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(54) Title: ANTI-GCGR ANTIBODIES AND USES THEREOF

Figure 1

(57) Abstract: The invention provides anti-GCGR antibodies and methods of using the same.

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ANTI-GCGR ANTIBODIES AND USES THEREOF

PRIORITY CLAIM

The present application claims the benefit of U.S. Provisional Patent Application Serial No. 61/549,255, filed on October 20, 2011, which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to anti-GCGR antibodies and methods of using the same.

BACKGROUND

There are three major types of diabetes mellitus, Type 1 diabetes mellitus (T1DM), Type 2 diabetes mellitus (T2DM) and Gestational diabetes mellitus (GDM). T1DM is an auto-immune disease where the body's immune system destroys the insulin-producing beta cells in the pancreas. Also known as juvenile-onset diabetes or insulin-dependent diabetes mellitus, T1DM accounts for 10-15% of all people with the disease. T2DM is the most common form of diabetes, affecting 85-90% of all people with diabetes. T2DM patients have a 2-4 fold increased risk of major adverse cardiovascular events. This type of diabetes is also known as late-onset diabetes or non-insulin dependent diabetes mellitus. Gestational diabetes mellitus is first diagnosed during pregnancy through an oral glucose tolerance test. While GDM usually returns to normal after the birth, the mother has a significant risk of developing permanent diabetes while the baby is more likely to develop obesity and impaired glucose tolerance and/or diabetes later in life.

T1DM is caused by insulin deficiency (insulin-dependent) and can be corrected by insulin replacement therapy. T2DM is a multifactorial disease and its treatment is not dependent on insulin therapy alone. Available medications for T2DM come in various classes — alpha-glucosidase inhibitors, amylin agonists, dipeptidyl-peptidase 4 (DPP-4) inhibitors, meglitinides, sulfonylureas and thiazolidinediones.

Glucagon is a 29-amino-acid peptide hormone that is synthesized mainly in pancreatic alpha-cells at the periphery of the islets of Langerhans. It is released in response to hypoglycemia i.e. a decrease in blood glucose levels induced by insulin.

The pancreas releases glucagon when blood sugar (glucose) levels fall too low. Glucagon is involved in several processes that result overall in the production of glucose and its release thus alleviating the hypoglycemic condition in the bloodstream. Glucagon causes the liver to convert stored glycogen into glucose, which is released into the bloodstream in a process known as glycogenolysis. As these hepatic glycogen stores become depleted, glucagon then encourages the liver to synthesize additional glucose by gluconeogenesis. Gluconeogenesis is the pathway by which non-hexose substrates such as amino acids are converted to glucose. As such, it provides another source of glucose for blood. Glucagon turns off glycolysis in the liver, causing glycolytic intermediates to be shuttled to gluconeogenesis. Glucagon also regulates the rate of glucose production through lipolysis.

T2DM patients typically exhibit an increased glucagon insulin ratio with a concomitant increase in blood glucose levels (Baron, Shafer et al. Diabetes 1987, Basu, Shah et al., J Investig Med 2004, others). Inhibiting glucagon secretion in T2DM patients significantly reduces blood glucose levels (somatostatin references, i.e. Baron, Shaeffer et al, Diabetes 1987; Raskin, Unger NEJM 1978, Gerich, Lorenzi et al, NEJM 1975).

Glucagon binds to and acitvates the glucagon receptor (GCGR), a 62 kDa class B G protein-coupled receptor. Members of this class are characterized by a large (~ 110 amino acids) N-terminal extracellular domain (ECD), a core seven alpha-helix transmembrane region and an intracellular carboxyl terminus. Both the ECD and the transmembrane (juxtamembrane) regions of these receptors are important determinants for peptide hormone recognition and binding.

To start the process of glycogenolysis, glucagon binds to the G protein-coupled glucagon receptor, located in the plasma membrane. The conformation change in the receptor activates G-proteins, a heterotrimeric protein with α, β, and γ subunits. When the G protein interacts with the receptor, it undergoes a conformational change that results in the replacement of the GDP molecