contiguous residues in a GLP-1 agonist or mimetibody Optionally, this subsequence of contiguous amino acids is at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250 or more amino
20 acids in length, or any range or value therein Further, the number of such subsequences can be any integer selected from the group consisting of from 1 to 20, such as at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more

[142] As those of skill will appreciate, the present invention includes at least one biologically active GLP-1 agonist or mimetibody or specified portion or variant of the
25 present invention Biologically active mimetibodies or specified portions or variants have a specific activity at least 20%, 30%, or 40%, and preferably at least 50%, 60%, or 70%, and most preferably at least 80%, 90%, or 95%-1000% of that of the native (non-synthetic), endogenous or related and known inserted or fused protein or specified portion or variant Methods of assaying and quantifying measures of enzymatic activity
30 and substrate specificity are well known to those of skill in the art

[143] In another aspect, the invention relates to human mimetibodies and ligand-binding fragments, as described herein, which are modified by the covalent attachment of an organic moiety. Such modification can produce a GLP-1 agonist or mimetibody or ligand-binding fragment with improved pharmacokinetic properties (e.g., increased *in vivo* serum half-life). The organic moiety can be a linear or branched hydrophilic polymeric group, fatty acid group, or fatty acid ester group. In particular embodiments, the hydrophilic polymeric group can have a molecular weight of about 800 to about 120,000 Daltons and can be a polyalkane glycol (e.g., polyethylene glycol (PEG), polypropylene glycol (PPG)), carbohydrate polymer, amino acid polymer or polyvinyl pyrrolidone, and the fatty acid or fatty acid ester group can comprise from about eight to about forty carbon atoms.

[144] The modified mimetibodies and ligand-binding fragments of the invention can comprise one or more organic moieties that are covalently bonded, directly or indirectly, to the GLP-1 agonist or mimetibody or specified portion or variant. Each organic moiety that is bonded to a GLP-1 agonist or mimetibody or ligand-binding fragment of the invention can independently be a hydrophilic polymeric group, a fatty acid group or a fatty acid ester group. As used herein, the term "fatty acid" encompasses monocarboxylic acids and dicarboxylic acids. A "hydrophilic polymeric group," as the term is used herein, refers to an organic polymer that is more soluble in water than in octane. For example, polylysine is more soluble in water than in octane. Thus, a GLP-1 agonist or mimetibody modified by the covalent attachment of polylysine is encompassed by the invention. Hydrophilic polymers suitable for modifying mimetibodies of the invention can be linear or branched and include, for example, polyalkane glycols (e.g., PEG, monomethoxy-polyethylene glycol (mPEG), PPG and the like), carbohydrates (e.g., dextran, cellulose, oligosaccharides, polysaccharides and the like), polymers of hydrophilic amino acids (e.g., polylysine, polyarginine, polyaspartate and the like), polyalkane oxides (e.g., polyethylene oxide, polypropylene oxide and the like) and polyvinyl pyrrolidone. Preferably, the hydrophilic polymer that modifies the GLP-1 agonist or mimetibody of the invention has a molecular weight of about 800 to about 150,000 Daltons as a separate molecular entity. For example, PEG₂₅₀₀, PEG₅₀₀₀, PEG₇₅₀₀, PEG₉₀₀₀, PEG₁₀₀₀₀, PEG₁₂₅₀₀, PEG₁₅₀₀₀, and PEG_{20,000}, wherein the subscript is the average molecular weight of the polymer in Daltons, can be used.

[145] The hydrophilic polymeric group can be substituted with one to about six alkyl, fatty acid or fatty acid ester groups. Hydrophilic polymers that are substituted with a fatty acid or fatty acid ester group can be prepared by employing suitable methods. For example, a polymer comprising an amine group can be coupled to a carboxylate of the fatty acid or fatty acid ester, and an activated carboxylate (e.g., activated with N,N-carbonyl diimidazole) on a fatty acid or fatty acid ester can be coupled to a hydroxyl group on a polymer.

[146] Fatty acids and fatty acid esters suitable for modifying mimetibodies of the invention can be saturated or can contain one or more units of unsaturation. Fatty acids that are suitable for modifying mimetibodies of the invention include, for example, n-dodecanoate (C₁₂, laurate), n-tetradecanoate (C₁₄, myristate), n-octadecanoate (C₁₈, stearate), n-eicosanoate (C₂₀, arachidate), n-docosanoate (C₂₂, behenate), n-triacontanoate (C₃₀), n-tetracontanoate (C₄₀), *cis*- Δ^9 -octadecanoate (C₁₈, oleate), all *cis*- $\Delta^{5,8,11,14}$ -eicosatetraenoate (C₂₀, arachidonate), octanedioic acid, tetradecanedioic acid, octadecanedioic acid, docosanedioic acid, and the like. Suitable fatty acid esters include monoesters of dicarboxylic acids that comprise a linear or branched lower alkyl group. The lower alkyl group can comprise from one to about twelve, preferably one to about six, carbon atoms.

[147] The modified human mimetibodies and ligand-binding fragments can be prepared using suitable methods, such as by reaction with one or more modifying agents. A "modifying agent" as the term is used herein, refers to a suitable organic group (e.g., hydrophilic polymer, a fatty acid, a fatty acid ester) that comprises an activating group. An "activating group" is a chemical moiety or functional group that can, under appropriate conditions, react with a second chemical group thereby forming a covalent bond between the modifying agent and the second chemical group. For example, amine-reactive activating groups include electrophilic groups such as tosylate, mesylate, halo (chloro, bromo, fluoro, iodo), N-hydroxysuccinimidyl esters (NHS), and the like. Activating groups that can react with thiols include, for example, maleimide, iodoacetyl, acryloyl, pyridyl disulfides, 5-thiol-2-nitrobenzoic acid thiol (TNB-thiol), and the like. An aldehyde functional group can be coupled to amine- or hydrazide-containing molecules, and an azide group can react with a trivalent phosphorous group to form phosphoramidate or phosphorimide linkages. Suitable methods to introduce

activating groups into molecules are known in the art (see for example, Hermanson, G. T., *Bioconjugate Techniques*, Academic Press San Diego, CA (1996)) An activating group can be bonded directly to the organic group (e.g., hydrophilic polymer, fatty acid, fatty acid ester), or through a linker moiety, for example a divalent C₁-C₁₂ group

5 wherein one or more carbon atoms can be replaced by a heteroatom such as oxygen, nitrogen or sulfur. Suitable linker moieties include, for example, tetraethylene glycol, -(CH₂)₃-, -NH-(CH₂)₆-NH-, -(CH₂)₂-NH- and -CH₂-O-CH₂-CH₂-O-CH₂-CH₂-O-CH₂-NH-

Modifying agents that comprise a linker moiety can be produced, for example, by reacting a mono-Boc-alkyldiamine (e.g., mono-Boc-ethylenediamine, mono-Boc-
10 diaminoheptane) with a fatty acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) to form an amide bond between the free amine and the fatty acid carboxylate. The Boc protecting group can be removed from the product by treatment with trifluoroacetic acid (TFA) to expose a primary amine that can be coupled to another carboxylate as described, or can be reacted with maleic anhydride and the
15 resulting product cyclized to produce an activated maleimido derivative of the fatty acid (See, for example, Thompson, *et al.*, WO 92/16221 the entire teachings of which are incorporated herein by reference.)

[148] The modified mimetibodies of the invention can be produced by reacting an human GLP-1 agonist or mimetibody or ligand-binding fragment with a modifying
20 agent. For example, the organic moieties can be bonded to the GLP-1 agonist or mimetibody in a non-site specific manner by employing an amine-reactive modifying agent, for example, an NHS ester of PEG. Modified human mimetibodies or ligand-binding fragments can also be prepared by reducing disulfide bonds (e.g., intra-chain disulfide bonds) of a GLP-1 agonist or mimetibody or ligand-binding fragment. The
25 reduced GLP-1 agonist or mimetibody or ligand-binding fragment can then be reacted with a thiol-reactive modifying agent to produce the modified GLP-1 agonist or mimetibody of the invention. Modified human mimetibodies and ligand-binding fragments comprising an organic moiety that is bonded to specific sites of a GLP-1 agonist or mimetibody or specified portion or variant of the present invention can be
30 prepared using suitable methods, such as reverse proteolysis (Fisch *et al.*, *Bioconjugate Chem.*, 3 147-153 (1992), Werlen *et al.*, *Bioconjugate Chem.*, 5 411-417 (1994), Kumaran *et al.*, *Protein Sci.* 6(10) 2233-2241 (1997), Itoh *et al.*, *Bioorg. Chem.*, 24(1)

59-68 (1996), Capellas *et al.*, *Biotechnol Bioeng*, 56(4):456-463 (1997)), and the methods described in Hermanson, G T, *Bioconjugate Techniques*, Academic Press: San Diego, CA (1996)

[149] **GLP-1 MIMETIBODY COMPOSITIONS.** The present invention also
 5 provides at least one GLP-1 agonist or mimetibody or specified portion or variant composition comprising at least one, at least two, at least three, at least four, at least five, at least six or more mimetibodies or specified portions or variants thereof, as described herein and/or as known in the art that are provided in a non-naturally occurring composition, mixture or form Such composition percentages are by weight,
 10 volume, concentration, molarity, or molality as liquid or dry solutions, mixtures, suspension, emulsions or colloids, as known in the art or as described herein

[150] Such compositions can comprise 0 00001-99 9999 percent by weight, volume, concentration, molarity, or molality as liquid, gas, or dry solutions, mixtures, suspension, emulsions or colloids, as known in the art or as described herein, on any
 15 range or value therein, such as but not limited to 0 00001, 0 00003, 0.00005, 0 00009, 0 0001, 0 0003, 0.0005, 0.0009, 0 001, 0 003, 0 005, 0 009, 0 01, 0 02, 0 03, 0 05, 0 09, 0 1, 0 2, 0.3, 0 4, 0 5, 0 6, 0 7, 0 8, 0 9, 1 0, 1 1, 1 2, 1 3, 1.4, 1 5, 1 6, 1 7, 1 8, 1 9, 2 0, 2 1, 2.2, 2 3, 2 4, 2 5, 2 6, 2 7, 2.8, 2.9, 3.0, 3 1, 3.2, 3 3, 3 4, 3 5, 3 6, 3 7, 3 8, 3.9, 4 0, 4 3, 4 5, 4 6, 4 7, 4 8, 4 9, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70,
 20 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99 1, 99.2, 99 3, 99 4, 99 5, 99 6, 99 7, 99 8, 99.9 % Such compositions of the present invention thus include but are not limited to 0 00001-100 mg/ml and/or 0 00001-100 mg/g

[151] The present invention provides at least one GLP-1 mimetobody composition,
 25 when administered in a therapeutically effective amount, promotes islet differentiation, increases β -cell mass, and/or increases insulin secretion, and reduces islet and/or insulin producing cell apoptosis, inhibits glucagon secretion, delays gastric emptying, and decreases food intake The present invention also provides at least one GLP-1 mimetibody composition, when administered in a therapeutically effective dose, delays
 30 the onset of or prevents diabetes in individuals at high risk to become diabetic. The present invention also provides at least one GLP-1 mimetibody composition, when administered in a therapeutically effective dose, modulates or treats hyperglycemia due

to a variety of metabolic disorders. The present invention further provides at least one GLP-1 mimetobody composition, when administered in a therapeutically effective dose, modulates or treats at least one pancreas malfunction caused by disease or disorder, including but not limited to, diabetes, pancreatitis, pancreatic tumors, pancreatic cancer,
 5 and associated symptoms.

[152] The GLP-1 mimitibody composition can optionally further comprise an effective amount of at least one compound or protein selected from at least one of a diabetes drug, an insulin metabolism related drug, a glucose metabolism related drug, an anti-infective drug, a cardiovascular (CV) system drug, a central nervous system (CNS)
 10 drug, an autonomic nervous system (ANS) drug, a respiratory tract drug, a gastrointestinal (GI) tract drug, a hormonal drug, a drug for fluid or electrolyte balance, a hematologic drug, an antineoplastic, an immunomodulation drug, an ophthalmic, otic or nasal drug, a topical drug, a nutritional drug or the like. Such drugs are well known in the art, including formulations, indications, dosing and administration for each presented
 15 herein (see e.g., Nursing 2001 Handbook of Drugs, 21st edition, Springhouse Corp., Springhouse, PA, 2001, Health Professional's Drug Guide 2001, ed., Shannon, Wilson, Stang, Prentice-Hall, Inc, Upper Saddle River, NJ, Pharmacotherapy Handbook, Wells et al., ed., Appleton & Lange, Stamford, CT, each entirely incorporated herein by reference)

[153] The diabetes related drug can be at least one of glitazones, insulin and derivatives, sulfonylureas, meglitinides, biguanides, alpha-glucosidase inhibitors, protein tyrosine phosphatase-1B, glycogen synthase kinase 3, gluconeogenesis inhibitors, pyruvate dehydrogenase kinase (PDH) inhibitors, lipolysis inhibitors, fat
 20 oxidation inhibitors, carnitine palmitoyltransferase I and/or II inhibitors, beta-3 adrenoceptor agonists, sodium and glucose cotransporter (SGLT) inhibitors, or compounds that act on one or more of at least one of autoimmune suppression, immune regulation, activation, proliferation, migration and/or suppressor cell function of T-cells, inhibition of T cell receptor/peptide/MHC-II interaction, Induction of T cell anergy, deletion of autoreactive T cells, reduction of trafficking across blood brain barrier,
 25 alteration of balance of pro-inflammatory (Th1) and immunomodulatory (Th2) cytokines, inhibition of matrix metalloprotease inhibitors, neuroprotection, reduction of gliosis, promotion of re-myelination

[154] The anti-infective drug can be at least one selected from amebicides or antiprotozoals, anthelmintics, antifungals, antimalarials, antituberculosics or antileprotics, aminoglycosides, penicillins, cephalosporins, tetracyclines, sulfonamides, fluoroquinolones, antivirals, macrolide anti-infectives and miscellaneous anti-infectives.

5 The CV drug can be at least one selected from inotropics, antiarrhythmics, antianginals, antihypertensives, antilipemics and miscellaneous cardiovascular drugs. The CNS drug can be at least one selected from nonnarcotic analgesics or at least one selected from antipyretics, nonsteroidal anti-inflammatory drugs, narcotic or opioid analgesics, sedative-hypnotics, anticonvulsants, antidepressants, antianxiety drugs, antipsychotics,

10 central nervous system stimulants, antiparkinsonians and miscellaneous central nervous system drugs. The ANS drug can be at least one selected from cholinergics (parasympathomimetics), anticholinergics, adrenergics (sympathomimetics), adrenergic blockers (sympatholytics), skeletal muscle relaxants and neuromuscular blockers. The respiratory tract drug can be at least one selected from antihistamines, bronchodilators,

15 expectorants or antitussives and miscellaneous respiratory drugs. The GI tract drug can be at least one selected from antacids, adsorbents, antiflatulents, digestive enzymes, gallstone solubilizers, antidiarrheals, laxatives, antiemetics and antiulcer drugs. The hormonal drug can be at least one selected from corticosteroids, androgens, anabolic steroids, estrogens, progestins, gonadotropins, antidiabetic drugs, at least one glucagon,

20 thyroid hormones, thyroid hormone antagonists, pituitary hormones and parathyroid-like drugs. The drug for fluid and electrolyte balance can be at least one selected from diuretics, electrolytes, replacement solutions, acidifiers and alkalizers. The hematologic drug can be at least one selected from hematinics, anticoagulants, blood derivatives and thrombolytic enzymes. The antineoplastics can be at least one selected

25 from alkylating drugs, antimetabolites, antibiotic antineoplastics, antineoplastics that alter hormone balance and miscellaneous antineoplastics. The immunomodulation drug can be at least one selected from immunosuppressants, vaccines, toxoids, antitoxins, antivenoms, immune serums and biological response modifiers. The ophthalmic, otic, and nasal drugs can be at least one selected from ophthalmic anti-infectives, ophthalmic

30 anti-inflammatories, miotics, mydriatics, ophthalmic vasoconstrictors and miscellaneous ophthalmics, otics, nasal drugs. The topical drug can be at least one selected from local anti-infectives, scabicides, pediculicides and topical corticosteroids. The nutritional

drug can be at least one selected from vitamins, minerals and calorics See, e.g., contents of *Nursing 2001 Drug Handbook*, *supra*

- [155] The at least one amebicide or antiprotozoal can be at least one selected from atovaquone, chloroquine hydrochloride, chloroquine phosphate, metronidazole, metronidazole hydrochloride and pentamidine isethionate The at least one anthelmintic can be at least one selected from mebendazole, pyrantel pamoate and thiabendazole The at least one antifungal can be at least one selected from amphotericin B, amphotericin B cholesteryl sulfate complex, amphotericin B lipid complex, amphotericin B liposomal, fluconazole, flucytosine, griseofulvin microsize, griseofulvin ultramicrosize, itraconazole, ketoconazole, nystatin and terbinafine hydrochloride The at least one antimalarial can be at least one selected from chloroquine hydrochloride, chloroquine phosphate, doxycycline, hydroxychloroquine sulfate, mefloquine hydrochloride, primaquine phosphate, pyrimethamine and pyrimethamine with sulfadoxine The at least one antituberculosic or antileprotic can be at least one selected from clofazimine, cycloserine, dapsone, ethambutol hydrochloride, isoniazid, pyrazinamide, rifabutin, rifampin, rifapentine and streptomycin sulfate The at least one aminoglycoside can be at least one selected from amikacin sulfate, gentamicin sulfate, neomycin sulfate, streptomycin sulfate and tobramycin sulfate The at least one penicillin can be at least one selected from amoxicillin/clavulanate potassium, amoxicillin trihydrate, ampicillin, ampicillin sodium, ampicillin trihydrate, ampicillin sodium/sulbactam sodium, cloxacillin sodium, dicloxacillin sodium, mezlocillin sodium, nafcillin sodium, oxacillin sodium, penicillin G benzathine, penicillin G potassium, penicillin G procaine, penicillin G sodium, penicillin V potassium, piperacillin sodium, piperacillin sodium/tazobactam sodium, ticarcillin disodium and ticarcillin disodium/clavulanate potassium The at least one cephalosporin can be at least one selected from at least one of cefaclor, cefadroxil, cefazolin sodium, cefdinir, cefepime hydrochloride, cefixime, cefmetazole sodium, cefonicid sodium, cefoperazone sodium, cefotaxime sodium, cefotetan disodium, cefoxitin sodium, cefpodoxime proxetil, cefprozil, ceftazidime, ceftibuten, ceftizoxime sodium, ceftriaxone sodium, cefuroxime axetil, cefuroxime sodium, cephalexin hydrochloride, cephalexin monohydrate, cephradine, loracarbef The at least one tetracycline can be at least one selected from demeclocycline hydrochloride, doxycycline calcium, doxycycline hyclate,

- doxycycline hydrochloride, doxycycline monohydrate, minocycline hydrochloride, tetracycline hydrochloride The at least one sulfonamide can be at least one selected from co-trimoxazole, sulfadiazine, sulfamethoxazole, sulfisoxazole, sulfisoxazole acetyl The at least one fluoroquinolone can be at least one selected from alatrofloxacin mesylate, ciprofloxacin, enoxacin, levofloxacin, lomefloxacin hydrochloride, nalidixic acid, norfloxacin, ofloxacin, sparfloxacin, trovafloxacin mesylate The at least one fluoroquinolone can be at least one selected from alatrofloxacin mesylate, ciprofloxacin, enoxacin, levofloxacin, lomefloxacin hydrochloride, nalidixic acid, norfloxacin, ofloxacin, sparfloxacin, trovafloxacin mesylate The at least one antiviral can be at least one selected from abacavir sulfate, acyclovir sodium, amantadine hydrochloride, amprenavir, cidofovir, delavirdine mesylate, didanosine, efavirenz, famciclovir, fomivirsen sodium, foscarnet sodium, ganciclovir, indinavir sulfate, lamivudine, lamivudine/zidovudine, nelfinavir mesylate, nevirapine, oseltamivir phosphate, ribavirin, rimantadine hydrochloride, ritonavir, saquinavir, saquinavir mesylate, stavudine, valacyclovir hydrochloride, zalcitabine, zanamivir, zidovudine The at least one macroline anti-infective can be at least one selected from azithromycin, clarithromycin, dirithromycin, erythromycin base, erythromycin estolate, erythromycin ethylsuccinate, erythromycin lactobionate, erythromycin stearate The at least one miscellaneous anti-infective can be at least one selected from aztreonam, bacitracin, chloramphenicol sodium succinate, clindamycin hydrochloride, clindamycin palmitate hydrochloride, clindamycin phosphate, imipenem and cilastatin sodium, meropenem, nitrofurantoin macrocrystals, nitrofurantoin microcrystals, quinupristin/dalfopristin, spectinomycin hydrochloride, trimethoprim, vancomycin hydrochloride (See, e.g., pp 24-214 of *Nursing 2001 Drug Handbook*)
- [156] The at least one inotropic can be at least one selected from amrinone lactate, digoxin, milrinone lactate The at least one antiarrhythmic can be at least one selected from adenosine, amiodarone hydrochloride, atropine sulfate, bretylium tosylate, diltiazem hydrochloride, disopyramide, disopyramide phosphate, esmolol hydrochloride, flecainide acetate, ibutilide fumarate, lidocaine hydrochloride, mexiletine hydrochloride, moricizine hydrochloride, phenytoin, phenytoin sodium, procainamide hydrochloride, propafenone hydrochloride, propranolol hydrochloride, quinidine bisulfate, quinidine gluconate, quinidine polygalacturonate, quinidine sulfate, sotalol,

tocamide hydrochloride, verapamil hydrochloride The at least one antianginal can be at
 least one selected from amlodipine besylate, amyl nitrite, bepridil hydrochloride,
 diltiazem hydrochloride, isosorbide dinitrate, isosorbide mononitrate, nadolol,
 nicardipine hydrochloride, nifedipine, nitroglycerin, propranolol hydrochloride,
 5 verapamil, verapamil hydrochloride The at least one antihypertensive can be at least
 one selected from acebutolol hydrochloride, amlodipine besylate, atenolol, benazepril
 hydrochloride, betaxolol hydrochloride, bisoprolol fumarate, candesartan cilexetil,
 captopril, carteolol hydrochloride, carvedilol, clonidine, clonidine hydrochloride,
 diazoxide, diltiazem hydrochloride, doxazosin mesylate, enalaprilat, enalapril maleate,
 10 eprosartan mesylate, felodipine, fenoldopam mesylate, fosinopril sodium, guanabenz
 acetate, guanadrel sulfate, guanfacine hydrochloride, hydralazine hydrochloride,
 irbesartan, isradipine, labetalol hydrochloride, lisinopril, losartan potassium, methyldopa,
 methyldopate hydrochloride, metoprolol succinate, metoprolol tartrate, minoxidil,
 moexipril hydrochloride, nadolol, nicardipine hydrochloride, nifedipine, nisoldipine,
 15 nitroprusside sodium, penbutolol sulfate, perindopril erbumine, phentolamine mesylate,
 pindolol, prazosin hydrochloride, propranolol hydrochloride, quinapril hydrochloride,
 ramipril, telmisartan, terazosin hydrochloride, timolol maleate,trandolapril, valsartan,
 verapamil hydrochloride The at least one antilipemic can be at least one selected from
 atorvastatin calcium, cerivastatin sodium, cholestyramine, colestipol hydrochloride,
 20 fenofibrate (micronized), fluvastatin sodium, gemfibrozil, lovastatin, niacin, pravastatin
 sodium, simvastatin The at least one miscellaneous CV drug can be at least one
 selected from abciximab, alprostadil, arbutamine hydrochloride, cilostazol, clopidogrel
 bisulfate, dipyridamole, eptifibatide, midodrine hydrochloride, pentoxifylline,
 ticlopidine hydrochloride, tirofiban hydrochloride (See, e g , pp 215-336 of *Nursing*
 25 *2001 Drug Handbook*)

[157] The at least one nonnarcotic analgesic or antipyretic can be at least one selected
 from acetaminophen, aspirin, choline magnesium trisalicylate, diflunisal, magnesium
 salicylate The at least one nonsteroidal anti-inflammatory drug can be at least one
 selected from celecoxib, diclofenac potassium, diclofenac sodium, etodolac, fenoprofen
 30 calcium, flurbiprofen, ibuprofen, indomethacin, indomethacin sodium trihydrate,
 ketoprofen, ketorolac tromethamine, nabumetone, naproxen, naproxen sodium,
 oxaprozin, piroxicam, rofecoxib, sulindac The at least one narcotic or opioid analgesic

can be at least one selected from alfentanil hydrochloride, buprenorphine hydrochloride, butorphanol tartrate, codeine phosphate, codeine sulfate, fentanyl citrate, fentanyl transdermal system, fentanyl transmucosal, hydromorphone hydrochloride, meperidine hydrochloride, methadone hydrochloride, morphine hydrochloride, morphine sulfate, morphine tartrate, nalbuphine hydrochloride, oxycodone hydrochloride, oxycodone pectinate, oxymorphone hydrochloride, pentazocine hydrochloride, pentazocine hydrochloride and naloxone hydrochloride, pentazocine lactate, propoxyphene hydrochloride, propoxyphene napsylate, remifentanil hydrochloride, sufentanil citrate, tramadol hydrochloride. The at least one sedative-hypnotic can be at least one selected from chloral hydrate, estazolam, flurazepam hydrochloride, pentobarbital, pentobarbital sodium, phenobarbital sodium, secobarbital sodium, temazepam, triazolam, zaleplon, zolpidem tartrate. The at least one anticonvulsant can be at least one selected from acetazolamide sodium, carbamazepine, clonazepam, clorazepate dipotassium, diazepam, divalproex sodium, ethosuximide, fosphenytoin sodium, gabapentin, lamotrigine, magnesium sulfate, phenobarbital, phenobarbital sodium, phenytoin, phenytoin sodium, phenytoin sodium (extended), primidone, tiagabine hydrochloride, topiramate, valproate sodium, valproic acid. The at least one antidepressant can be at least one selected from amitriptyline hydrochloride, amitriptyline pamoate, amoxapine, bupropion hydrochloride, citalopram hydrobromide, clomipramine hydrochloride, desipramine hydrochloride, doxepin hydrochloride, fluoxetine hydrochloride, imipramine hydrochloride, imipramine pamoate, mirtazapine, nefazodone hydrochloride, nortriptyline hydrochloride, paroxetine hydrochloride, phenelzine sulfate, sertraline hydrochloride, tranylcypromine sulfate, trimipramine maleate, venlafaxine hydrochloride. The at least one antianxiety drug can be at least one selected from alprazolam, buspirone hydrochloride, chlordiazepoxide, chlordiazepoxide hydrochloride, clorazepate dipotassium, diazepam, doxepin hydrochloride, hydroxyzine embonate, hydroxyzine hydrochloride, hydroxyzine pamoate, lorazepam, mephrobamate, midazolam hydrochloride, oxazepam. The at least one antipsychotic drug can be at least one selected from chlorpromazine hydrochloride, clozapine, fluphenazine decanoate, fluphenazine enanthate, fluphenazine hydrochloride, haloperidol, haloperidol decanoate, haloperidol lactate, loxapine hydrochloride, loxapine succinate, mesoridazine besylate, molindone hydrochloride, olanzapine, perphenazine,

pimozide, prochlorperazine, quetiapine fumarate, risperidone, thioridazine hydrochloride, thiothixene, thiothixene hydrochloride, trifluoperazine hydrochloride. The at least one central nervous system stimulant can be at least one selected from amphetamine sulfate, caffeine, dextroamphetamine sulfate, doxapram hydrochloride, 5 methamphetamine hydrochloride, methylphenidate hydrochloride, modafinil, pemoline, phentermine hydrochloride. The at least one antiparkinsonian can be at least one selected from amantadine hydrochloride, benzotropine mesylate, biperiden hydrochloride, biperiden lactate, bromocriptine mesylate, carbidopa-levodopa, entacapone, levodopa, pergolide mesylate, pramipexole dihydrochloride, ropinirole 10 hydrochloride, selegiline hydrochloride, tolcapone, trihexyphenidyl hydrochloride. The at least one miscellaneous central nervous system drug can be at least one selected from bupropion hydrochloride, donepezil hydrochloride, droperidol, fluvoxamine maleate, lithium carbonate, lithium citrate, naratriptan hydrochloride, nicotine polacrilex, nicotine transdermal system, propofol, rizatriptan benzoate, sibutramine hydrochloride 15 monohydrate, sumatriptan succinate, tacrine hydrochloride, zolmitriptan (See, e.g., pp 337-530 of *Nursing 2001 Drug Handbook*)

[158] The at least one cholinergic (e.g., parasympathomimetic) can be at least one selected from bethanechol chloride, edrophonium chloride, neostigmine bromide, neostigmine methylsulfate, physostigmine salicylate, pyridostigmine bromide. The at 20 least one anticholinergics can be at least one selected from atropine sulfate, dicyclomine hydrochloride, glycopyrrolate, hyoscyamine, hyoscyamine sulfate, propantheline bromide, scopolamine, scopolamine butylbromide, scopolamine hydrobromide. The at least one adrenergics (sympathomimetics) can be at least one selected from dobutamine hydrochloride, dopamine hydrochloride, metaraminol bitartrate, norepinephrine 25 bitartrate, phenylephrine hydrochloride, pseudoephedrine hydrochloride, pseudoephedrine sulfate. The at least one adrenergic blocker (sympatholytic) can be at least one selected from dihydroergotamine mesylate, ergotamine tartrate, methysergide maleate, propranolol hydrochloride. The at least one skeletal muscle relaxant can be at least one selected from baclofen, carisoprodol, chlorzoxazone, cyclobenzaprine 30 hydrochloride, dantrolene sodium, methocarbamol, tizanidine hydrochloride. The at least one neuromuscular blockers can be at least one selected from atracurium besylate, cisatracurium besylate, doxacurium chloride, mivacurium chloride, pancuronium

bromide, pipecuronium bromide, rapacuronium bromide, rocuronium bromide, succinylcholine chloride, tubocurarine chloride, vecuronium bromide (See, e.g., pp 531-84 of *Nursing 2001 Drug Handbook*)

- [159] The at least one antihistamine can be at least one selected from
- 5 brompheniramine maleate, cetirizine hydrochloride, chlorpheniramine maleate, clemastine fumarate, cyproheptadine hydrochloride, diphenhydramine hydrochloride, fexofenadine hydrochloride, loratadine, promethazine hydrochloride, promethazine theoclate, triprolidine hydrochloride. The at least one bronchodilators can be at least one selected from albuterol, albuterol sulfate, aminophylline, atropine sulfate, ephedrine
 - 10 sulfate, epinephrine, epinephrine bitartrate, epinephrine hydrochloride, ipratropium bromide, isoproterenol, isoproterenol hydrochloride, isoproterenol sulfate, levalbuterol hydrochloride, metaproterenol sulfate, oxtriphylline, pirbuterol acetate, salmeterol xinafoate, terbutaline sulfate, theophylline The at least one expectorants or antitussives can be at least one selected from benzonatate, codeine phosphate, codeine sulfate,
 - 15 dextromethorphan hydrobromide, diphenhydramine hydrochloride, guaifenesin, hydromorphone hydrochloride The at least one miscellaneous respiratory drug can be at least one selected from acetylcysteine, beclomethasone dipropionate, beractant, budesonide, calfactant, cromolyn sodium, dornase alfa, epoprostenol sodium, flunisolide, fluticasone propionate, montelukast sodium, nedocromil sodium,
 - 20 palivizumab, triamcinolone acetonide, zafirlukast, zileuton. (See, e.g., pp 585-642 of *Nursing 2001 Drug Handbook*)

- [160] The at least one antacid, adsorbents, or antiflatulents can be at least one selected from aluminum carbonate, aluminum hydroxide, calcium carbonate, magaldrate, magnesium hydroxide, magnesium oxide, simethicone, and sodium bicarbonate The at
- 25 least one digestive enzyme or gallstone solubilizers can be at least one selected from pancreatin, pancrelipase, and ursodiol The at least one antidiarrheal can be at least one selected from attapulgit, bismuth subsalicylate, calcium polycarbophil, diphenoxylate hydrochloride or atropine sulfate, loperamide, octreotide acetate, opium tincture, opium tincture (camphorated) The at least one laxative can be at least one selected from
 - 30 bisocodyl, calcium polycarbophil, cascara sagrada, cascara sagrada aromatic fluidextract, cascara sagrada fluidextract, castor oil, docusate calcium, docusate sodium, glycerin, lactulose, magnesium citrate, magnesium hydroxide, magnesium sulfate,

methylcellulose, mineral oil, polyethylene glycol or electrolyte solution, psyllium,
 senna, sodium phosphates. The at least one antiemetic can be at least one selected from
 chlorpromazine hydrochloride, dimenhydrinate, dolasetron mesylate, dronabinol,
 granisetron hydrochloride, meclizine hydrochloride, metoclopramide hydrochloride,
 5 ondansetron hydrochloride, perphenazine, prochlorperazine, prochlorperazine edisylate,
 prochlorperazine maleate, promethazine hydrochloride, scopolamine, thiethylperazine
 maleate, trimethobenzamide hydrochloride The at least one antiulcer drug can be at
 least one selected from cimetidine, cimetidine hydrochloride, famotidine, lansoprazole,
 misoprostol, nizatidine, omeprazole, rabeprozole sodium, ranitidine bismuth citrate,
 10 ranitidine hydrochloride, sucralfate (See, e g., pp. 643-95 of *Nursing 2001 Drug
 Handbook*) The at least one corticosteroids can be at least one selected from
 betamethasone, betamethasone acetate or betamethasone sodium phosphate,
 betamethasone sodium phosphate, cortisone acetate, dexamethasone, dexamethasone
 acetate, dexamethasone sodium phosphate, fludrocortisone acetate, hydrocortisone,
 15 hydrocortisone acetate, hydrocortisone cypionate, hydrocortisone sodium phosphate,
 hydrocortisone sodium succinate, methylprednisolone, methylprednisolone acetate,
 methylprednisolone sodium succinate, prednisolone, prednisolone acetate, prednisolone
 sodium phosphate, prednisolone tebutate, prednisone, triamcinolone, triamcinolone
 acetonide, triamcinolone diacetate The at least one androgen or anabolic steroids can
 20 be at least one selected from danazol, fluoxymesterone, methyltestosterone, nandrolone
 decanoate, nandrolone phenpropionate, testosterone, testosterone cypionate, testosterone
 enanthate, testosterone propionate, testosterone transdermal system The at least one
 estrogen or progestin can be at least one selected from esterified estrogens, estradiol,
 estradiol cypionate, estradiol/norethindrone acetate transdermal system, estradiol
 25 valerate, estrogens (conjugated), estropipate, ethinyl estradiol, ethinyl estradiol and
 desogestrel, ethinyl estradiol and ethynodiol diacetate, ethinyl estradiol and desogestrel,
 ethinyl estradiol and ethynodiol diacetate, ethinyl estradiol and levonorgestrel, ethinyl
 estradiol and norethindrone, ethinyl estradiol and norethindrone acetate, ethinyl estradiol
 and norgestimate, ethinyl estradiol and norgestrel, ethinyl estradiol and norethindrone
 30 and acetate and ferrous fumarate, levonorgestrel, medroxyprogesterone acetate,
 mestranol and norethindron, norethindrone, norethindrone acetate, norgestrel,
 progesterone The at least one gonadotropin can be at least one selected from

- ganirelix acetate, gonadoreline acetate, histrelin acetate, menotropins. The at least one antidiabetic or glucagon can be at least one selected from acarbose, chlorpropamide, glimepiride, glipizide, glucagon, glyburide, insulins, metformin hydrochloride, miglitol, pioglitazone hydrochloride, repaglinide, rosiglitazone maleate, troglitazone. The at least one thyroid hormone can be at least one selected from levothyroxine sodium, liothyronine sodium, liotrix, thyroid. The at least one thyroid hormone antagonist can be at least one selected from methimazole, potassium iodide, potassium iodide (saturated solution), propylthiouracil, radioactive iodine (sodium iodide ¹³¹I), strong iodine solution. The at least one pituitary hormone can be at least one selected from corticotropin, cosyntropin, desmopressin acetate, leuprolide acetate, repository corticotropin, somatrem, somatropin, vasopressin. The at least one parathyroid-like drug can be at least one selected from calcifediol, calcitonin (human), calcitonin (salmon), calcitriol, dihydrotachysterol, etidronate disodium. (See, e.g., pp. 696-796 of *Nursing 2001 Drug Handbook*)
- 15 [161] The at least one diuretic can be at least one selected from acetazolamide, acetazolamide sodium, amiloride hydrochloride, bumetanide, chlorthalidone, ethacrynate sodium, ethacrynic acid, furosemide, hydrochlorothiazide, indapamide, mannitol, metolazone, spironolactone, torsemide, triamterene, urea. The at least one electrolyte or replacement solution can be at least one selected from calcium acetate, calcium carbonate, calcium chloride, calcium citrate, calcium gluconate, calcium gluceptate, calcium gluconate, calcium lactate, calcium phosphate (dibasic), calcium phosphate (tribasic), dextran (high-molecular-weight), dextran (low-molecular-weight), hetastarch, magnesium chloride, magnesium sulfate, potassium acetate, potassium bicarbonate, potassium chloride, potassium gluconate, Ringer's injection, Ringer's injection (lactated), sodium chloride. The at least one acidifier or alkalizer can be at least one selected from sodium bicarbonate, sodium lactate, tromethamine. (See, e.g., pp. 797-833 of *Nursing 2001 Drug Handbook*)
- 20 [162] The at least one hematonic can be at least one selected from ferrous fumarate, ferrous gluconate, ferrous sulfate, ferrous sulfate (dried), iron dextran, iron sorbitol, polysaccharide-iron complex, sodium ferric gluconate complex. The at least one anticoagulant can be at least one selected from ardeparin sodium, dalteparin sodium, danaparoid sodium, enoxaparin sodium, heparin calcium, heparin sodium, warfarin
- 25 30

sodium The at least one blood derivative can be at least one selected from albumin 5%, albumin 25%, antihemophilic factor, anti-inhibitor coagulant complex, antithrombin III (human), factor IX (human), factor IX complex, plasma protein fractions The at least one thrombolytic enzyme can be at least one selected from alteplase, anistreplase, reteplase (recombinant), streptokinase, urokinase (See, e.g., pp. 834-66 of *Nursing 2001 Drug Handbook*)

[163] The at least one alkylating drug can be at least one selected from busulfan, carboplatin, carmustine, chlorambucil, cisplatin, cyclophosphamide, ifosfamide, lomustine, mechlorethamine hydrochloride, melphalan, melphalan hydrochloride, streptozocin, temozolomide, thiotepe. The at least one antimetabolite can be at least one selected from capecitabine, cladribine, cytarabine, floxuridine, fludarabine phosphate, fluorouracil, hydroxyurea, mercaptopurine, methotrexate, methotrexate sodium, thioguanine. The at least one antibiotic antineoplastic can be at least one selected from bleomycin sulfate, dactinomycin, daunorubicin citrate liposomal, daunorubicin hydrochloride, doxorubicin hydrochloride, doxorubicin hydrochloride liposomal, epirubicin hydrochloride, idarubicin hydrochloride, mitomycin, pentostatin, plicamycin, valrubicin The at least one antineoplastics that alter hormone balance can be at least one selected from anastrozole, bicalutamide, estramustine phosphate sodium, exemestane, flutamide, goserelin acetate, letrozole, leuprolide acetate, megestrol acetate, nilutamide, tamoxifen citrate, testolactone, toremifene citrate. The at least one miscellaneous antineoplastic can be at least one selected from asparaginase, bacillus Calmette-Guerin (BCG) (live intravesical), dacarbazine, docetaxel, etoposide, etoposide phosphate, gemcitabine hydrochloride, irinotecan hydrochloride, mitotane, mitoxantrone hydrochloride, paclitaxel, pegaspargase, porfimer sodium, procarbazine hydrochloride, rituximab, teniposide, topotecan hydrochloride, trastuzumab, tretinoin, vinblastine sulfate, vincristine sulfate, vinorelbine tartrate (See, e.g., pp. 867-963 of *Nursing 2001 Drug Handbook*)

[164] The at least one immunosuppressant can be at least one selected from azathioprine, basiliximab, cyclosporine, daclizumab, lymphocyte immune globulin, muromonab-CD3, mycophenolate mofetil, mycophenolate mofetil hydrochloride, sirolimus, tacrolimus The at least one vaccine or toxoid can be at least one selected from BCG vaccine, cholera vaccine, diphtheria and tetanus toxoids (adsorbed),

- diphtheria and tetanus toxoids and acellular pertussis vaccine adsorbed, diphtheria and tetanus toxoids and whole-cell pertussis vaccine, *Haemophilus b* conjugate vaccines, hepatitis A vaccine (inactivated), hepatitis B vaccine (recombinant), influenza virus vaccine 1999-2000 trivalent types A & B (purified surface antigen), influenza virus vaccine 1999-2000 trivalent types A & B (subvirion or purified subvirion), influenza virus vaccine 1999-2000 trivalent types A & B (whole virion), Japanese encephalitis virus vaccine (inactivated), Lyme disease vaccine (recombinant OspA), measles and mumps and rubella virus vaccine (live), measles and mumps and rubella virus vaccine (live attenuated), measles virus vaccine (live attenuated), meningococcal polysaccharide vaccine, mumps virus vaccine (live), plague vaccine, pneumococcal vaccine (polyvalent), poliovirus vaccine (inactivated), poliovirus vaccine (live, oral, trivalent), rabies vaccine (adsorbed), rabies vaccine (human diploid cell), rubella and mumps virus vaccine (live), rubella virus vaccine (live, attenuated), tetanus toxoid (adsorbed), tetanus toxoid (fluid), typhoid vaccine (oral), typhoid vaccine (parenteral), typhoid Vi polysaccharide vaccine, varicella virus vaccine, yellow fever vaccine. The at least one antitoxin or antivenin can be at least one selected from black widow spider antivenin, Crotalidae antivenom (polyvalent), diphtheria antitoxin (equine), *Micrurus fulvius* antivenin) The at least one immune serum can be at least one selected from cytomegalovirus immune globulin (intravenous), hepatitis B immune globulin (human), immune globulin intramuscular, immune globulin intravenous, rabies immune globulin (human), respiratory syncytial virus immune globulin intravenous (human), Rh₀(D) immune globulin (human), Rh₀(D) immune globulin intravenous (human), tetanus immune globulin (human), varicella-zoster immune globulin The at least one biological response modifiers can be at least one selected from aldesleukin, GLP-1etin alfa, filgrastim, glatiramer acetate for injection, interferon alfacon-1, interferon alfa-2a (recombinant), interferon alfa-2b (recombinant), interferon beta-1a, interferon beta-1b (recombinant), interferon gamma-1b, levamisole hydrochloride, oprelvekin, sargramostim (See, e g , pp 964-1040 of *Nursing 2001 Drug Handbook*)
- [165] The at least one ophthalmic anti-infectives can be selected from bacitracin, chloramphenicol, ciprofloxacin hydrochloride, erythromycin, gentamicin sulfate, ofloxacin 0.3%, polymyxin B sulfate, sulfacetamide sodium 10%, sulfacetamide sodium 15%, sulfacetamide sodium 30%, tobramycin, vidarabine The at least one ophthalmic

- anti-inflammatories can be at least one selected from dexamethasone, dexamethasone sodium phosphate, diclofenac sodium 0.1%, fluorometholone, flurbiprofen sodium, ketorolac tromethamine, prednisolone acetate (suspension) prednisolone sodium phosphate (solution) The at least one miotic can be at least one selected from
- 5 acetylcholine chloride, carbachol (intraocular), carbachol (topical), echothiophate iodide, pilocarpine, pilocarpine hydrochloride, pilocarpine nitrate. The at least one mydriatic can be at least one selected from atropine sulfate, cyclopentolate hydrochloride, epinephrine hydrochloride, epinephryl borate, homatropine hydrobromide, phenylephrine hydrochloride, scopolamine hydrobromide, tropicamide
- 10 The at least one ophthalmic vasoconstrictors can be at least one selected from naphazoline hydrochloride, oxymetazoline hydrochloride, tetrahydrozoline hydrochloride The at least one miscellaneous ophthalmics can be at least one selected from apraclonidine hydrochloride, betaxolol hydrochloride, brimonidine tartrate, carteolol hydrochloride, dipivefrin hydrochloride, dorzolamide hydrochloride,
- 15 emedastine difumarate, fluorescein sodium, ketotifen fumarate, latanoprost, levobunolol hydrochloride, metipranolol hydrochloride, sodium chloride (hypertonic), timolol maleate. The at least one otic can be at least one selected from boric acid, carbamide peroxide, chloramphenicol, triethanolamine polypeptide oleate-condensate The at least one nasal drug can be at least one selected from beclomethasone dipropionate,
- 20 budesonide, ephedrine sulfate, epinephrine hydrochloride, flunisolide, fluticasone propionate, naphazoline hydrochloride, oxymetazoline hydrochloride, phenylephrine hydrochloride, tetrahydrozoline hydrochloride, triamcinolone acetonide, xylometazoline hydrochloride (See, e.g., pp. 1041-97 of *Nursing 2001 Drug Handbook*)
- [166] The at least one local anti-infectives can be at least one selected from acyclovir,
- 25 amphotericin B, azelaic acid cream, bacitracin, butoconazole nitrate, clindamycin phosphate, clotrimazole, econazole nitrate, erythromycin, gentamicin sulfate, ketoconazole, mafenide acetate, metronidazole (topical), miconazole nitrate, mupirocin, naftifine hydrochloride, neomycin sulfate, nitrofurazone, nystatin, silver sulfadiazine, terbinafine hydrochloride, terconazole, tetracycline hydrochloride, tioconazole,
- 30 tolnaftate The at least one scabicide or pediculicide can be at least one selected from crotamiton, lindane, permethrin, and pyrethrins The at least one topical corticosteroid can be at least one selected from betamethasone dipropionate, betamethasone valerate,

clobetasol propionate, desonide, desoximetasone, dexamethasone, dexamethasone sodium phosphate, diflorasone diacetate, fluocinolone acetonide, fluocinonide, flurandrenolide, fluticasone propionate, halcionide, hydrocortisone, hydrocortisone acetate, hydrocortisone butyrate, hydrocortisone valerate, mometasone furoate,

5 triamcinolone acetonide (See, e.g., pp. 1098-1136 of *Nursing 2001 Drug Handbook*)

[167] The at least one vitamin or mineral can be at least one selected from vitamin A, vitamin B complex, cyanocobalamin, folic acid, hydroxocobalamin, leucovorin calcium, niacin, niacinamide, pyridoxine hydrochloride, riboflavin, thiamine hydrochloride, vitamin C, vitamin D, cholecalciferol, ergocalciferol, vitamin D analogue,

10 doxercalciferol, paricalcitol, vitamin E, vitamin K analogue, phytonadione, sodium fluoride, sodium fluoride (topical), trace elements, chromium, copper, iodine, manganese, selenium, zinc. The at least one caloric can be at least one selected from amino acid infusions (crystalline), amino acid infusions in dextrose, amino acid infusions with electrolytes, amino acid infusions with electrolytes in dextrose, amino acid infusions for hepatic failure, amino acid infusions for high metabolic stress, amino acid infusions for renal failure, dextrose, fat emulsions, medium-chain triglycerides
15 (See, e.g., pp. 1137-63 of *Nursing 2001 Drug Handbook*)

[168] The present invention also provides at least one of any suitable and/or effective amount of a composition or pharmaceutical composition comprising at least one GLP-1 agonist or mimetibody or specified portion or variant, optionally further comprise an effective amount of at least one further compound, protein or composition selected from at least one TNF antagonist (e.g., but not limited to a TNF chemical or protein antagonist, TNF monoclonal or polyclonal antibody or fragment, a soluble TNF receptor (e.g., p55, p70 or p85) or fragment, fusion polypeptides thereof, or a small molecule

25 TNF antagonist, e.g., TNF binding protein I or II (TBP-1 or TBP-II), nerelimonmab, infliximab, entercept, CDP-571, CDP-870, afelimomab, lenercept, and the like), an antirheumatic (e.g., methotrexate, auranofin, aurothioglucose, azathioprine, etanercept, gold sodium thiomalate, hydroxychloroquine sulfate, leflunomide, sulfasalazine), a muscle relaxant, a narcotic, a non-steroid inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial
30 (e.g., aminoglycoside, an antifungal, an antiparasitic, an antiviral, a carbapenem, cephalosporin, a fluroquinolone, a macrolide, a penicillin, a sulfonamide, a tetracycline,

another antimicrobial), an antipsoriatic, a corticosteroid, an anabolic steroid, a diabetes related agent, a mineral, a nutritional, a thyroid agent, a vitamin, a calcium related hormone, an antidiarrheal, an antitussive, an antiemetic, an antiulcer, a laxative, an anticoagulant, an erythropoietin (e.g., epoetin alpha), a filgrastim (e.g., G-CSF, Neupogen), a sargramostim (GM-CSF, Leukine), an immunization, an immunoglobulin, an immunosuppressive (e.g., basiliximab, cyclosporine, daclizumab), a growth hormone, a hormone replacement drug, an estrogen receptor modulator, a mydriatic, a cycloplegic, an alkylating agent, an antimetabolite, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, antimanic agent, an antipsychotic, an anxiolytic, a hypnotic, a sympathomimetic, a stimulant, donepezil, tacrine, an asthma medication, a beta agonist, an inhaled steroid, a leukotriene inhibitor, a methylxanthine, a cromolyn, an epinephrine or analog, dornase alpha (Pulmozyme), a cytokine or a cytokine antagonist. Non-limiting examples of such cytokines include, but are not limited to, any of IL-1 to IL-23. Suitable dosages are well known in the art. See, e.g., Wells et al., eds., Pharmacotherapy Handbook, 2nd Edition, Appleton and Lange, Stamford, CT (2000); PDR Pharmacopoeia, Tarascon Pocket Pharmacopoeia 2000, Deluxe Edition, Tarascon Publishing, Loma Linda, CA (2000), each of which references are entirely incorporated herein by reference.

[169] Such compositions can also include toxin molecules that are associated, bound, co-formulated or co-administered with at least one antibody or polypeptide of the present invention. The toxin can optionally act to selectively kill the pathologic cell or tissue. The pathologic cell can be a cancer or other cell. Such toxins can be, but are not limited to, purified or recombinant toxin or toxin fragment comprising at least one functional cytotoxic domain of toxin, e.g., selected from at least one of ricin, diphtheria toxin, a venom toxin, or a bacterial toxin. The term toxin also includes both endotoxins and exotoxins produced by any naturally occurring, mutant or recombinant bacteria or viruses which may cause any pathological condition in humans and other mammals, including toxin shock, which can result in death. Such toxins may include, but are not limited to, enterotoxigenic *E. coli* heat-labile enterotoxin (LT), heat-stable enterotoxin (ST), *Shigella* cytotoxin, *Aeromonas* enterotoxins, toxic shock syndrome toxin-1 (TSST-1), Staphylococcal enterotoxin A (SEA), B (SEB), or C (SEC), Streptococcal enterotoxins and the like. Such bacteria include, but are not limited to, strains of a

species of enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (e.g., strains of serotype 0157:H7), *Staphylococcus* species (e.g., *Staphylococcus aureus*, *Staphylococcus pyogenes*), *Shigella* species (e.g., *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii*, and *Shigella sonnei*), *Salmonella* species (e.g., *Salmonella typhi*,
 5 *Salmonella cholerae-suis*, *Salmonella enteritidis*), *Clostridium* species (e.g., *Clostridium perfringens*, *Clostridium difficile*, *Clostridium botulinum*), *Camphlobacter* species (e.g., *Camphlobacter jejuni*, *Camphlobacter fetus*), *Heliobacter* species, (e.g., *Heliobacter pylori*), *Aeromonas* species (e.g., *Aeromonas sobria*, *Aeromonas hydrophila*, *Aeromonas caviae*), *Pleisomonas shigelloides*, *Yersinia enterocolitica*, *Vibrios* species
 10 (e.g., *Vibrios cholerae*, *Vibrios parahemolyticus*), *Klebsiella* species, *Pseudomonas aeruginosa*, and *Streptococci*. See, e.g., Stein, ed., INTERNAL MEDICINE, 3rd ed., pp 1-13, Little, Brown and Co., Boston, (1990), Evans et al., eds., Bacterial Infections of Humans: Epidemiology and Control, 2d. Ed., pp 239-254, Plenum Medical Book Co., New York (1991), Mandell et al., Principles and Practice of Infectious Diseases, 3d Ed.,
 15 Churchill Livingstone, New York (1990), Berkow et al., eds., *The Merck Manual*, 16th edition, Merck and Co., Rahway, N.J., 1992; Wood et al., FEMS Microbiology Immunology, 76:121-134 (1991), Marrack et al., Science, 248:705-711 (1990), the contents of which references are incorporated entirely herein by reference

[170] GLP-1 agonist or mimetibody or specified portion or variant compositions of
 20 the present invention can further comprise at least one of any suitable auxiliary, such as, but not limited to, diluent, binder, stabilizer, buffers, salts, lipophilic solvents, preservative, adjuvant or the like. Pharmaceutically acceptable auxiliaries are preferred. Non-limiting examples of, and methods of preparing such sterile solutions are well known in the art, such as, but limited to, Gennaro, Ed., *Remington's Pharmaceutical*
 25 *Sciences*, 18th Edition, Mack Publishing Co. (Easton, PA) 1990. Pharmaceutically acceptable carriers can be routinely selected that are suitable for the mode of administration, solubility and/or stability of the GLP-1 agonist or mimetibody composition as well known in the art or as described herein.

[171] Pharmaceutical excipients and additives useful in the present composition
 30 include but are not limited to proteins, peptides, amino acids, lipids, and carbohydrates (e.g., sugars, including monosaccharides, di-, tri-, tetra-, and oligosaccharides, derivatized sugars such as alditols, aldonic acids, esterified sugars and the like, and

polysaccharides or sugar polymers), which can be present singly or in combination, comprising alone or in combination 1-99.99% by weight or volume. Exemplary protein excipients include serum albumin such as human serum albumin (HSA), recombinant human albumin (rHA), gelatin, casein, and the like. Representative amino acid/GLP-1 agonist or mimetibody or specified portion or variant components, which can also function in a buffering capacity, include alanine, glycine, arginine, betaine, histidine, glutamic acid, aspartic acid, cysteine, lysine, leucine, isoleucine, valine, methionine, phenylalanine, aspartame, and the like. One preferred amino acid is glycine.

[172] Carbohydrate excipients suitable for use in the invention include, for example, monosaccharides such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like, disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like, polysaccharides, such as raffinose, melezitose, maltodextrins, dextrans, starches, and the like, and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol sorbitol (glucitol), myoinositol and the like. Preferred carbohydrate excipients for use in the present invention are mannitol, trehalose, and raffinose.

[173] GLP-1 agonist or mimetibody compositions can also include a buffer or a pH adjusting agent, typically, the buffer is a salt prepared from an organic acid or base. Representative buffers include organic acid salts such as salts of citric acid, ascorbic acid, gluconic acid, carbonic acid, tartaric acid, succinic acid, acetic acid, or phthalic acid, Tris, tromethamine hydrochloride, or phosphate buffers. Preferred buffers for use in the present compositions are organic acid salts such as citrate.

[174] Additionally, the GLP-1 agonist or mimetibody or specified portion or variant compositions of the invention can include polymeric excipients/additives such as polyvinylpyrrolidones, ficolls (a polymeric sugar), dextrans (e.g., cyclodextrins, such as 2-hydroxypropyl- β -cyclodextrin), polyethylene glycols, flavoring agents, antimicrobial agents, sweeteners, antioxidants, antistatic agents, surfactants (e.g., polysorbates such as "TWEEN 20" and "TWEEN 80"), lipids (e.g., phospholipids, fatty acids), steroids (e.g., cholesterol), and chelating agents (e.g., EDTA).

[175] These and additional known pharmaceutical excipients and/or additives suitable for use in the GLP-1 agonist or mimetibody compositions according to the invention are known in the art, e.g., as listed in "Remington: The Science & Practice of Pharmacy",

19th ed., Williams & Williams, (1995), and in the "Physician's Desk Reference", 52nd ed., Medical Economics, Montvale, NJ (1998), the disclosures of which are entirely incorporated herein by reference Preferred carrier or excipient materials are carbohydrates (e.g., saccharides and alditols) and buffers (e.g., citrate) or polymeric

5 agents

[176] **Formulations.** As noted above, the invention provides for stable formulations, which can preferably include a suitable buffer with saline or a chosen salt, as well as optional preserved solutions and formulations containing a preservative as well as multi-use preserved formulations suitable for pharmaceutical or veterinary use, comprising at least one GLP-1 agonist or mimetibody or specified portion or variant in a pharmaceutically acceptable formulation Preserved formulations contain at least one known preservative or optionally selected from the group consisting of at least one phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, phenylmercuric nitrite, phenoxyethanol, formaldehyde, chlorobutanol, magnesium chloride (e.g., hexahydrate), alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof in an aqueous diluent Any suitable concentration or mixture can be used as known in the art, such as 0.001-5%, or any range or value therein, such as, but not limited to 0.001, 0.003, 0.005, 0.009, 0.01, 0.02, 0.03, 0.05, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.3, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0 or any range or value therein Non-limiting examples include, no preservative, 0.1-2% m-cresol (e.g., 0.2, 0.3, 0.4, 0.5, 0.9, 1.0%), 0.1-3% benzyl alcohol (e.g., 0.5, 0.9, 1.1, 1.5, 1.9, 2.0, 2.5%), 0.001-0.5% thimerosal (e.g., 0.005, 0.01), 0.001-2.0% phenol (e.g., 0.05, 0.25, 0.28, 0.5, 0.9, 1.0%), 0.0005-1.0% alkylparaben(s) (e.g., 0.00075, 0.0009, 0.001, 0.002, 0.005, 0.0075, 0.009, 0.01, 0.02, 0.05, 0.075, 0.09, 0.1, 0.2, 0.3, 0.5, 0.75, 0.9, 1.0%), and the like

[177] As noted above, the invention provides an article of manufacture, comprising packaging material and at least one vial comprising a solution of at least one GLP-1 agonist or mimetibody or specified portion or variant with the prescribed buffers and/or preservatives, optionally in an aqueous diluent, wherein said packaging material comprises a label that indicates that such solution can be held over a period of 1, 2, 3, 4,

5, 6, 9, 12, 18, 20, 24, 30, 36, 40, 48, 54, 60, 66, 72 hours or greater. The invention further comprises an article of manufacture, comprising packaging material, a first vial comprising lyophilized at least one GLP-1 agonist or mimetibody or specified portion or variant, and a second vial comprising an aqueous diluent of prescribed buffer or
5 preservative, wherein said packaging material comprises a label that instructs a patient to reconstitute the at least one GLP-1 agonist or mimetibody or specified portion or variant in the aqueous diluent to form a solution that can be held over a period of twenty-four hours or greater.

[178] The at least one GLP-1 agonist or mimetibody or specified portion or variant
10 used in accordance with the present invention can be produced by recombinant means, including from mammalian cell or transgenic preparations, or can be purified from other biological sources, as described herein or as known in the art.

[179] The range of amounts of at least one GLP-1 agonist or mimetibody or specified portion or variant in the product of the present invention includes amounts yielding upon
15 reconstitution, if in a wet/dry system, concentrations from about 10 µg/ml to about 1000 mg/ml, although lower and higher concentrations are operable and are dependent on the intended delivery vehicle, e.g., solution formulations will differ from transdermal patch, pulmonary, transmucosal, or osmotic or micro pump methods.

[180] Preferably, the aqueous diluent optionally further comprises a pharmaceutically
20 acceptable preservative. Preferred preservatives include those selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof. The concentration of preservative used in the formulation is a concentration sufficient to
25 yield an anti-microbial effect. Such concentrations are dependent on the preservative selected and are readily determined by the skilled artisan.

[181] Other excipients, e.g., isotonicity agents, buffers, antioxidants, preservative enhancers, can be optionally and preferably added to the diluent. An isotonicity agent, such as glycerin, is commonly used at known concentrations. A physiologically
30 tolerated buffer is preferably added to provide improved pH control. The formulations can cover a wide range of pHs, such as from about pH 4 to about pH 10, and preferred

ranges from about pH 5 to about pH 9, and a most preferred range of about 6.0 to about 8.0. Preferably the formulations of the present invention have pH between about 6.8 and about 7.8. Preferred buffers include phosphate buffers, most preferably sodium phosphate, particularly phosphate buffered saline (PBS).

- 5 [182] Other additives, such as pharmaceutically acceptable solubilizers like Tween 20 (polyoxyethylene (20) sorbitan monolaurate), Tween 40 (polyoxyethylene (20) sorbitan monopalmitate), Tween 80 (polyoxyethylene (20) sorbitan monooleate), Pluronic F68 (polyoxyethylene polyoxypropylene block copolymers), and PEG (polyethylene glycol) or non-ionic surfactants such as polysorbate 20 or 80 or
- 10 poloxamer 184 or 188, Pluronic® polyols, other block co-polymers, and chelators such as EDTA and EGTA can optionally be added to the formulations or compositions to reduce aggregation. These additives are particularly useful if a pump or plastic container is used to administer the formulation. The presence of pharmaceutically acceptable surfactant mitigates the propensity for the protein to aggregate.
- 15 [183] The formulations of the present invention can be prepared by a process which comprises mixing at least one GLP-1 agonist or mimetibody or specified portion or variant and a preservative selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkylparaben, (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate
- 20 and thimerosal or mixtures thereof in an aqueous diluent. Mixing the at least one GLP-1 agonist or mimetibody or specified portion or variant and preservative in an aqueous diluent is carried out using conventional dissolution and mixing procedures. To prepare a suitable formulation, for example, a measured amount of at least one GLP-1 agonist or mimetibody or specified portion or variant in buffered solution is combined with the
- 25 desired preservative in a buffered solution in quantities sufficient to provide the protein and preservative at the desired concentrations. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the temperature and pH at which the formulation is prepared, are all factors that may be optimized for the concentration and
- 30 means of administration used.
- [184] The claimed formulations can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized at least one GLP-1 agonist or mimetibody or

specified portion or variant that is reconstituted with a second vial containing water, a preservative and/or excipients, preferably a phosphate buffer and/or saline and a chosen salt, in an aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times and can suffice for a single or multiple
5 cycles of patient treatment and thus can provide a more convenient treatment regimen than currently available

[185] The present claimed articles of manufacture are useful for administration over a period of immediately to twenty-four hours or greater. Accordingly, the presently claimed articles of manufacture offer significant advantages to the patient. Formulations
10 of the invention can optionally be safely stored at temperatures of from about 2 to about 40°C and retain the biological activity of the protein for extended periods of time, thus, allowing a package label indicating that the solution can be held and/or used over a period of 6, 12, 18, 24, 36, 48, 72, or 96 hours or greater. If preserved diluent is used, such label can include use up to at least one of 1-12 months, one-half, one and a half,
15 and/or two years

[186] The solutions of at least one GLP-1 agonist or mimetibody or specified portion or variant in the invention can be prepared by a process that comprises mixing at least one GLP-1 agonist or mimetibody or specified portion or variant in an aqueous diluent. Mixing is carried out using conventional dissolution and mixing procedures. To prepare
20 a suitable diluent, for example, a measured amount of at least one GLP-1 agonist or mimetibody or specified portion or variant in water or buffer is combined in quantities sufficient to provide the protein and optionally a preservative or buffer at the desired concentrations. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional
25 additives are used, the temperature and pH at which the formulation is prepared, are all factors that may be optimized for the concentration and means of administration used

[187] The claimed products can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized at least one GLP-1 agonist or mimetibody or specified portion or variant that is reconstituted with a second vial containing the
30 aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times and can suffice for a single or multiple cycles of patient treatment and thus provides a more convenient treatment regimen than currently available

- [188] The claimed products can be provided indirectly to patients by providing to pharmacies, clinics, or other such institutions and facilities, clear solutions or dual vials comprising a vial of lyophilized at least one GLP-1 agonist or mimetibody or specified portion or variant that is reconstituted with a second vial containing the aqueous diluent
- 5 The clear solution in this case can be up to one liter or even larger in size, providing a large reservoir from which smaller portions of the at least one GLP-1 agonist or mimetibody or specified portion or variant solution can be retrieved one or multiple times for transfer into smaller vials and provided by the pharmacy or clinic to their customers and/or patients
- 10 [189] Recognized devices comprising these single vial systems include those pen-injector devices for delivery of a solution such as Humaject[®], NovoPen[®], B-D[®] Pen, AutoPen[®], and OptiPen[®]. Recognized devices comprising a dual vial system include those pen-injector systems for reconstituting a lyophilized drug in a cartridge for delivery of the reconstituted solution such as the HumatroPen[®]
- 15 [190] The products presently claimed include packaging material. The packaging material provides, in addition to the information required by the regulatory agencies, the conditions under which the product can be used. The packaging material of the present invention provides instructions to the patient to reconstitute the at least one GLP-1 agonist or mimetibody or specified portion or variant in the aqueous diluent to form a
- 20 solution and to use the solution over a period of 2-24 hours or greater for the two-vial, wet/dry, product. For the single-vial solution product, the label indicates that such solution can be used over a period of 2-96 hours or greater. The presently claimed products are useful for human pharmaceutical product use
- [191] The formulations of the present invention can be prepared by a process that
- 25 comprises mixing at least one GLP-1 agonist or mimetibody or specified portion or variant and a selected buffer, preferably a phosphate buffer containing saline or a chosen salt. Mixing the at least one GLP-1 agonist or mimetibody or specified portion or variant and buffer in an aqueous diluent is carried out using conventional dissolution and mixing procedures. To prepare a suitable formulation, for example, a measured
- 30 amount of at least one GLP-1 agonist or mimetibody or specified portion or variant in water or buffer is combined with the desired buffering agent in water in quantities

sufficient to provide the protein and buffer at the desired concentrations. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the temperature and pH at which the formulation is prepared, are all factors that can be optimized for the concentration and means of administration used.

[192] The claimed stable or preserved formulations can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized at least one GLP-1 agonist or mimetibody or specified portion or variant that is reconstituted with a second vial containing a preservative or buffer and excipients in an aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times and can suffice for a single or multiple cycles of patient treatment and thus provides a more convenient treatment regimen than currently available.

[193] At least one GLP-1 agonist or mimetibody or specified portion or variant in either the stable or preserved formulations or solutions described herein, can be administered to a patient in accordance with the present invention via a variety of delivery methods including SC or IM injection; transdermal, pulmonary, transmucosal, implant, osmotic pump, cartridge, micro pump, or other means appreciated by the skilled artisan, as well-known in the art.

[194] **Therapeutic Applications.** The present invention provides a method of increasing the function of pancreas, comprising administering an effective amount of at least one GLP-1 agonist or mimetibody composition to a cell, tissue, organ, or individual in need thereof. The GLP-1 agonist or mimetibody may promote islet differentiation, increase β -cell mass, and/or increase insulin secretion. The GLP-1 agonist or mimetibody can be administered *in vitro*, *ex vivo*, or *in vivo*.

[195] For example, GLP-1 agonist or mimetibody treatment can be used in pancreas or islet transplantation patients or other cell therapies involving insulin-producing cells. Cell therapies may be delivered to the patient intravenously, subcutaneously, intramuscularly, or intraperitoneally, with or without the support of a device designed to enhance cell survival or prevent immune rejection. It can also be used in patients following surgical removal of a portion of the pancreas. GLP-1 agonist or mimetibody can be administered to living pancreas or islet donors to increase β -cell mass and

function of pancreas prior to or after the procedure. It can also be used to stimulate the proliferation of β -cells *in vitro* prior to the transplantation, thereby increasing the β -cell mass and preventing the apoptosis of the islets once transplanted. It can further be used in culture of stem, progenitor, or precursor of insulin-producing cells to stimulate the differentiation and proliferation and for the prevention of apoptosis.

[196] The present invention provides a method delaying the onset of or preventing diabetes in individuals at high risk to become diabetic, comprising administering an effective amount of at least one GLP-1 agonist or mimetibody composition to an individual in need thereof.

[197] The present invention provides a method for modulating or treating at least one pancreas malfunction caused disease or disorder, comprising administering an effective amount of at least one GLP-1 agonist or mimetibody composition to an individual in need thereof. The conditions and diseases suitable for treatment using the methods of the present invention include but are not limited to diabetes, such as type 1 diabetes, type 2 diabetes, gestational diabetes, or Mature Onset of Diabetes in the Young (MODY), including the associated signs and symptoms, such as but not limited to, insulin resistance, hyperglycemia, hypoglycemia, Cushing's syndrome, acanthosis nigricans, lipoatrophy diabetes, retinopathy, nephropathy, polyneuropathy, mononeuropathy, autonomic neuropathy, ulcers, foot ulcers, joint problems, infections (e.g., fungal or bacterial), and the like. See, e.g., the Merck Manual, 12th-17th Editions, Merck & Company, Rahway, NJ (1972, 1977, 1982, 1987, 1992, 1999), Pharmacotherapy Handbook, Wells et al., eds., Second Edition, Appleton and Lange, Stamford, Conn. (1998, 2001), each entirely incorporated by reference. Other non-limiting pancreatic disorders include pancreatitis, pancreatic tumors, or pancreatic cancer.

[198] The present invention provides a method for modulating or treating at least one metabolic disorder that results in hyperglycemia. Non-limiting examples of such disorders include cirrhosis and impaired glucose tolerance associated with hypertension. The GLP-1 agonist or mimetibody treatment can also be used in conjunction with other medications known to induce hyperglycemia and/or diabetes. Non-limiting examples of such medications include immunosuppressive drugs such as cyclosporine or FK-506.

given in organ transplantation, protease inhibitors prescribed for patients with AIDS, and atypical antipsychotics used in the treatment of schizophrenia.

[199] The present invention also provides a method for modulating or treating at least one cardiovascular disease in a cell, tissue, organ, animal, or patient, including, but not limited to, at least one of cardiac stun syndrome, myocardial infarction, congestive heart failure, stroke, ischemic stroke, hemorrhage, arteriosclerosis, atherosclerosis, diabetic atherosclerotic disease, hypertension, arterial hypertension, renovascular hypertension, syncope, shock, syphilis of the cardiovascular system, heart failure, cor pulmonale, primary pulmonary hypertension, cardiac arrhythmias, atrial ectopic beats, atrial flutter, atrial fibrillation (sustained or paroxysmal), chaotic or multifocal atrial tachycardia, regular narrow QRS tachycardia, specific arrhythmias, ventricular fibrillation, His bundle arrhythmias, atrioventricular block, bundle branch block, myocardial ischemic disorders, coronary artery disease, angina pectoris, myocardial infarction, cardiomyopathy, dilated congestive cardiomyopathy, restrictive cardiomyopathy, valvular heart diseases, endocarditis, pericardial disease, cardiac tumors, aortic and peripheral aneurysms, aortic dissection, inflammation of the aorta, occlusion of the abdominal aorta and its branches, peripheral vascular disorders, occlusive arterial disorders, peripheral atherosclerotic disease, thromboangitis obliterans, functional peripheral arterial disorders, Raynaud's phenomenon and disease, acrocyanosis, erythromelalgia, venous diseases, venous thrombosis, varicose veins, arteriovenous fistula, lymphedema, lipedema, unstable angina, reperfusion injury, post pump syndrome, ischemia-reperfusion injury, and the like. Such a method can optionally comprise administering an effective amount of a composition or pharmaceutical composition comprising at least one GLP-1 agonist or mimetibody or specified portion or variant to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy.

[200] Any method of the present invention can comprise administering an effective amount of a composition or pharmaceutical composition comprising at least one GLP-1 agonist or mimetibody or specified portion or variant to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy. Such a method can optionally further comprise co-administration or combination therapy, wherein the administering of said at least one GLP-1 agonist or mimetibody, specified portion or variant thereof, further comprises administering, before concurrently, and/or after, at least one selected

- from at least one of a diabetes or insulin metabolism related drug, a TNF antagonist (e.g., but not limited to a TNF antibody or fragment, a soluble TNF receptor or fragment, fusion proteins thereof, or a small molecule TNF antagonist), an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug
- 5 (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial (e.g., aminoglycoside, an antifungal, an antiparasitic, an antiviral, a carbapenem, cephalosporin, a fluorquinolone, a macrolide, a penicillin, a sulfonamide, a tetracycline, another antimicrobial), an antipsoriatic, a corticosteroid, an anabolic steroid, a diabetes related agent, a mineral, a nutritional, a thyroid agent, a
- 10 vitamin, a calcium related hormone, an antidiarrheal, an antitussive, an antiemetic, an antiulcer, a laxative, an anticoagulant, an erythropoietin (e.g., GLP-1etin alpha), a filgrastim (e.g., G-CSF, Neupogen), a sargramostim (GM-CSF, Leukine), an immunization, an immunoglobulin, an immunosuppressive (e.g., basiliximab, cyclosporine, daclizumab), a growth hormone, a hormone replacement drug, an estrogen
- 15 receptor modulator, a mydriatic, a cycloplegic, an alkylating agent, an antimetabolite, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, antimanic agent, an antipsychotic, an anxiolytic, a hypnotic, a sympathomimetic, a stimulant, donepezil, tacrine, an asthma medication, a beta agonist, an inhaled steroid, a leukotriene inhibitor, a methylxanthine, a cromolyn, an epinephrine or analog, dornase alpha (Pulmozyme), a
- 20 cytokine or a cytokine antagonist Suitable dosages are well known in the art See, e.g., Wells et al., eds., Pharmacotherapy Handbook, 2nd Edition, Appleton and Lange, Stamford, CT (2000), PDR Pharmacopoeia, Tarascon Pocket Pharmacopoeia 2000, Deluxe Edition, Tarascon Publishing, Loma Linda, CA (2000), each of which references are entirely incorporated herein by reference
- 25 [201] TNF antagonists suitable for compositions, combination therapy, co-administration, devices and/or methods of the present invention (further comprising at least one antibody, specified portion and variant thereof, of the present invention), include, but are not limited to, anti-TNF antibodies, ligand-binding fragments thereof, and receptor molecules which bind specifically to TNF, compounds which prevent
- 30 and/or inhibit TNF synthesis, TNF release or its action on target cells, such as thalidomide, tenidap, phosphodiesterase inhibitors (e.g., pentoxifylline and rolipram), A2b adenosine receptor agonists and A2b adenosine receptor enhancers, compounds

which prevent and/or inhibit TNF receptor signalling, such as mitogen activated protein (MAP) kinase inhibitors; compounds which block and/or inhibit membrane TNF cleavage, such as metalloproteinase inhibitors, compounds which block and/or inhibit TNF activity, such as angiotensin converting enzyme (ACE) inhibitors (e g., captopril),
5 and compounds which block and/or inhibit TNF production and/or synthesis, such as MAP kinase inhibitors

[202] As used herein, a "tumor necrosis factor antibody," "TNF antibody," "TNF α antibody," or fragment and the like decreases, blocks, inhibits, abrogates or interferes with TNF α activity *in vitro*, *in situ* and/or preferably *in vivo*. For example, a suitable
10 TNF human antibody of the present invention can bind TNF α and includes anti-TNF antibodies, antigen-binding fragments thereof, and specified mutants or domains thereof that bind specifically to TNF α . A suitable TNF antibody or fragment can also decrease block, abrogate, interfere, prevent and/or inhibit TNF RNA, DNA or protein synthesis, TNF release, TNF receptor signaling, membrane TNF cleavage, TNF activity, TNF
15 production and/or synthesis

[203] Chimeric antibody cA2 consists of the antigen binding variable region of the high-affinity neutralizing mouse anti-human TNF α IgG1 antibody, designated A2, and the constant regions of a human IgG1, kappa immunoglobulin. The human IgG1 Fc region improves allogeneic antibody effector function, increases the circulating serum
20 half-life and decreases the immunogenicity of the antibody. The avidity and epitope specificity of the chimeric antibody cA2 is derived from the variable region of the murine antibody A2. In a particular embodiment, a preferred source for nucleic acids encoding the variable region of the murine antibody A2 is the A2 hybridoma cell line

[204] Chimeric A2 (cA2) neutralizes the cytotoxic effect of both natural and
25 recombinant human TNF α in a dose dependent manner. From binding assays of chimeric antibody cA2 and recombinant human TNF α , the affinity constant of chimeric antibody cA2 was calculated to be $1.04 \times 10^{10} \text{ M}^{-1}$. Preferred methods for determining monoclonal antibody specificity and affinity by competitive inhibition can be found in Harlow, *et al*, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press,
30 Cold Spring Harbor, New York, 1988; Colligan *et al*, eds, *Current Protocols in Immunology*, Greene Publishing Assoc. and Wiley Interscience, New York, (1992-

2005), Kozbor *et al* , *Immunol Today*, 4 72-79 (1983), Ausubel *et al.*, eds *Current Protocols in Molecular Biology*, Wiley Interscience, New York (1987-2005); and Muller, *Meth. Enzymol.*, 92.589-601 (1983), which references are entirely incorporated herein by reference

- 5 [205] In a particular embodiment, murine monoclonal antibody A2 is produced by a cell line designated c134A Chimeric antibody cA2 is produced by a cell line designated c168A

Additional examples of monoclonal anti-TNF antibodies that can be used in the present invention are described in the art (see, e g , U.S Patent No 5,231,024, Moller, A. *et al.*,
 10 *Cytokine* 2(3) 162-169 (1990), U S. Application No. 07/943,852 (filed September 11, 1992), Rathjen *et al.*, International Publication No WO 91/02078 (published February 21, 1991), Rubin *et al.*, GLP-1 Patent Publication No. 0 218 868 (published April 22, 1987), Yone *et al* , GLP-1 Patent Publication No 0 288 088 (October 26, 1988), Liang, *et al* , *Biochem Biophys Res Comm.* 137:847-854 (1986), Meager, *et al* , *Hybridoma*
 15 6 305-311 (1987), Fendly *et al* , *Hybridoma* 6 359-369 (1987), Bringman, *et al.*, *Hybridoma* 6:489-507 (1987); and Hirai, *et al* , *J Immunol Meth* 96 57-62 (1987), which references are entirely incorporated herein by reference)

[206] **TNF Receptor Molecules.** Preferred TNF receptor molecules useful in the present invention are those that bind TNF α with high affinity (see, e g , Feldmann *et al* ,
 20 International Publication No WO 92/07076 (published April 30, 1992), Schall *et al* , *Cell* 61.361-370 (1990), and Loetscher *et al* , *Cell* 61 351-359 (1990), which references are entirely incorporated herein by reference) and optionally possess low immunogenicity In particular, the 55 kDa (p55 TNF-R) and the 75 kDa (p75 TNF-R) TNF cell surface receptors are useful in the present invention Truncated forms of these
 25 receptors, comprising the extracellular domains (ECD) of the receptors or functional portions thereof (see, e g., Corcoran *et al* , *Eur J. Biochem* 223 831-840 (1994)), are also useful in the present invention Truncated forms of the TNF receptors, comprising the ECD, have been detected in urine and serum as 30 kDa and 40 kDa TNF α inhibitory binding proteins (Engelmann, H *et al* , *J. Biol Chem* 265 1531-1536 (1990)) TNF
 30 receptor multimeric molecules and TNF immunoreceptor fusion molecules, and derivatives and fragments or portions thereof, are additional examples of TNF receptor

molecules which are useful in the methods and compositions of the present invention. The TNF receptor molecules which can be used in the invention are characterized by their ability to treat patients for extended periods with good to excellent alleviation of symptoms and low toxicity. Low immunogenicity and/or high affinity, as well as other
5 undefined properties, may contribute to the therapeutic results achieved.

[207] TNF receptor multimeric molecules useful in the present invention comprise all or a functional portion of the ECD of two or more TNF receptors linked via one or more polypeptide linkers or other nonpeptide linkers, such as polyethylene glycol (PEG). The multimeric molecules can further comprise a signal peptide of a secreted protein to
10 direct expression of the multimeric molecule. These multimeric molecules and methods for their production have been described in U.S. Application No. 08/437,533 (filed May 9, 1995), the content of which is entirely incorporated herein by reference.

[208] TNF immunoreceptor fusion molecules useful in the methods and compositions of the present invention comprise at least one portion of one or more immunoglobulin
15 molecules and all or a functional portion of one or more TNF receptors. These immunoreceptor fusion molecules can be assembled as monomers, or hetero- or homo-multimers. The immunoreceptor fusion molecules can also be monovalent or multivalent. An example of such a TNF immunoreceptor fusion molecule is TNF receptor/IgG fusion protein. TNF immunoreceptor fusion molecules and methods for
20 their production have been described in the art (Lesslauer *et al.*, *Eur. J. Immunol.* 21: 2883-2886 (1991), Ashkenazi *et al.*, *Proc. Natl. Acad. Sci. USA* 88: 10535-10539 (1991), Peppel *et al.*, *J. Exp. Med.* 174: 1483-1489 (1991), Kolls *et al.*, *Proc. Natl. Acad. Sci. USA* 91: 215-219 (1994), Butler *et al.*, *Cytokine* 6(6): 616-623 (1994), Baker *et al.*, *Eur. J. Immunol.* 24: 2040-2048 (1994), Beutler *et al.*, U.S. Patent No. 5,447,851, and
25 U.S. Application No. 08/442,133 (filed May 16, 1995), each of which references are entirely incorporated herein by reference). Methods for producing immunoreceptor fusion molecules can also be found in Capon *et al.*, U.S. Patent No. 5,116,964, Capon *et al.*, U.S. Patent No. 5,225,538, and Capon *et al.*, *Nature* 337: 525-531 (1989), which references are entirely incorporated herein by reference.

[209] A functional equivalent, derivative, fragment or region of TNF receptor molecule refers to the portion of the TNF receptor molecule, or the portion of the TNF receptor molecule sequence which encodes TNF receptor molecule, that is of sufficient

size and sequences to functionally resemble TNF receptor molecules that can be used in the present invention (e.g., bind TNF α with high affinity and possess low immunogenicity). A functional equivalent of TNF receptor molecule also includes modified TNF receptor molecules that functionally resemble TNF receptor molecules that can be used in the present invention (e.g., bind TNF α with high affinity and possess low immunogenicity). For example, a functional equivalent of TNF receptor molecule can contain a "SILENT" codon or one or more amino acid substitutions, deletions or additions (e.g., substitution of one acidic amino acid for another acidic amino acid, or substitution of one codon encoding the same or different hydrophobic amino acid for another codon encoding a hydrophobic amino acid). See Ausubel *et al*, *Current Protocols in Molecular Biology*, Greene Publishing Assoc and Wiley-Interscience, New York (1987-2005)

[210] Cytokines include, but are not limited to all known cytokines. See, e.g., CopewithCytokines.com. Cytokine antagonists include, but are not limited to, any antibody, fragment or mimetic, any soluble receptor, fragment or mimetic, any small molecule antagonist, or any combination thereof.

[211] Any method of the present invention can comprise a method for treating a protein mediated disorder, comprising administering an effective amount of a composition or pharmaceutical composition comprising at least one GLP-1 agonist or mimetibody or specified portion or variant to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy. Such a method can optionally further comprise co-administration or combination therapy for treating such immune diseases, wherein the administering of said at least one GLP-1 agonist or mimetibody, specified portion or variant thereof, further comprises administering, before concurrently, and/or after, at least one selected from at least one other cytokines such as IL-3, -6 and -11, stem cell factor, G-CSF and GM-CSF.

[212] Typically, treatment of pathologic conditions is effected by administering an effective amount or dosage of at least one GLP-1 agonist or mimetibody composition that total, on average, a range from at least about 0.01 to 500 milligrams of at least one GLP-1 agonist or mimetibody or specified portion or variant /kilogram of patient per dose, and preferably from at least about 0.1 to 100 milligrams GLP-1 agonist or

- mimetic body or specified portion or variant /kilogram of patient per single or multiple administration, depending upon the specific activity of contained in the composition. Alternatively, the effective serum concentration can comprise 0.1-5000 µg/ml serum concentration per single or multiple administration. Suitable dosages are known to medical practitioners and will, of course, depend upon the particular disease state, specific activity of the composition being administered, and the particular patient undergoing treatment. In some instances, to achieve the desired therapeutic amount, it can be necessary to provide for repeated administration, *i.e.*, repeated individual administrations of a particular monitored or metered dose, where the individual administrations are repeated until the desired daily dose or effect is achieved.
- [213] Preferred doses can optionally include 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, and/or 30 mg/kg/administration, or any range, value or fraction thereof, or to achieve a serum concentration of 0.1, 0.5, 0.9, 1.0, 1.1, 1.2, 1.5, 1.9, 2.0, 2.5, 2.9, 3.0, 3.5, 3.9, 4.0, 4.5, 4.9, 5.0, 5.5, 5.9, 6.0, 6.5, 6.9, 7.0, 7.5, 7.9, 8.0, 8.5, 8.9, 9.0, 9.5, 9.9, 10, 10.5, 10.9, 11, 11.5, 11.9, 12, 12.5, 12.9, 13.0, 13.5, 13.9, 14.0, 14.5, 14.9, 15.0, 15.5, 15.9, 16, 16.5, 16.9, 17, 17.5, 17.9, 18, 18.5, 18.9, 19, 19.5, 19.9, 20, 20.5, 20.9, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 96, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, and/or 5000 µg/ml serum concentration per single or multiple administration, or any range, value or fraction thereof.
- [214] Alternatively, the dosage administered can vary depending upon known factors, such as the pharmacodynamic characteristics of the particular agent, and its mode and route of administration, age, health, and weight of the recipient, nature and extent of symptoms, kind of concurrent treatment, frequency of treatment, and the effect desired. Usually a dosage of active ingredient can be about 0.1 to 100 milligrams per kilogram of body weight. Ordinarily 0.1 to 50, and preferably 0.1 to 10 milligrams per kilogram per administration or in sustained release form is effective to obtain desired results.

- [215] As a non-limiting example, treatment of humans or animals can be provided as a one-time or periodic dosage of at least one GLP-1 agonist or mimetibody or specified portion or variant of the present invention 0.01 to 100 mg/kg, such as 0.5, 0.9, 1.0, 1.1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90 or 100 mg/kg, per day, on at least one of day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, or alternatively, at least one of week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20, or any combination thereof, using single, infusion or repeated doses.
- 10 [216] Dosage forms (composition) suitable for internal administration generally contain from about 0.0001 milligram to about 500 milligrams of active ingredient per unit or container. In these pharmaceutical compositions the active ingredient will ordinarily be present in an amount of about 0.5-95% by weight based on the total weight of the composition
- 15 [217] For parenteral administration, the GLP-1 agonist or mimetibody or specified portion or variant can be formulated as a solution, suspension, emulsion or lyophilized powder in association, or separately provided, with a pharmaceutically acceptable parenteral vehicle. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Liposomes and nonaqueous vehicles
20 such as fixed oils may also be used. The vehicle or lyophilized powder may contain additives that maintain isotonicity (e.g., sodium chloride, mannitol) and chemical stability (e.g., buffers and preservatives). The formulation is sterilized by known or suitable techniques
- [218] Suitable pharmaceutical carriers are described in the most recent edition of
25 Remington's Pharmaceutical Sciences, A. Osol, a standard reference text in this field
- [219] **Therapeutic Administration.** Many known and developed modes of can be used for administering pharmaceutically effective amounts of at least one GLP-1 agonist or mimetibody or specified portion or variant according to the present invention. A GLP-1 agonist or mimetibody of the present invention can be delivered in a carrier, as a
30 solution, emulsion, colloid, or suspension, or as a powder, using any of a variety of

devices and methods suitable for administration by inhalation or other modes described here within or known in the art

[220] **Parenteral Formulations and Administration.** Formulations for parenteral administration can contain as common excipients sterile water or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes and the like. Aqueous or oily suspensions for injection can be prepared by using an appropriate emulsifier or humidifier and a suspending agent, according to known methods. Agents for injection can be a non-toxic, non-orally administrable diluting agent such as aqueous solution or a sterile injectable solution or suspension in a solvent. As the usable vehicle or solvent, water, Ringer's solution, isotonic saline, etc. are allowed, as an ordinary solvent, or suspending solvent, sterile involatile oil can be used. For these purposes, any kind of involatile oil and fatty acid can be used, including natural or synthetic or semisynthetic fatty oils or fatty acids, natural or synthetic or semisynthetic mono- or di- or tri-glycerides. Parental administration is known in the art and includes, but is not limited to, conventional means of injections, a gas pressured needle-less injection device as described in U S Pat. No. 5,851,198, and a laser perforator device as described in U S Pat. No. 5,839,446 entirely incorporated herein by reference.

[221] **Alternative Delivery.** The invention further relates to the administration of at least one GLP-1 agonist or mimetibody or specified portion or variant by parenteral, subcutaneous, intramuscular, intravenous, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal means. Protein, GLP-1 agonist or mimetibody or specified portion or variant compositions can be prepared for use for parenteral (subcutaneous, intramuscular or intravenous) administration particularly in the form of liquid solutions or suspensions, for use in vaginal or rectal administration particularly in semisolid forms such as creams and suppositories, for buccal, or sublingual administration particularly in the form of tablets or capsules, or intranasally particularly in the form of powders, nasal drops or aerosols or certain agents, or transdermally particularly in the form of a gel, ointment, lotion, suspension or patch delivery system (e.g., but not limited to, Macroflux™ by Alza, California, USA, or any other known methods, devices or techniques) with chemical enhancers such as dimethyl sulfoxide to either modify the skin structure or to increase the drug concentration in the transdermal patch (Junginger,

et al In "Drug Permeation Enhancement", Hsieh, D S., Eds , pp 59-90 (Marcel Dekker, Inc New York 1994, entirely incorporated herein by reference), or with oxidizing agents that enable the application of formulations containing proteins and peptides onto the skin (WO 98/53847), or applications of electric fields to create transient transport pathways such as electroporation, or to increase the mobility of charged drugs through the skin such as iontophoresis, or application of ultrasound such as sonophoresis (U.S. Pat Nos. 4,309,989 and 4,767,402) (the above publications and patents being entirely incorporated herein by reference)

[222] **Pulmonary/Nasal Administration.** For pulmonary administration, preferably at least one GLP-1 agonist or mimetibody or specified portion or variant composition is delivered in a particle size effective for reaching the lower airways of the lung or sinuses According to the invention, at least one GLP-1 agonist or mimetibody or specified portion or variant can be delivered by any of a variety of inhalation or nasal devices known in the art for administration of a therapeutic agent by inhalation These devices capable of depositing aerosolized formulations in the sinus cavity or alveoli of a patient include metered dose inhalers, nebulizers, dry powder generators, sprayers, and the like Other devices suitable for directing the pulmonary or nasal administration of GLP-1 agonist or mimetibody or specified portion or variants are also known in the art All such devices can use of formulations suitable for the administration for the dispensing of GLP-1 agonist or mimetibody or specified portion or variant in an aerosol Such aerosols can be comprised of either solutions (both aqueous and non aqueous) or solid particles Metered dose inhalers like the Ventolin[®] metered dose inhaler, typically use a propellant gas and require actuation during inspiration (See, e g , WO 94/16970, WO 98/35888) Dry powder inhalers like Turbuhaler[™] (Astra), Rotahaler[®] (Glaxo), Diskus[®] (Glaxo), Spiros[™] inhaler (Dura), devices marketed by Inhale Therapeutics, and the Spinhaler[®] powder inhaler (Fisons), use breath-actuation of a mixed powder (US 4668218 Astra, EP 237507 Astra, WO 97/25086 Glaxo, WO 94/08552 Dura, US 5458135 Inhale, WO 94/06498 Fisons, entirely incorporated herein by reference) Nebulizers like AERx[™] Aradigm, the Ultravent[®] nebulizer (Mallinckrodt), and the Acorn II[®] nebulizer (Marquest Medical Products) (US 5404871 Aradigm, WO 97/22376), the above references entirely incorporated herein by reference, produce aerosols from solutions, while metered dose inhalers, dry powder inhalers, etc generate

small particle aerosols. These specific examples of commercially available inhalation devices are intended to be a representative of specific devices suitable for the practice of this invention, and are not intended as limiting the scope of the invention. Preferably, a composition comprising at least one GLP-1 agonist or mimetibody or specified portion or variant is delivered by a dry powder inhaler or a sprayer. There are several desirable features of an inhalation device for administering at least one GLP-1 agonist or mimetibody or specified portion or variant of the present invention. For example, delivery by the inhalation device is advantageously reliable, reproducible, and accurate. The inhalation device can optionally deliver small dry particles, e.g. less than about 10 μm , preferably about 1-5 μm , for good respirability.

[223] **Administration of GLP-1 agonist or mimetibody or specified portion or variant Compositions as a Spray.** A spray including GLP-1 agonist or mimetibody or specified portion or variant composition protein can be produced by forcing a suspension or solution of at least one GLP-1 agonist or mimetibody or specified portion or variant through a nozzle under pressure. The nozzle size and configuration, the applied pressure, and the liquid feed rate can be chosen to achieve the desired output and particle size. An electrospray can be produced, for example, by an electric field in connection with a capillary or nozzle feed. Advantageously, particles of at least one GLP-1 agonist or mimetibody or specified portion or variant composition protein delivered by a sprayer have a particle size less than about 10 μm , preferably in the range of about 1 μm to about 5 μm , and most preferably about 2 μm to about 3 μm .

[224] Formulations of at least one GLP-1 agonist or mimetibody or specified portion or variant composition protein suitable for use with a sprayer typically include GLP-1 agonist or mimetibody or specified portion or variant composition protein in an aqueous solution at a concentration of about 1 mg to about 20 mg of at least one GLP-1 agonist or mimetibody or specified portion or variant composition protein per ml of solution. The formulation can include agents such as an excipient, a buffer, an isotonicity agent, a preservative, a surfactant, and, preferably, zinc. The formulation can also include an excipient or agent for stabilization of the GLP-1 agonist or mimetibody or specified portion or variant composition protein, such as a buffer, a reducing agent, a bulk protein, or a carbohydrate. Bulk proteins useful in formulating GLP-1 agonist or mimetibody or specified portion or variant composition proteins include albumin, protamine, or the

like Typical carbohydrates useful in formulating GLP-1 agonist or mimetibody or specified portion or variant composition proteins include sucrose, mannitol, lactose, trehalose, glucose, or the like The GLP-1 agonist or mimetibody or specified portion or variant composition protein formulation can also include a surfactant, which can reduce or prevent surface-induced aggregation of the GLP-1 agonist or mimetibody or specified portion or variant composition protein caused by atomization of the solution in forming an aerosol Various conventional surfactants can be employed, such as polyoxyethylene fatty acid esters and alcohols, and polyoxyethylene sorbitol fatty acid esters Amounts will generally range between 0.001 and 14% by weight of the formulation Especially preferred surfactants for purposes of this invention are polyoxyethylene sorbitan monooleate, polysorbate 80, polysorbate 20, or the like Additional agents known in the art for formulation of a protein such as mimetibodies, or specified portions or variants, can also be included in the formulation

[225] **Administration of GLP-1 agonist or mimetibody or specified portion or variant compositions by a Nebulizer.** GLP-1 agonist or mimetibody or specified portion or variant composition protein can be administered by a nebulizer, such as jet nebulizer or an ultrasonic nebulizer Typically, in a jet nebulizer, a compressed air source is used to create a high-velocity air jet through an orifice As the gas expands beyond the nozzle, a low-pressure region is created, which draws a solution of GLP-1 agonist or mimetibody or specified portion or variant composition protein through a capillary tube connected to a liquid reservoir The liquid stream from the capillary tube is sheared into unstable filaments and droplets as it exits the tube, creating the aerosol. A range of configurations, flow rates, and baffle types can be employed to achieve the desired performance characteristics from a given jet nebulizer In an ultrasonic nebulizer, high-frequency electrical energy is used to create vibrational, mechanical energy, typically employing a piezoelectric transducer This energy is transmitted to the formulation of the GLP-1 agonist or mimetibody or specified portion or variant composition protein either directly or through a coupling fluid, creating an aerosol including the GLP-1 agonist or mimetibody or specified portion or variant composition protein Advantageously, particles of GLP-1 agonist or mimetibody or specified portion or variant composition protein delivered by a nebulizer have a particle size less than

about 10 μm , preferably in the range of about 1 μm to about 5 μm , and most preferably about 2 μm to about 3 μm .

[226] Formulations of at least one GLP-1 agonist or mimetibody or specified portion or variant suitable for use with a nebulizer, either jet or ultrasonic, typically include

5 GLP-1 agonist or mimetibody or specified portion or variant composition protein in an aqueous solution at a concentration of about 1 mg to about 20 mg of at least one GLP-1 agonist or mimetibody or specified portion or variant protein per ml of solution. The formulation can include agents such as an excipient, a buffer, an isotonicity agent, a preservative, a surfactant, and, preferably, zinc. The formulation can also include an

10 excipient or agent for stabilization of the at least one GLP-1 agonist or mimetibody or specified portion or variant composition protein, such as a buffer, a reducing agent, a bulk protein, or a carbohydrate. Bulk proteins useful in formulating at least one GLP-1 agonist or mimetibody or specified portion or variant composition proteins include albumin, protamine, or the like. Typical carbohydrates useful in formulating at least one

15 GLP-1 agonist or mimetibody or specified portion or variant include sucrose, mannitol, lactose, trehalose, glucose, or the like. The at least one GLP-1 agonist or mimetibody or specified portion or variant formulation can also include a surfactant, which can reduce or prevent surface-induced aggregation of the at least one GLP-1 agonist or mimetibody or specified portion or variant caused by atomization of the solution in forming an

20 aerosol. Various conventional surfactants can be employed, such as polyoxyethylene fatty acid esters and alcohols, and polyoxyethylene sorbitol fatty acid esters. Amounts will generally range between 0.001 and 4% (14% above) by weight of the formulation. Especially preferred surfactants for purposes of this invention are polyoxyethylene sorbitan mono-oleate, polysorbate 80, polysorbate 20, or the like. Additional agents

25 known in the art for formulation of a protein such as at least one GLP-1 agonist or mimetibody or specified portion or variant protein can also be included in the formulation.

[227] **Administration of GLP-1 agonist or mimetibody or specified portion or variant compositions By A Metered Dose Inhaler.** In a metered dose inhaler (MDI),

30 a propellant, at least one GLP-1 agonist or mimetibody or specified portion or variant, and any excipients or other additives are contained in a canister as a mixture including a liquefied compressed gas. Actuation of the metering valve releases the mixture as an

aerosol, preferably containing particles in the size range of less than about 10 μm , preferably about 1 μm to about 5 μm , and most preferably about 2 μm to about 3 μm . The desired aerosol particle size can be obtained by employing a formulation of GLP-1 agonist or mimetibody or specified portion or variant composition protein produced by various methods known to those of skill in the art, including jet-milling, spray drying, critical point condensation, or the like Preferred metered dose inhalers include those manufactured by 3M or Glaxo and employing a hydrofluorocarbon propellant

[228] Formulations of at least one GLP-1 agonist or mimetibody or specified portion or variant for use with a metered-dose inhaler device will generally include a finely divided powder containing at least one GLP-1 agonist or mimetibody or specified portion or variant as a suspension in a non-aqueous medium, for example, suspended in a propellant with the aid of a surfactant The propellant can be any conventional material employed for this purpose, such as chlorofluorocarbon, a hydrochlorofluorocarbon, a hydrofluorocarbon, or a hydrocarbon, including trichlorofluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol and 1,1,1,2-tetrafluoroethane, HFA-134a (hydrofluroalkane-134a), HFA-227 (hydrofluroalkane-227), or the like Preferably the propellant is a hydrofluorocarbon The surfactant can be chosen to stabilize the at least one GLP-1 agonist or mimetibody or specified portion or variant as a suspension in the propellant, to protect the active agent against chemical degradation, and the like Suitable surfactants include sorbitan trioleate, soya lecithin, oleic acid, or the like In some cases solution aerosols are preferred using solvents such as ethanol. Additional agents known in the art for formulation of a protein such as protein can also be included in the formulation

[229] One of ordinary skill in the art will recognize that the methods of the current invention can be achieved by pulmonary administration of at least one GLP-1 agonist or mimetibody or specified portion or variant compositions via devices not described herein

[230] **Mucosal Formulations and Administration.** For absorption through mucosal surfaces, compositions and methods of administering at least one GLP-1 agonist or mimetibody or specified portion or variant include an emulsion comprising a plurality of submicron particles, a mucoadhesive macromolecule, a bioactive peptide, and an

aqueous continuous phase, which promotes absorption through mucosal surfaces by achieving mucoadhesion of the emulsion particles (U.S. Pat. Nos. 5,514,670) Mucous surfaces suitable for application of the emulsions of the present invention can include corneal, conjunctival, buccal, sublingual, nasal, vaginal, pulmonary, stomachic, intestinal, and rectal routes of administration Formulations for vaginal or rectal administration, e.g. suppositories, can contain as excipients, for example, polyalkyleneglycols, vaseline, cocoa butter, and the like Formulations for intranasal administration can be solid and contain as excipients, for example, lactose or can be aqueous or oily solutions of nasal drops. For buccal administration excipients include sugars, calcium stearate, magnesium stearate, pregelatinized starch, and the like (U.S. Pat. Nos. 5,849,695).

[231] **Oral Formulations and Administration.** Formulations for oral rely on the co-administration of adjuvants (e.g., resorcinols and nonionic surfactants such as polyoxyethylene oleyl ether and n-hexadecylpolyethylene ether) to increase artificially the permeability of the intestinal walls, as well as the co-administration of enzymatic inhibitors (e.g., pancreatic trypsin inhibitors, diisopropylfluorophosphate (DFF) and trasylol) to inhibit enzymatic degradation. The active constituent compound of the solid-type dosage form for oral administration can be mixed with at least one additive, including sucrose, lactose, cellulose, mannitol, trehalose, raffinose, maltitol, dextran, starches, agar, arginates, chitins, chitosans, pectins, gum tragacanth, gum arabic, gelatin, collagen, casein, albumin, synthetic or semisynthetic polymer, and glyceride. These dosage forms can also contain other type(s) of additives, e.g., inactive diluting agent, lubricant such as magnesium stearate, paraben, preserving agent such as sorbic acid, ascorbic acid, alpha-tocopherol, antioxidant such as cysteine, disintegrator, binder, thickener, buffering agent, sweetening agent, flavoring agent, perfuming agent, etc

[232] Tablets and pills can be further processed into enteric-coated preparations The liquid preparations for oral administration include emulsion, syrup, elixir, suspension and solution preparations allowable for medical use These preparations may contain inactive diluting agents ordinarily used in said field, e.g., water Liposomes have also been described as drug delivery systems for insulin and heparin (U.S. Pat. No. 4,239,754) More recently, microspheres of artificial polymers of mixed amino acids (proteinoids) have been used to deliver pharmaceuticals (U.S. Pat. No. 4,925,673)

Furthermore, carrier compounds described in U.S. Pat. No 5,879,681 and U S. Pat. No 5,587,753 are used to deliver biologically active agents orally are known in the art

[233] **Transdermal Formulations and Administration.** For transdermal administration, the at least one GLP-1 agonist or mimetibody or specified portion or
5 variant is encapsulated in a delivery device such as a liposome or polymeric nanoparticles, microparticle, microcapsule, or microspheres (referred to collectively as microparticles unless otherwise stated) A number of suitable devices are known, including microparticles made of synthetic polymers such as polyhydroxy acids such as polylactic acid, polyglycolic acid and copolymers thereof, polyorthoesters,
10 polyanhydrides, and polyphosphazenes, and natural polymers such as collagen, polyamino acids, albumin and other proteins, alginate and other polysaccharides, and combinations thereof (U S Pat Nos 5,814,599).

[234] **Prolonged Administration and Formulations.** It can be sometimes desirable to deliver the compounds of the present invention to the subject over prolonged periods
15 of time, for example, for periods of one week to one year from a single administration Various slow release, depot or implant dosage forms can be utilized For example, a dosage form can contain a pharmaceutically acceptable non-toxic salt of the compounds that has a low degree of solubility in body fluids, for example, (a) an acid addition salt with a polybasic acid such as phosphoric acid, sulfuric acid, citric acid, tartaric acid,
20 tannic acid, pamoic acid, alginic acid, polyglutamic acid, naphthalene mono- or di-sulfonic acids, polygalacturonic acid, and the like, (b) a salt with a polyvalent metal cation such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium and the like, or with an organic cation formed from e g , N,N'-dibenzyl-ethylenediamine or ethylenediamine, or (c) combinations of (a) and (b) e g a
25 zinc tannate salt Additionally, the compounds of the present invention or, preferably, a relatively insoluble salt such as those just described, can be formulated in a gel, for example, an aluminum monostearate gel with, e g. sesame oil, suitable for injection Particularly preferred salts are zinc salts, zinc tannate salts, pamoate salts, and the like Another type of slow release depot formulation for injection would contain the
30 compound or salt dispersed for encapsulated in a slow degrading, non-toxic, non-antigenic polymer such as a polylactic acid/polyglycolic acid polymer for example as described in U.S Pat No 3,773,919. The compounds or, preferably, relatively insoluble

salts such as those described above can also be formulated in cholesterol matrix silastic pellets, particularly for use in animals. Additional slow release, depot or implant formulations, e.g. gas or liquid liposomes are known in the literature (U.S. Pat. Nos. 5,770,222 and "Sustained and Controlled Release Drug Delivery Systems", J. R.

5 Robinson ed, Marcel Dekker, Inc, N.Y., 1978)

[235] Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

[236] **Example 1: Cloning and Expression of a GLP-1 agonist or mimetibody in**

10 **Mammalian Cells.** A typical mammalian expression vector contains at least one promoter element, which mediates the initiation of transcription of mRNA, the GLP-1 agonist or mimetibody or specified portion or variant coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences.
15 flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRS) from Retroviruses, e.g., RSV, HTLV, HIV and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter). Suitable expression vectors for use in practicing the present invention
20 include, for example, vectors such as pIRES1neo, pRetro-Off, pRetro-On, PLXSN, or pLNCX (Clontech Labs, Palo Alto, CA), pcDNA3.1 (+/-), pcDNA/Zeo (+/-) or pcDNA3.1/Hygro (+/-) (Invitrogen), PSVL and PMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109). Mammalian host cells that could be used include human HeLa 293, H9 and Jurkat cells,
25 mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV 1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

[237] Alternatively, the gene can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, or hygromycin allows the identification and isolation of the
30 transfected cells.

- [238] The transfected gene can also be amplified to express large amounts of the encoded GLP-1 agonist or mimetibody or specified portion or variant. The DHFR (dihydrofolate reductase) marker is useful to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful
- 5 selection marker is the enzyme glutamine synthase (GS) (Murphy, et al, Biochem J 227:277-279 (1991), Bebbington, et al, Bio/Technology 10:169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often
- 10 used for the production of GLP-1 agonist or mimetibody or specified portion or variants.
- [239] The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen, et al, Molec. Cell Biol. 5 438-447 (1985)) plus a fragment of the CMV-enhancer (Boshart, et al, Cell 41 521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718,
- 15 facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.
- [240] **Cloning and Expression in CHO Cells.** The vector pC4 is used for the expression of GLP-1 agonist or mimetibody or specified portion or variant. Plasmid pC4 is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). The
- 20 plasmid contains the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary- or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (e.g., alpha minus MEM, Life Technologies, Gaithersburg, MD) supplemented with the chemotherapeutic agent methotrexate. The amplification of the DHFR genes in cells
- 25 resistant to methotrexate (MTX) has been well documented (see, e.g., F. W. Alt, et al, J Biol Chem 253 1357-1370 (1978), J. L. Hamlin and C. Ma, Biochem et Biophys Acta 1097 107-143 (1990), and M. J. Page and M. A. Sydenham, Biotechnology 9 64-68 (1991)). Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the
- 30 DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art that this approach can be used to develop cell lines carrying more than 1,000 copies of the amplified gene(s). Subsequently, when the

methotrexate is withdrawn, cell lines are obtained that contain the amplified gene integrated into one or more chromosome(s) of the host cell

[241] Plasmid pC4 contains for expressing the gene of interest the strong promoter of the long terminal repeat (LTR) of the Rous Sarcoma Virus (Cullen, et al., *Molec Cell Biol* 5:438-447 (1985)) plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart, et al, *Cell* 41:521-530 (1985)) Downstream of the promoter are BamHI, XbaI, and Asp718 restriction enzyme cleavage sites that allow integration of the genes. Behind these cloning sites the plasmid contains the 3' intron and polyadenylation site of the rat preproinsulin gene Other high efficiency promoters can also be used for the expression, e g , the human b-actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g , HIV and HTLVII Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the GLP-1 in a regulated way in mammalian cells (M Gossen, and H Bujard, *Proc Natl Acad Sci USA* 89 5547-5551 (1992)) For the polyadenylation of the mRNA other signals, e g., from the human growth hormone or globin genes can be used as well. Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418 or hygromycin It is advantageous to use more than one selectable marker in the beginning, e g , G418 plus methotrexate

[242] The plasmid pC4 is digested with restriction enzymes and then dephosphorylated using calf intestinal phosphatase by procedures known in the art The vector is then isolated from a 1% agarose gel

[243] The DNA sequence encoding the complete GLP-1 mimetibody or specified portion or variant is used, corresponding to HC and LC variable regions of a GLP-1 mimetibody of the present invention, according to known method steps Isolated nucleic acid encoding a suitable human constant region (i e , HC and LC regions) is also used in this construct

[244] The isolated variable and constant region encoding DNA and the dephosphorylated vector are then ligated with T4 DNA ligase E coli HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis

[245] Chinese hamster ovary (CHO) cells lacking an active DHFR gene are used for transfection. 5 µg of the expression plasmid pC4 is cotransfected with 0.5 µg of the plasmid pSV2-neo using lipofectin. The plasmid pSV2neo contains a dominant selectable marker, the neo gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 µg /ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 µg /ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 mM, 2 mM, 5 mM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained that grow at a concentration of 100 - 200 mM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reverse phase HPLC analysis.

[246] **Example 2: Non-Limiting Example of a GLP-1 mimetibody of the Invention.** GLP-1 is a 37-amino acid peptide secreted from the L-cells of the intestine following an oral glucose challenge. A mimetibody construct incorporating a biologically active GLP-1 (7-37) peptide, variant or derivative is expected to prolong the *in vivo* lifetime of the peptide and provide a novel therapy for lowering blood glucose in Type 2 diabetic patients. Peptides encoding the native GLP-1 (7-37) peptide or a DPP-IV resistant analogue can be incorporated into the mimetibody scaffold. Several of these molecules have been made, and the resulting mimetibodies have demonstrated activity in functional *in vitro* cell-based assays. It should be noted that different *in vitro* assays and *in vivo* models can be used in these studies and the potencies may not be comparable to each other or to results presented herein.

[247] To generate GLP-1 mimetibody variants, the GLP-1 peptide, the linker, the hinge, or the CH2 and CH3 sequences in the mimetibody could be deleted, added, substituted, mutated or modified to improve expression, potency, stability, or effector functions.

[248] The wild-type GLP-1 sequence as well as DPP-IV resistant GLP-1 variants, such as GLP-1 (A2S) or GLP-1 (A2G) can be incorporated into a mimetibody scaffold. Mutations of the peptide could be made to improve the properties of a GLP-1 mimetibody. For example mutations in the amino terminal residues may improve signaling while mutations in the helical domain may stabilize the helix and thereby improve binding to the receptor and/or stability of the mimetibody.

[249] The length and composition of the linker could be mutated to vary the flexibility or stability of the attachment between the GLP-1 peptide and the Fc region. Different isotypes could be incorporated into the hinge region of the molecule. In addition, mutations could be made within the hinge region of the mimetibody to stabilize the molecule. For example, the human IgG4 hinge could be mutated to make the Ser²²⁸->Pro variant, to stabilize the interchain disulfide bonds in the mimetibody. Variations within the Fc portion of the mimetibody could be made to improve the stability of the molecule and to change effector functions such as FcR binding. For example, one could use human or murine isotypes (or variations of these molecules) such as IgG4 with Ala/Ala mutations.

[250] **GLP-1 mimetibody of the Present Invention.** A specific, non-limiting, example of this invention is the GLP-1 mimetibody construct (SEQ ID NO.2) according to Formula (I)

20 ((P(n)-L(o)-V(p)-H(q)-CH₂(r)-CH₃(s))(t),

[251] where P is a single copy of the bioactive GLP-1 peptide (7-36), L is a tandem repeat of either Gly-Ser or Gly-Gly-Gly-Ser flexible linker, V is the C-terminal of V_H sequence, i.e., the J region of a naturally occurring IgG, H is the complete IgG1 hinge region and CH₂ & CH₃ are of the IgG1 isotype subclass. It is expected that the half-life of this construct will be many times that of the GLP-1 peptide alone or its variant or derivative and similar to that of an IgG.

[252] In addition to the basic structure described above, variants with potentially favorable biological characteristics are described. These include constructs that may have a decreased tendency to self-associate, reduced immune effector functions or decreased immunogenicity. Other modifications that confer desired characteristics such as improved conformation of the biologically active peptide, and transfer across the

blood-brain barrier are envisioned. The proposed variants and modifications may be combined in any fashion to yield constructs with desired activities

[253] Using recombinant DNA methods, the GLP-1 peptide was inserted into an intermediate vector between an immunoglobulin signal peptide and a human J sequence.

5 This was done using complementary synthetic oligonucleotides with ends compatible with the restriction sites present in the vector. These oligonucleotides comprised coding sequences for the GLP-1 peptide, and a flexible linker composed of two GGGS repeats. A restriction fragment containing the above-mentioned functional elements was then transferred into an expression vector. This vector contained the anti-CD4
10 immunoglobulin promoter and enhancer, and the coding sequence for the human IgG1 hinge sequence, HC constant region 2 (CH2) and constant region 3 (CH3) as well as the necessary elements for plasmid replication and selection in bacteria and selection for stable expressers in mammalian cells.

[254] This plasmid was introduced into the HEK293E cells and expression of the wt
15 GLP-1 mimetibody was achieved in transiently transfected cells. Purification of GLP-1 mimetibody was accomplished by standard protein A and Superose 12 affinity chromatography, yielding approximately 1.5 mg/L of transfected cells. This protein was the starting material for the experiments described below.

[255] The amino acid sequence of GLP-1 mimetibody is shown in Figure 1
20 Functional domains are annotated above the peptide coding sequence. It is thought that the J sequence will provide even more flexibility to allow the GLP-1 dimer to assume the proper conformation, and allow the dimer to protrude from the globular structure of the immunoglobulin and penetrate into the cleft between two GLP-1 receptors. There are three cysteines in the IgG1 hinge region. The first would normally pair to the
25 immunoglobulin light chain (LC) and the other two participate in interchain bonds between two HCs. CH2 and CH3 regions constitute the bulk of the protein. One of the reasons that immunoglobulins are believed to have a long serum half-life is their ability to bind the FcRn that extends the serum half-life by returning pinocytosed immunoglobulin back to the extracellular space. The binding site of the FcRn overlaps
30 the junction of the CH2 and CH3 regions (Sheilds et al, 2001, J Biol Chem, vol 276 (9), 6591-6604)

- [256] It is well known that two IgG heavy chains are assembled during cellular processing via disulfide bonds between cysteines located in the hinge region to form a homodimer. It is expected that this will also occur between the modified peptides to form the assembled GLP-1 mimetibody construct. In addition, it is expected that the intrachain disulfide bond between the two cysteines in the GLP-1 peptide will also form. The expected structure of GLP-1 mimetibody contains two GLP-1 peptides. The spatial arrangement of the peptides at the N-terminus along with the flexibility of adjoining sequences should allow the peptides to form the bioactive dimer.
- [257] **Example 3: FACS Binding Assay.** The activity of GLP-1 mimetibody was tested in an *in vitro* FACS binding assay. To determine whether the GLP-1 mimetibody binds GLP-1R, HEK293 cells (1×10^6 cells) over-expressing GLP-1R were incubated with GLP-1 mimetibody (20 nM) for 2 hours at 4°C. The cells were washed, and a fluorescently labeled secondary detection antibody (1 µg/mL goat anti-human IgG, Fc gamma specific) was added for 30 minutes at 4°C. The fluorescence intensity of the cells was monitored via flow cytometry. As shown in Figure 2, GLP-1 mimetibody binds to HEK293 cells over-expressing GLP-1R (Figure 2A) but not to control HEK293 cells (Figure 2B). This binding is specific as GLP-1 peptide analogue (A2S) is able to compete with GLP-1 mimetibody in the binding (Figure 2C).
- [258] **Example 4: cAMP Assay** The binding of GLP-1 to GLP-1R results in a dose-dependent increase in the signaling molecule, 3',5'-cyclic AMP (cAMP). cAMP can be measured with an *in vitro* assay in cells expressing the GLP-1R (Applied Biosystems). Briefly, RINm cells (1×10^5 cells) were incubated with increasing concentrations of GLP-1 peptide (0-30 nM) or GLP-1 mimetibody (0-100 nM). The cells were lysed, and the amount of cAMP was determined using a competitive assay that employs an alkaline-phosphatase labeled cAMP conjugate and a chemiluminescent substrate (Tropix® CDPD®). The concentration dependent cAMP activity for the wt GLP-1 mimetibody (Figure 3A) is comparable to the GLP-1 peptide (Figure 3B) ($EC_{50}=11$ nM vs. 0.4 nM, respectively). In a similar experiment, GLP-1 (A2G) mimetibody in IgG4 scaffold (Figure 3C) and GLP-1 (A2S) mimetibody in IgG4 scaffold (Figure 3D) both increased cAMP levels in RINm cells to a significantly higher level than wt GLP-1 mimetibody in IgG4 scaffold did (Figure 3E).

[259] **Example 5: DPP-IV cleavage assay.** Since GLP-1 is rapidly inactivated by DPP-IV, an *in vitro* assay was established to quantitate intact (i.e. uncleaved) GLP-1 mimetibody. Briefly, GLP-1 mimetibody or peptide (1-2 nM) was incubated at room temperature with DPP-IV (1 µg/mL, R&D Systems). After various times (0, 5, 10, 15, 20, 30, 40 minutes), a DPP-IV inhibitor (100 µM, Linco) was added to quench the reaction. The amount of intact GLP-1 mimetibody or peptide was measured using the GLP-1 Active ELISA (Linco) and the GLP-1 mimetibody or peptides for the respective standard curves. Figure 4 shows that the GLP-1 mimetibody was significantly more resistant to cleavage by DPP-IV, relative to the GLP-1 peptide.

10 [260] **Example 6: Human Serum stability assay.** The stability of the GLP-1 mimetibody in serum was also measured to ensure that other serum proteases were not able to cleave and inactivate the GLP-1 mimetibody. Briefly, GLP-1 peptide or the GLP-1 mimetibody (30 nM) was incubated in human serum at 37°C. After various times, samples were evaluated to determine the amount of intact peptide or mimetibody. The reactions were quenched with a DPP-IV inhibitor (100 µM, Linco), and the samples were analyzed using the GLP-1 Active ELISA from Linco. Figure 5 shows that < 5% of the GLP-1 peptide remained intact after 4 hours while > 90% of the GLP-1 mimetibody remained intact after 24 hours.

[261] **Example 7: GLP-1 mimetibody causes insulin secretion in RINm cells.** To test the effect of GLP-1 mimetibody in insulin secretion, RINm cells were treated with increasing concentrations of GLP-1 (7-36) peptide (0-5 nM) (Figure 6A), exendin-4 peptide (0-5 nM) (Figure 6A), or various GLP-1 mimetibodies (5 or 50 nM) and the amount of insulin secreted was measured via ELISA. All GLP-1 mimetibodies tested had activities in stimulating insulin secretion in RINm cells. At 50 nM, the mimetibodies had activities comparable to that of the wide-type GLP-1 (7-36) peptide (Figure 6B).

[262] **Example 8: Pharmacokinetics of GLP-1 mimetibody.** To measure the pharmacokinetics of three GLP-1 mimetibodies (wt, A2S, and A2G), C57Blk/6 mice were intravenously dosed with 0.062 mg/kg GLP-1 peptide or 1.0 mg/kg A2S GLP-1 mimetibody. Plasma was obtained via cardiac puncture after sacrificing mice at different time points. Various ELISAs were used to measure Fc, total mimetibody, active

mimetibody, and active peptide as they were metabolized over time in the animal. The intact GLP-1 peptide was quantitated using the GLP-1 Active ELISA from Linco. The intact GLP-1 mimetibody was captured with an antibody specific to the N-terminus of the GLP-1 peptide (Linco) and was detected with an antibody specific to the Fc. As shown in Figure 12, levels of GLP-1 peptide were no longer detectable 15 minutes after the injection (Figure 12A) whereas the GLP-1 A2S mimetibody molecule was detected for over 72 hours post injection (Figure 12B).

[263] **Example 9: GLP-1 mimetibody lowers glucose level in db/db mice.** Six week old db/db mice were fasted for two hours and then dosed intravenously with vehicle, GLP-1 peptide, or GLP-1 (A2S) mimetibody. Blood glucose was monitored 0.5, 1, 2, 3, and 4 hours post-dosing. The GLP-1 peptide lowered blood glucose at 30 minutes, but by 60 minutes, the blood glucose began to increase again likely due to the short half-life of the GLP-1 peptide (Figure 7A). In comparison, GLP-1 (A2S) mimetibody at a dose 100-fold lower than the GLP-1 peptide dose induced a decrease in blood glucose throughout the entire 4 hour period (Figure 7A). Results shown in Figure 7B indicate that the GLP-1 mimetibody was effective in lowering the fasting blood glucose of the diabetic animals compared to control animals at doses ranging from 0.019 to 1.9 nmol. In addition, consistent with the mechanism of action for GLP-1, the maximum glucose lowering effect achieved at 0.19 nmol was not enhanced by a higher dose of the GLP-1 mimetibody (1.9 nmol). The maximum glucose lowering effect maintained blood glucose values within the normal range.

[264] **Example 10: The effects of GLP-1 mimetibody on the proliferation of rat islets.** Ten male Sprague-Dawley rats weighing approximately 250 grams were anesthetized with Nembutal (50mg/kg IP). Following confirmation of unresponsiveness by toe pinch, a midline incision was made and the common bile duct isolated and its entrance to the duodenum clamped. The common bile duct was cut just distal to its bifurcation to the liver. Ten mL of Liberase™ RI (0.25 mg/mL) dissolved in Hanks Balanced Salt Solution (HBSS) was injected into the common bile duct via PE-50 connected to a 10 cc syringe causing the pancreas to distend with the Liberase™ solution. Following infusion, the pancreas was excised from the animal and placed into a 50 mL test tube and stored on ice until the pancreas was infused and collected from all animals.

[265] The 50 mL test tube containing the infused pancreas was placed in a 37°C waterbath and allowed to incubate for 20 minutes. Following incubation the tube was removed from the waterbath and shaken and tube filled with cold HBSS supplemented (HBSS+) with 10% Newborn Calf Serum (NCS), 5 mM glucose, 10 mM HEPES, 100U/100 µg penicillin/streptomycin (P/S). The contents were filtered through a 425 µm stainless steel sieve and collected into 2 x 50 mL tubes. The filter was rinsed with HBSS+ and the tubes centrifuged. The pancreatic digest was applied to a discontinuous polysucrose density gradient. Ten mL of 1.08 gm/mL polysucrose solution is added to the pellet and suspended. Five mL of each of 1.096, 1.068, and 1.037 gm/mL polysucrose solutions are then added sequentially to finish the discontinuous gradient. The tubes are then centrifuged at 2000 rpm for 10 minutes at 4°C with no brake applied. Following centrifugation the interfaces containing the islets were collected and washed. The isolated islets were cultured in CMRL-1066 media supplemented (CMRL+) with 10% Fetal Bovine Serum (FBS), 2mM glutamine, 25 mM HEPES, and 100U/100µg penicillin/streptomycin (P/S) overnight. The next day the islets were hand-picked and incubated in the presence of three different GLP-1 mimetibody constructs for eight hours in triplicate. Negative control islets were not treated and exendin-4 was used as a positive control. Following the incubation tritiated thymidine was added to the cultures and returned to 37°C culture for 24-48 hours. Following the incubation period the islets were washed and the tritiated thymidine incorporation was counted on a beta counter. As shown in Figure 8A, GLP-1 mimetibodies tested increased thymidine incorporation above the control to the levels comparable to those of exendin-4 at both concentrations tested (5 nM and 50 nM). It suggests that each of the GLP-1 mimetibodies is able to increase proliferation of rat islets.

[266] GLP-1 A2S mimetibody was further used to test the dose effect on the rat islet proliferation. Isolated and cultured islets were treated with at increasing doses (0.05 – 500 nM), and thymidine incorporation was measured. Figure 8B shows that the A2S mimetibody (0.05 – 500 nM) increased thymidine incorporation above the control, suggesting that the mimetibody is able to increase proliferation of the rat islets at all concentrations tested.

[267] Example 11: The effects of GLP-1 mimetibody on the proliferation of non-human primate (NHP) islets and human islets

NHP islets were isolated using an enzymatic digestion and density gradient purifications as described in detail in Ranuncoli et al. Cell Transplant (2002) 9, 3, 409-414. Human islets were isolated using an enzymatic digestion and density gradient purification described in detail in Ricordi et al. Diabetes (1998) 37, 4, 413-420. The islets were isolated and cultured overnight in Miami Media 1 (University of Miami, Miami, FL). Tritiated thymidine incorporation was evaluated as described in Example 10. GLP-1 A2S mimetibody stimulated the proliferation of NHP islets to the level similar to that of exendin-4 (Figure 9).

Similarly, it also stimulated the proliferation of human islets to the level comparable to that of exendin-4 (Figure 10A). Figure 10B demonstrated that A2S mimetibody was effective at concentrations ranging from 0.05 nM to 500 nM.

[268] Example 12: The effects of GLP-1 mimetibody on insulin secretion of rat islets

Rat islets were isolated according to the protocol described in Example 10.

Islets were cultured in medium containing either 3.5 or 15 mM glucose and the insulin secreted by the islets was measured using ELISA. The results show that multiple constructs of the GLP-1 mimetibody potentiate insulin secretion in response to glucose (Figure 11).

[269] Example 13: Stability of GLP-1 mimetibody is species specific.

GLP-1 A2S mimetibody was incubated in the whole blood from mice, rats, cynomolgus monkeys, and humans. At various time points, samples were removed and assayed by ELISA for the amount of intact mimetibody. The stability of the mimetibody was significantly greater in blood from cynomolgus monkey or human serum than in either rat or mouse blood (Figure 13). After 18 hours incubation in mouse (Figure 13A) or rat blood (Figure 13B), most of the mimetibody was no longer intact. In contrast, nearly 100% of the mimetibody was intact after a 24-hour incubation in cynomolgus (Figure 13C) or human blood (Figure 13D).

[270] Example 14: Improved pharmacokinetics of GLP-1 mimetibody in cynomolgus monkeys.

Cynomolgus monkeys were injected intravenously with 1.0 mg/kg of four GLP-1 mimetibody constructs and serum samples were taken at different time points from 10 minutes to 5 days following dosing. Serum samples were evaluated by ELISA to quantify intact mimetibody. As illustrated in Figure 14, all four

mimetibody exhibit a rapid distribution phase, followed by a slower clearance phase. Pharmacokinetic constants were calculated for each of the constructs to indicate a $T_{1/2}$ of approximately 3 days with similar exposure determined by AUC from $T = 0$ to $T = 120$ hours.

- 5 [271] **Example 15: Effects of GLP-1 mimetibody treatment on blood glucose following a glucose challenge in mice.** Eight-week old diabetic db/db mice were fasted for 12 hours. Six hours into the fast, the mice were injected subcutaneously with the A2S mimetibody construct at doses ranging from 0.02 to 2 mg/kg. Six hours after GLP-1 mimetibody dosing and twelve hours of fasting, mice received 1 g/kg oral
10 glucose and their blood glucose was measured at 0, 15, 30, 60, 90, 120, 150, and 180 minutes. As shown in Figure 15A, the GLP-1 A2S mimetibody was effective at all doses in lowering the blood glucose as compared to the vehicle control. Similar experiments were performed on C57/Bl6 mice maintained on a high fat diet and fasted for 12 hours. As shown in Figure 15B, the GLP-1 A2S mimetibody was also effective
15 at all doses in lowering the blood glucose as compared to the vehicle control.

- [272] **Example 16: GLP-1 MMB Dose-Dependently Inhibits Cytokine-Induced Apoptosis.** RIN-m cells were seeded at 50,000 cells/well in 96 well plates and incubated at 37°C overnight. The following day, cells were treated with a dose range of GLP-1 MMB (serially diluted from 100nM) for 30 minutes before the addition of the
20 cytokines TNF α (10ng/mL) or IL-1 β (4ng/mL). The plates were incubated at 37°C for 16 hours. To assay apoptosis the Cell Death ELISA-Plus assay kit (Roche Applied Science, Cat No. 11920685001) was used. Media was aspirated from the wells, 200 μ L of lysis buffer was added to each well, and the plate was incubated for 30 minutes at room temperature. After incubation, 20 μ L of lysate was transferred to a streptavidin
25 coated 96 well microtiter plate. 80 μ L of immunoreagent (1:20 dilutions of the anti-histone-biotin and anti-DNA-peroxidase in the incubation buffer provided) was also added to each well. The 100 μ L suspension was then incubated at room temperature for 2 hours, shaking gently. After 2 hours, the plates were washed three times with incubation buffer and development solution was added to the plates. Absorbance was
30 read at 405nm after approximately five minutes. The data indicate that GLP-1 MMB protect RIN-m cells from apoptosis induced by TNF- α or IL-1 β (Figure 16).

[273] In type 1 diabetes (T1D), there is a specific destruction of the insulin secreting pancreatic beta cells. Although the exact molecular mechanisms underlying beta cell destruction are not known, sera from T1D patients have been shown to promote apoptosis suggesting that apoptosis plays a role in the onset of diabetes. By treating a patient with recent onset, one in the honeymoon period, and even a patient predisposed to become diabetic with the GLP-1 MMB, apoptosis could be inhibited and β -cell mass preserved.

[274] In T1D, β -cell apoptosis may play a role in diabetes development in two distinct phases of the disease. First, developmental wave of islet apoptosis may affect the T-cell biology that ultimately impact on the onset of autoimmune diabetes. Secondly, islet apoptosis is also the mode of cell death that finally results in the massive destruction of β -cells. The use of the GLP-1 MMB to inhibit β -cell apoptosis could therefore be useful in the prevention of the autoimmune onset of diabetes or the progression onto overt diabetes by preserving β -cell mass.

[275] T1D is a result from an irreversible loss of insulin-secreting beta cells. However, insulin secretion is detectable in some people with long-standing T1D, indicating either a small population of surviving beta cells or continued renewal of beta cells subject to ongoing autoimmune destruction. It has been shown that T1D have β -cells that continually undergo apoptosis following replication. Beta cell apoptosis is twice as frequent in T1D as in control subjects. Most people with long-standing T1D have beta cells that continue to be destroyed. The mechanisms underlying increased beta cell death may involve both ongoing autoimmunity and glucose toxicity. The presence of beta cells despite ongoing apoptosis implies, by definition, that concomitant new beta cell formation must be occurring, even after long-standing T1D. We conclude that T1D may be prevented or reversed by targeted GLP-1 MMB inhibition of β -cell destruction.

[276] In multi-organ donors, islets in the pancreas are subject to apoptosis and necrosis during the events that lead to brain death for cadaveric organ donors that continue during their clinical course prior to organ donation. In addition, islets are subject to apoptosis during the islet isolation process in preparation of the insulin-producing cells for implantation. Furthermore, when islets are transplanted into the

liver of a diabetic recipient, there is a cascade of cytokine activation specifically TNF α , IL1B, and γ INF. The use of GLP -1 MMB in islet transplantation in the donor, during the isolation process, and during and following transplantation reduces the apoptosis and improve islet function. It also reduces the number of islets required for clinical benefit and provides an opportunity for the use of living donors in islet transplantation.

[277] Example 17. GLP-1 MMB Increases Glucose-Dependent Insulin Secretion

in INS-1E Cells. INS-1E cells were plated in 24-well plates at a cell density of 200,000 cells/well in RPMI 1640 + 10% FBS + 1% L-glutamine + 1% Sodium

Pyruvate + 1% Non-essential Amino Acids + 50 μ M β -Mercaptoethanol media. The

cells were allowed to grow for 7 days at 37°C and 5% CO₂ and were fed on day 3 or 4.

The cells were washed twice with 0.4 ml of KRBH buffer/3 mM glucose and allowed to incubate in this buffer for 30-60 minutes. The media was removed from the cells

and 0.4 ml of test article in KRBH with the appropriate amount of glucose was added per well. Twenty microliters of supernatant was removed per well for the T=0 time

point and following incubation for 60 minutes at 37°C at 5% CO₂ for insulin

measurements. Insulin concentrations were determined using a rat standard curve

(12.8 - 0.1 ng/ml) with the Ultra Sensitive Rat Insulin ELISA kit (Crystal Chem). Five microliters of a 1:50 dilution of the time point supernatants were used in the assay in

order to obtain values that fell on the standard curve. ELISA plates were read at OD₄₅₀

subtracting the OD₆₃₀ on a Molecular Devices SpectraMax 340PC plate reader. As

shown in Figure 17, the GLP-1 MMB increased insulin secretion in a dose-dependent manner but only in the presence of elevated glucose.

[278] Example 18. GLP-1 MMB Increases Glucose-Dependent Insulin Secretion in Rat and Human Islets.

Rat islets were obtained by collagenase digestion followed

by Ficoll gradient purification. Human islets were isolated using an enzymatic

digestion and density gradient purification described in detail in Ricordi et al. Diabetes

(1998) 37, 4, 413-420. Islets were plated overnight in CMRL-1066 (Gibco), pen/strep

(5,000 units/5,000 μ g), 10% FBS, 1% L-Glutamine 25 mM HEPES, pH 7.2-7.4 media in

a 20 x 100 mm petri dish, and cultured at 37°C in 5% CO₂ for 18 to 24 hours. The

following day, islets were swirled to center of dish and collected into a 50 mL conical

tube. The islets were allowed to settle to the bottom of the tube by gravity for

approximately 10-15 minutes. The supernatant was removed, Functionality/ Viability

media (MediaTech, cat#99-786-CV) was added and islets were allowed to settle to the bottom of the tube by gravity. The islets were washed for a total of three times. The supernatant was removed and the islets were resuspended at a density of 20 islets per mL in Functionality/ Viability media containing 1% BSA, 1% L-Glutamine, 1% pen/strep and 0.5 mM glucose solution. One mL of cell suspension (~20 islets) was added to each well of a 24 well plate and incubated for 2 hrs at 37°C in 5% CO₂. After 2 hrs, 1 mL of Functionality/Viability media was added containing 1% BSA, 1% L-Glutamine, 1% pen/strep, GLP-1 MMB (50 nM final concentrations, 100 nM initial concentrations) and glucose at a final concentration of 3.5 mM (n=12) or 15 mM (n=12). Twenty-five µL of supernatant was collected as time = 0 (baseline of insulin and 4 hours after the addition of the treatment. The supernatant was stored at -20°C until the insulin ELISA was performed. Rat insulin was quantitated using the Crystal Chem (Downers Grove, IL) Ultra-Sensitive ELISA Assay kit (cat # 90060). Human insulin was quantitated using the Human Insulin ELISA kit from Linco Research (St Charles, MI, cat#EZHI-14K). Figure 18 shows that GLP-1 MMB significantly increases insulin secretion from rat (Figure 18A) and human islets (Figure 18B).

[279] Diabetes, both type 1 and type 2, is ultimately due to an insufficient amount of insulin being secreted by an individual's β -cells. The ability to enhance the insulin secretion of individuals with diabetes by treating them with GLP-1 MMB would improve or eliminate their diabetic condition. Importantly, the increase in insulin secretion is glucose dependant, only increasing in the presence of elevated glucose levels. Therefore, the risk of a patient experiencing dangerous hypoglycemic episodes is minimized with the use of the GLP-1 MMB.

[280] Likewise, an increase in insulin secretion would also benefit an islet transplantation procedure. By increasing the insulin secreting capacity of transplanted islets, one could effectively reduce the number of islets required to provide clinical benefit. With the limited amount of cadaveric pancreas available to support islet transplantation, the use of the GLP-1 MMB would significantly increase the number of patients that could benefit from an islet transplantation procedure. Furthermore, the ability to increase insulin secretion could extend the donor population to living donors without the risk of depleting them of their insulin secreting capacity which may,

without intervention with the GLP-1 MMB, lead to the onset of diabetes in donors due to insufficient insulin secreting capacity

[281] Example 19. The effects of GLP-1 MMB on the blood glucose of normal

mice. Normal C57BLK/6 mice were randomized into two groups with each group having 13 animals. Group1 received daily intraperitoneal (IP) doses of PBS vehicle or GLP-1 MMB (0.5 mg/kg). Both groups were treated for 10 days. The mice were monitored daily for body weight and blood glucose was monitored (LifeScan, CA) for 32 days at which point the study was terminated.

Mice receiving GLP-1 MMB treatment showed lower blood glucose during the course of the 10-day treatment as compared to the PBS treated mice (Figure 19). Blood glucose returned to the level of the PBS group once treatment was terminated. Both groups showed identical body weight gain throughout the course of the study (data not shown).

[283] Hyperglycemia is present in both with both type 1 and type 2 diabetics and is responsible for the progression of the devastating secondary complications of the disease. The ability of the GLP-1 MMB to reduce blood glucose without adversely affecting the overall health of the recipients (as illustrated by the progressive weight gain identical to non-treated controls above) would be beneficial to both type 1 and type 2 patients.

[284] Example 20. GLP-1 MMB Protects Decline of Diabetic Mouse β -cells.

Mice (12 week old db/db) were fasted overnight. On day 1 of the study, mice (4 groups, n=5/group) were dosed s.c. with GLP-1 MMB (1.5 mg/kg) or a negative control mimetibody. On day 4 of the study, fasting blood glucose was measured and an OGTT (oral glucose tolerance test) was performed. Blood glucose levels were measured at 15, 30, 60, 90, 120, 150 and 180 min using tail vein blood. Mice were euthanized and pancreata were removed and fixed by immersion in 10% neutral buffered formalin. The pancreas from each mouse was embedded in paraffin and sectioned for staining by H&E and immunohistochemistry. Morphometric analysis of insulin staining showed a significantly higher intensity of insulin staining in GLP-1 MMB treated mice versus controls. The experiment was repeated with the mice sacrificed 14 days after dosing to evaluate the longevity of the single dose of GLP-1

MMB. Again, morphometric analysis of the insulin staining showed a higher intensity of insulin staining in the GLP-1 MMB treated animals two weeks following a single dose. The intensity of insulin staining is directly related to the amount of stored insulin within the islets suggesting that GLP-1 MMB treatment increases insulin secretion and storage in the islets of this diabetic animal model.

[285] In type 2 diabetes, the insufficient insulin synthesis and storage leads to hyperglycemia which is responsible for the onset and progression of secondary complications of the disease. The ability of the GLP-1 MMB to increase insulin synthesis and storage would reduce the hyperglycemia in type 2 patients thereby limiting the onset and progression of secondary complications.

[286] **Example 21. The effects of GLP-1 MMB on the efficacy of a marginal mass transplant of human islets in diabetic immunocompromised mice.** Athymic nu/nu (nude) mice were purchased from Harlan Laboratories. Animals were housed in Virus Antibody Free rooms in micro-isolated cages with free access to sterilized food and water. All experiments were conducted in accordance with the standards set forth in the *Guide for the Care and Use of Laboratory Animals*.

[287] Diabetes was induced by intravenous administration of streptozotocin (200mg/kg) freshly dissolved in citrate buffer. Induction of diabetes was confirmed by monitoring non-fasting glycemic values after induction and only animals with values ≥ 350 mg/dL were used as recipients. After transplantation of a marginal islet mass (≈ 50 IEQ/g body weight), meaning a mass that is not sufficient to immediately render the recipient normoglycemic, the animals were monitored for graft function by measuring non-fasting glycemic values daily in the first week post-transplant, and up to 3 times a week thereafter. Interventions that improve islet engraftment, viability, and function will reduce the time to achieve normoglycemia in animals transplanted with a marginal islet mass. Two groups of recipients of a marginal human islet mass were included, one group received daily IP injections of GLP-1 MMB (0.5 mg/kg) and the control group received vehicle IP injections.

[288] Animals were considered euglycemic when non-fasting glycemic values were ≤ 200 mg/dL on at least 2 consecutive readings. Additionally, graft function was assessed by the means of glucose tolerance tests (1.5 g/kg) performed in treated and

control animals after overnight fasting at >30 days post-transplantation, in order to study glucose clearance after glucose challenge. Animals achieving normoglycemia underwent graft removal in order to exclude residual function of the native pancreatic islets.

- 5 [289] As shown in Figure 20, GLP-1 MMB treated mice achieved normoglycemia more rapidly relative to the control mice following the transplant of a marginal mass of islets. In addition, GLP-1 MMB treated mice demonstrated a more efficient clearance of a glucose challenge. These data strongly suggest that GLP-1 MMB treatment improves the engraftment, viability and function of transplanted human islets and
- 10 supports the use of GLP-1 MMB in both donors (including living and brain dead) and recipients undergoing an islet transplant procedure. These results demonstrate the beneficial effects GLP-1 MMB has on human islets transplanted into a diabetic recipient. Furthermore, the data suggests that treatment with the GLP-1 MMB improves islet engraftment, survival, and function and would be a valuable adjunct
- 15 therapy in an islet transplantation setting.

- [290] **Example 22. The effects of GLP-1 MMB on the insulin secretion in response to glucose in nonhuman primate islets.** Nonhuman primate islets were isolated using an enzymatic digestion and density gradient purification and were divided into two groups and cultured at 37°C in humidified mixed 95%air/5% CO₂ in
- 20 non-tissue culture treated 175 cm² flasks (Corning, MA) at a density of ≈ 20,000 IE. One group of islets was cultured alone and the other group was cultured in MM1 supplemented with 50 nM GLP-1 MMB.

- [291] Following two days of culture, the islets were collected and a sample of islets (2 from control and 1 from GLP-1 MMB groups) was subjected to a dynamic stimulation
- 25 assay to evaluate the effects of glucose concentration on the insulin secretion of the islets. Islets were pre-perfused in a chromatography column (Bio-gel Fine 45–90 nm, Bio-Rad) with a buffer containing 125-mM NaCl, 5.9-mM KCl, 1.28-mM CaCl₂, 1.2-mM Mg Cl₂, 25-mM HEPES, 0.1% bovine serum albumin and 3-mM glucose for 20 min, at 37°C. Islets were perfused in the same buffer for 10 min and then sequentially
- 30 exposed to 11-mM and 3-mM glucose. Fractions of the perfusate were collected every

2 min during perfusate with 3-mM glucose, and every minute during stimulation. The concentration of insulin in each fraction was assayed by ELISA.

[292] Islets incubated in the presence of the GLP-1 MMB demonstrated a significant increase in glucose stimulated insulin secretion as opposed to non-treated controls.

5 (Figure 21). This data strongly supports the positive effects GLP-1 MMB has in vitro on insulin synthesis and secretion and further supports its use prior to islet transplantation. Increasing the potency of the islets prior to transplantation with the GLP-1 MMB should result in a reduced islet number required to achieve the same therapeutic effects in an individual with type-1 diabetes undergoing an islet transplant procedure. Furthermore, one can predict from these results that the treatment of an
10 individual with GLP-1 MMB will increase islet potency in vivo. This further supports the treatment of both donors (including living and brain dead) and recipients of an islet transplant to increase the function from their marginal mass of islets.

[293] **Example 23. The effects of GLP-1 MMB on allogeneic islet engraftment**

15 **and long-term survival in a cynomolgus monkey marginal mass model.** The use of a non-human primate marginal mass model enables one to study transplant approaches that have the potential to allow for reversal of diabetes with a reduced number of islets. In the marginal mass model, approximately 5,000 IEQ/kg, or half the number (usually 10,000 IEQ/kg) required to achieve normoglycemia, are transplanted. By utilizing
20 islets obtained from the same allogeneic donor one eliminates donor and isolation variables. By transplanting pairs of monkeys that have similar pre-transplant insulin requirements, it is possible to observe enhanced islet engraftment in the monkey treated with an agent designed to enhance islet function and limit early islet loss as compared to the monkey that does not receive the agent.

25 [294] Recipients were 2-3 year old cynomolgus monkeys of Mauritian origin, donors of the same strain were > 4 years of age. Diabetes was induced with 1250 mg/m² streptozotocin. Diabetic animals were treated with NPH insulin before the morning meal and with NPH/Lantus before the afternoon meal in an attempt to maintain blood glucose levels between 150-300 mg/dl. Four weeks after diabetes induction, the
30 monkeys underwent a glucagon challenge to verify that no stimulated c-peptide was produced.

[295] The donor organ was harvested and islets isolated via the semi-automated method referenced in Example A and Example B, followed by 2 overnight culture periods, first at 37°C and then at 22°C. Islets for the control monkey were cultured in standard MM1 media and islets for the experimental monkeys were cultured in MM1 supplemented with 50 nM GLP-1 MMB. Monkeys underwent a mini-laparotomy and islet transplantation was performed via the portal vein into the liver. A pair of monkeys received $\approx 5,000$ IEQ/kg from the same donor (cultured as described above), one monkey received GLP-1 MMB (0.2 mg/kg) subcutaneously two times per week and the control monkey did not. For both the control and GLP-1 MMB treated monkeys, rejection was prevented with steroid free immune suppression (abbreviated as SFIS, consisting of low dose FK506, high dose rapamycin, anti-IL2R induction therapy), as is clinically employed. Animals were monitored twice a day to determine fasting and post-prandial blood glucose levels (heel stick, glucometer) and treated with insulin as needed to maintain blood glucose in the 100-200 mg/dl range post-transplant. Fasting c-peptide was determined every other week and intravenous glucose tolerance testing (IVGTT) was done every 8 weeks to assess graft function.

[296] GLP-1 MMB treated animal had superior glucose control with a significant reduction in exogenous insulin requirements (Figure 22A). The improved glucose control was further illustrated by the rapid reduction in the HgbA1c in the GLP-1 MMB treated animal as compared to the non-treated control (Figure 22B). This study further supports the utilization of GLP-1 MMB in a type 1 diabetic undergoing an islet transplant procedure.

[297] **Advantages:** The use of this novel molecule as a therapeutic to treat type 2 diabetes provides several advantages over other GLP-1 analogues. For example, it is likely to prolong the half-life of the GLP-1 peptide. Also, the wild-type GLP-1 peptide in the mimetibody scaffold is resistant to protease degradation, specifically DPP-IV. This may allow for treatment with the wild-type GLP-1 peptide rather than a mutant peptide. Since GLP-1 is a native peptide, there may be less immune response in patients treated with a GLP-1 mimetibody than in patients treated with a mutated GLP-1 analogue. In addition, the large size of the GLP-1 mimetibody may preclude it from crossing the blood brain barrier. This may offer an advantage since nausea and anxiety have been associated with GLP-1 engaging the GLP-1R in the brain. Furthermore, the

mimetibody platform results in expression of two peptides on each mimetibody molecule. This may allow the GLP-1 peptides to interact with each other, forming a dimeric ligand that could increase affinity to the cell surface GLP-1 receptor.

5 The examples provided above demonstrate the positive effects that multiple constructs of the GLP-1 MMB have on the proliferation of islets, the amount of insulin secreted by the islets in response to elevated glucose levels, and the improved blood glucose of diabetic animals treated with the GLP-1 MMB. It is also shown that the GLP-1 MMB has superior pharmacokinetic properties when compared to the native GLP-1 peptide or its longer lasting analogues. In addition, the improved pharmacokinetics shown in
10 primates versus rodents suggest that the benefits of GLP-1 MMB treatment shown in the rodent models of diabetes may actually underestimate the clinical benefits the GLP-1 MMB will have when used in patients.

The hyperglycemia experienced by all patients with diabetes and other metabolic disorders is directly related to the relative deficiency of insulin. By increasing the
15 number of insulin producing cells and their insulin secretion potential, the amount of insulin that is produced is significantly increased. Therefore, GLP-1 MMB therapy is useful in any situation where an increase in insulin production would improve the clinical condition or prognosis of a patient. The improved pharmacokinetic properties of the GLP-1 MMB will make the treatment much more clinically acceptable and
20 convenient and therefore is a significant improvement over existing GLP-1-like therapies. GLP-1 MMB therapy is beneficial to all patients experiencing hyperglycemia, whether it is due to diabetes or other metabolic disorders. It is also useful in the pre-diabetic stage to increase the mass of islets and delay the clinical onset of diabetes in those patients determined to be predisposed to the disease. Likewise, it is
25 beneficial in the transplant setting, both whole organ and islets, to both donors and recipients. It is also useful in the in vitro setting, to increase the proliferation of insulin-producing cells prior to their transplantation and to stimulate the differentiation of insulin producing progenitors.

The examples above provide evidence that the GLP-1 MMB of the present invention
30 has many uses in the treatment of diabetes and other metabolic disorders resulting in hyperglycemia. The examples are not intended to set limitations on the potential clinical uses of the GLP-1 MMB as it will be obvious to those skilled in the art that the

GLP-1 MMB of the current invention has properties that make it an ideal antidiabetic agent for indications well beyond those set forth in the examples

[298] It will be clear that the invention can be practiced otherwise than as particularly described in the foregoing description and examples

- 5 Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the present invention

WHAT IS CLAIMED IS

- 1 . A method for diagnosing or treating an GLP-1 diabetes related condition in a cell, tissue, organ or animal, comprising contacting or administering a composition comprising an effective amount of at least one GLP-1 agonist or mimetibody nucleic acid, polypeptide or antibody with, or to, said cell, tissue, organ or animal, wherein said at least one GLP-1 CH1 deleted mimetibody nucleic acid comprises at least one polynucleotide encoding the amino acid sequence of SEQ ID NOS 2 or 4, or a polynucleotide complementary thereto
- 2 . A method for diagnosing or treating an GLP-1 diabetes related condition in a cell, tissue, organ or animal, comprising contacting or administering a composition comprising an effective amount of at least one GLP-1 agonist or mimetibody nucleic acid, polypeptide or antibody with, or to, said cell, tissue, organ or animal, wherein said at least one GLP-1 CH1 deleted mimetibody nucleic acid comprises at least one polynucleotide encoding the amino acid sequence comprising at least one selected from SEQ ID NOS 7-14, or a polynucleotide complementary thereto
- 3 . A method for diagnosing or treating an GLP-1 diabetes related condition in a cell, tissue, organ or animal, comprising contacting or administering a composition comprising an effective amount of at least one GLP-1 agonist or mimetibody nucleic acid, polypeptide or antibody with, or to, said cell, tissue, organ or animal, wherein said at least one GLP-1 CH1 deleted mimetibody nucleic acid comprises at least one polynucleotide encoding P or a polypeptide according to Formula (I)
$$(Pep(n)-L(o)-V(p)-H(q)-CH_2(r)-CH_3(s))(t),$$
wherein P is at least one bioactive GLP-1 peptide, variant or derivative, L is at least one linker sequence, which can be a polypeptide that provides structural flexibility by allowing the mimetibody to have alternative orientations and binding properties, V is at least one portion of a C-terminus of an immunoglobulin variable region, H is at least a portion of an immunoglobulin variable hinge region, CH₂ is at least a portion of an immunoglobulin CH₂ constant region, CH₃ is at least a portion of an immunoglobulin CH₃ constant region, n is an integer from 1 to 10, and o, p, q, r, s, and t can be independently an integer from 0 to 10
- 4 . A method for diagnosing or treating an GLP-1 diabetes related condition in a cell, tissue, organ or animal, comprising contacting or administering a composition comprising an effective amount of at least one GLP-1 agonist or mimetibody nucleic acid, polypeptide or antibody with, or to, said cell, tissue, organ or animal, wherein said

at least one GLP-1 CH1 deleted mimetibody comprises all of the contiguous amino acids of SEQ ID NOS 2 or 4.

5 . A method for diagnosing or treating an GLP-1 diabetes related condition in a cell, tissue, organ or animal, comprising contacting or administering a composition comprising an effective amount of at least one GLP-1 agonist or mimetibody nucleic acid, polypeptide or antibody with, or to, said cell, tissue, organ or animal, wherein said at least one GLP-1 CH1 deleted mimetibody or agonist comprises all of the contiguous amino acids of at least one of SEQ ID NOS 7-14.

6 . A method for diagnosing or treating an GLP-1 diabetes related condition in a cell, tissue, organ or animal, comprising contacting or administering a composition comprising an effective amount of at least one GLP-1 agonist or mimetibody nucleic acid, polypeptide or antibody with, or to, said cell, tissue, organ or animal, wherein said at least one GLP-1 CH1 deleted mimetibody comprises P or a polypeptide according to Formula (I)

(Pep(n)-L(o)-V(p)-H(q)-CH₂(r)-CH₃(s))(t),

wherein P is at least one bioactive GLP-1 peptide selected from SEQ ID NO 1 and 6, L is selected from GS, GGS, GGGS (SEQ ID NO 16), GSGGGS (SEQ ID NO 17), GGSGGGS (SEQ ID NO 18), GGSGGGSGG (SEQ ID NO 19) and GGGSGGGSGG (SEQ ID NO 20), V is selected from GTLVTVSS (SEQ ID NO 21), GTLVAVSS (SEQ ID NO 22), GTAVTVSS (SEQ ID NO 23), TVSS (SEQ ID NO 24), and AVSS (SEQ ID NO 25), H is EPKSCDKTHTCPPCPAPELLGGP (SEQ ID NO 26), CH₂ is SVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK (SEQ ID NO 43), CH₃ is GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTTPVLDSGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO 44), n is an integer from 1 to 10, and o, p, q, r, s, and t can be independently an integer from 0 to 10

7 . A method for diagnosing or treating an GLP-1 diabetes related condition in a cell, tissue, organ or animal, comprising contacting or administering a composition comprising an effective amount of at least one GLP-1 agonist or mimetibody nucleic acid, polypeptide or antibody with, or to, said cell, tissue, organ or animal, wherein said at least one GLP-1 CH1 deleted mimetibody comprises P or a polypeptide according to Formula (I)

(Pep(n)-L(o)-V(p)-H(q)-CH₂(r)-CH₃(s))(t),

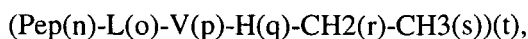
wherein P is at least one bioactive GLP-1 peptide of SEQ ID NO 6, L is selected from GS, GGS, GGG (SEQ ID NO 16), GSGGG (SEQ ID NO 17), GSGGGG (SEQ ID NO 18), GSGGGSGG (SEQ ID NO 19) and GSGGGSGG (SEQ ID NO 20), V is selected from
 5 GTLVTVSS (SEQ ID NO 21), GTLVAVSS (SEQ ID NO 22), GTAVTVSS (SEQ ID NO 23), TVSS (SEQ ID NO 24), and AVSS (SEQ ID NO 25), H is ESKYGPPCPSCPAPEFLGGP (SEQ ID NO 27), CH₂ is
 SVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF
 NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK (SEQ ID NO 45), CH₃
 10 is
 GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCVMHEALHNHYTQKSLSLGLGK (SEQ ID NO 46), n is an integer from 1 to 10, and o, p, q, r, s, and t can be independently an integer from 0 to 10

- 15 8 . A method for diagnosing or treating an GLP-1 diabetes related condition in a cell, tissue, organ or animal, comprising contacting or administering a composition comprising an effective amount of at least one GLP-1 agonist or mimetibody nucleic acid, polypeptide or antibody with, or to, said cell, tissue, organ or animal, wherein said at least one GLP-1 CH1 deleted mimetibody comprises P or a polypeptide according to
 20 Formula (I)

(Pep(n)-L(o)-V(p)-H(q)-CH₂(r)-CH₃(s))(t),

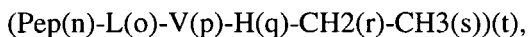
- wherein P is at least one bioactive GLP-1 peptide of SEQ ID NO 6, L is selected from GS, GGS, GGG (SEQ ID NO 16), GSGGG (SEQ ID NO 17), GSGGGG (SEQ ID NO 18), GSGGGSGG (SEQ ID NO 19) and GSGGGSGG (SEQ ID NO 20), V is selected from
 25 GTLVTVSS (SEQ ID NO 21), GTLVAVSS (SEQ ID NO 22), GTAVTVSS (SEQ ID NO 23), TVSS (SEQ ID NO 24), and AVSS (SEQ ID NO 25), H is ESKYGPPCPPCPAPEAAGGP (SEQ ID NO 28), CH₂ is
 SVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF
 NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK (SEQ ID NO 45), CH₃
 30 is
 GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCVMHEALHNHYTQKSLSLGLGK (SEQ ID NO 46), n is an integer from 1 to 10, and o, p, q, r, s, and t can be independently an integer from 0 to 10

9 . A method for diagnosing or treating an GLP-1 diabetes related condition in a cell, tissue, organ or animal, comprising contacting or administering a composition comprising an effective amount of at least one GLP-1 agonist or mimetibody nucleic acid, polypeptide or antibody with, or to, said cell, tissue, organ or animal, wherein said
 5 at least one GLP-1 CH1 deleted mimetibody comprises P or a polypeptide according to Formula (I)



wherein P is at least one bioactive GLP-1 peptide, variant or derivative, L is at least one linker sequence, which can be a polypeptide that provides structural flexibility by allowing
 10 the mimetibody to have alternative orientations and binding properties, V is at least one portion of a C-terminus of an immunoglobulin variable region, H is at least a portion of an immunoglobulin variable hinge region, CH2 is
 SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
 NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
 15 (SEQ ID NO 43), CH3 is
 GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
 PENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
 PGK (SEQ ID NO 44), n is an integer from 1 to 10, and o, p, q, r, s, and t can be independently an integer from 0 to 10

20 10 . A method for diagnosing or treating an GLP-1 diabetes related condition in a cell, tissue, organ or animal, comprising contacting or administering a composition comprising an effective amount of at least one GLP-1 agonist or mimetibody nucleic acid, polypeptide or antibody with, or to, said cell, tissue, organ or animal, wherein said
 at least one GLP-1 CH1 deleted mimetibody comprises P or a polypeptide according to
 25 Formula (I)



wherein P is at least one bioactive GLP-1 peptide, variant or derivative, L is at least one linker sequence, which can be a polypeptide that provides structural flexibility by allowing
 the mimetibody to have alternative orientations and binding properties, V is at least one portion
 30 of a C-terminus of an immunoglobulin variable region, H is at least a portion of an immunoglobulin variable hinge region, CH2 is
 SVFLFPPKPKDTLMISRTPEVTCVVVDVVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF
 NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK (SEQ ID NO 45), CH3

15

GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVL
DSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLGLGK (SEQ ID

NO 46), n is an integer from 1 to 10, and o, p, q, r, s, and t can be independently an integer

5 from 0 to 10

11. A method for diagnosing or treating an GLP-1 diabetes related

condition in a cell, tissue, organ or animal, comprising contacting or administering a

composition comprising an effective amount of at least one GLP-1 agonist or mimetibody

nucleic acid, polypeptide or antibody with, or to, said cell, tissue, organ or animal, wherein said

10 at least one GLP-1 CH1 deleted mimetibody comprises P or a polypeptide according to

Formula (I)

$$(\text{Pep}(n)\text{-L}(o)\text{-V}(p)\text{-H}(q)\text{-CH}_2(r)\text{-CH}_3(s))(t),$$

wherein P is at least one bioactive GLP-1 peptide of SEQ ID NO 6, L is at least one linker

sequence, which can be a polypeptide that provides structural flexibility by allowing the

15 mimetibody to have alternative orientations and binding properties, V is at least one portion of

a C-terminus of an immunoglobulin variable region, H is at least a portion of an

immunoglobulin variable hinge region, CH₂ is at least a portion of an immunoglobulin CH₂

constant region, CH₃ is at least a portion of an immunoglobulin CH₃ constant region, n is an

integer from 1 to 10, and o, p, q, r, s, and t can be independently an integer from 0 to 10

20 12. A method for diagnosing or treating an GLP-1 diabetes related

condition in a cell, tissue, organ or animal, comprising contacting or administering a

composition comprising an effective amount of at least one GLP-1 agonist or mimetibody

nucleic acid, polypeptide or antibody with, or to, said cell, tissue, organ or animal, wherein said

at least one GLP-1 CH1 deleted mimetibody comprises P or a polypeptide according to

25 Formula (I)

$$(\text{Pep}(n)\text{-L}(o)\text{-V}(p)\text{-H}(q)\text{-CH}_2(r)\text{-CH}_3(s))(t),$$

wherein P is at least one bioactive GLP-1 peptide, variant or derivative, L is selected from GS,

GGs, GGS (SEQ ID NO 16), GSGGS (SEQ ID NO 17), GSGGGS (SEQ ID NO 18),

GSGGGSGG (SEQ ID NO 19) and GGGSGGGSGG (SEQ ID NO 20), V is at least one

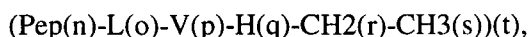
30 portion of a C-terminus of an immunoglobulin variable region, H is at least a portion of an

immunoglobulin variable hinge region, CH₂ is at least a portion of an immunoglobulin CH₂

constant region, CH₃ is at least a portion of an immunoglobulin CH₃ constant region, n is an

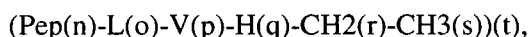
integer from 1 to 10, and o, p, q, r, s, and t can be independently an integer from 0 to 10

13 . A method for diagnosing or treating an GLP-1 diabetes related condition in a cell, tissue, organ or animal, comprising contacting or administering a composition comprising an effective amount of at least one GLP-1 agonist or mimetibody nucleic acid, polypeptide or antibody with, or to, said cell, tissue, organ or animal, wherein said
 5 at least one GLP-1 CH1 deleted mimetibody comprises P or a polypeptide according to Formula (I)



wherein P is at least one bioactive GLP-1 peptide, variant or derivative, L is at least one linker sequence, which can be a polypeptide that provides structural flexibility by allowing
 10 the mimetibody to have alternative orientations and binding properties, V is selected from GTLVTVSS (SEQ ID NO 21), GTLVAVSS (SEQ ID NO 22), GTAVTVSS (SEQ ID NO 23), TVSS (SEQ ID NO 24), and AVSS (SEQ ID NO 25), H is at least a portion of an immunoglobulin variable hinge region, CH2 is at least a portion of an immunoglobulin CH2 constant region, CH3 is at least a portion of an immunoglobulin CH3 constant region, n is an
 15 integer from 1 to 10, and o, p, q, r, s, and t can be independently an integer from 0 to 10

14 . A method for diagnosing or treating an GLP-1 diabetes related condition in a cell, tissue, organ or animal, comprising contacting or administering a composition comprising an effective amount of at least one GLP-1 agonist or mimetibody nucleic acid, polypeptide or antibody with, or to, said cell, tissue, organ or animal, wherein said
 20 at least one GLP-1 CH1 deleted mimetibody comprises P or a polypeptide according to Formula (I)



wherein P is at least one bioactive GLP-1 peptide, variant or derivative, L is at least one linker sequence, which can be a polypeptide that provides structural flexibility by allowing
 25 the mimetibody to have alternative orientations and binding properties, V is at least one portion of a C-terminus of an immunoglobulin variable region, H is selected from EPKSCDKTHTCPPCPAPELLGGP (SEQ ID NO 26), ESKYGPPCPSCPAPEFLGGP (SEQ ID NO 27), and ESKYGPPCPCPAPEAAGGP (SEQ ID NO 28), CH2 is at least a portion of an immunoglobulin CH2 constant region, CH3 is at least a portion of an immunoglobulin CH3
 30 constant region, n is an integer from 1 to 10, and o, p, q, r, s, and t can be independently an integer from 0 to 10

15 . A method for diagnosing or treating an GLP-1 diabetes related condition in a cell, tissue, organ or animal, comprising contacting or administering a

composition comprising an effective amount of at least one GLP-1 agonist or mimetibody nucleic acid, polypeptide or antibody with, or to, said cell, tissue, organ or animal, wherein said at least one GLP-1 CH1 deleted mimetibody comprises P or a polypeptide according to Formula (I)

5 (Pep(n)-L(o)-V(p)-H(q)-CH₂(r)-CH₃(s))(t),

wherein P is at least one bioactive GLP-1 peptide, variant or derivative, L is at least one linker sequence, which can be a polypeptide that provides structural flexibility by allowing the mimetibody to have alternative orientations and binding properties, V is at least one portion of a C-terminus of an immunoglobulin variable region, H is selected from

10 EPKSADKTHTCPPCPAPEAAGGP (SEQ ID NO 29), EPKSADKTHTCPPCPAPELAGGP (SEQ ID NO 30), EPKSADKTHTCPPCPAPEALGGP (SEQ ID NO 31), EPKSADKTHTCPPCPAPELEGGP (SEQ ID NO 32), EPKSSDKTHTCPPCPAPEFLGGP (SEQ ID NO 33), EPKSADKTHACPPCPAPELLGGP (SEQ ID NO 34), EPKSADKAHTCPPCPAPELLGGP (SEQ ID NO 35), and

15 EPKSADKTHTCPPCPAPELLGGP (SEQ ID NO 36), ADKTHTCPPCPAPELLGGP (SEQ ID NO 37), THTCPPCPAPELLGGP (SEQ ID NO 38), ESKYGPPCPSCPAPEAAGGP (SEQ ID NO 39), ESKYGPPCPAPELLGGP (SEQ ID NO 40), CPPCPAPELLGGP (SEQ ID NO 41), and CPPCPAPEAAGGP (SEQ ID NO 42), CH₂ is at least a portion of an immunoglobulin CH₂ constant region, CH₃ is at least a portion of an immunoglobulin CH₃

20 constant region, n is an integer from 1 to 10, and o, p, q, r, s, and t can be independently an integer from 0 to 10

16 . A method for diagnosing or treating an GLP-1 diabetes related condition in a cell, tissue, organ or animal, comprising contacting or administering a composition comprising an effective amount of at least one GLP-1 agonist or mimetibody

25 nucleic acid, polypeptide or antibody with, or to, said cell, tissue, organ or animal, wherein said at least one GLP-1 CH1 deleted mimetibody comprises P or a polypeptide according to Formula (I)

(Pep(n)-L(o)-V(p)-H(q)-CH₂(r)-CH₃(s))(t),

wherein P is at least one bioactive GLP-1 peptide, variant or derivative, L is at least one

30 linker sequence, which can be a polypeptide that provides structural flexibility by allowing the mimetibody to have alternative orientations and binding properties, V is at least one portion of a C-terminus of an immunoglobulin variable region, H is at least a portion of an immunoglobulin variable hinge region, CH₂ is at least a portion of an immunoglobulin CH₂

constant region, CH3 is at least a portion of an immunoglobulin CH3 constant region, n is an integer from 1 to 10, and o, p, q, r, s, and t can be independently an integer from 0 to 10

17 . A GLP-1 CH1 deleted mimetibody nucleic acid or GLP-1 CH1 deleted mimetibody polypeptide according to claim 1 wherein said polypeptide has at least one activity of at least one P polypeptide according to Formula I

18 . A method according to claim 1, wherein said method further comprises administering at least one composition comprising an therapeutically effective amount of at least one compound, composition or polypeptide selected from at least one of a diabetes drug, an insulin metabolism related drug, a detectable label or reporter, a TNF antagonist, an anti-infective drug, a cardiovascular (CV) system drug, a central nervous system (CNS) drug, an autonomic nervous system (ANS) drug, a respiratory tract drug, a gastrointestinal (GI) tract drug, a hormonal drug, a drug for fluid or electrolyte balance, a hematologic drug, an antineoplastic, an immunomodulation drug, an ophthalmic, otic or nasal drug, a topical drug, a nutritional drug, a cytokine, or a cytokine antagonist

19 . A method according to claim 1, wherein said effective amount is 0 001-50 mg of GLP-1 CH1 deleted mimetibody or agonist, 0 000001-500 mg of said GLP-1 CH1 deleted mimetibody or agonist, or 0 0001-100µg of said GLP-1 CH1 deleted mimetibody or agonist nucleic acid per kilogram, or equivalent concentration of said cells, tissue, organ or animal

20 . A method according to to claim 1, wherein said contacting or said administering is by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, intralesional, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal

21 . A method according to claim 1, wherein said GLP-1 related condition is a metabolic disorder

22 . A method according to claim 21, wherein said metabolic disorder is hyperglycemia

23 . A method according to claim 21, wherein said metabolic disorder is diabetes

24 . A method according to claims 21, wherein said effective amount treats said metabolic disorder by lowering blood glucose levels in an animal in need thereof

5 25 . A method according to claim 21, wherein said effective amount treats said metabolic disorder by increasing insulin secretion from insulin producing cells

26 . A method according to claim 21, wherein said effective amount treats said metabolic disorder by preventing apoptosis of insulin producing cells

10 27 . A method according to claim 21, wherein said effective amount treats said metabolic disorder increasing the proliferation of insulin producing cells

28 . A method according to claim 1, wherein said GLP-1 related condition is related to diabetes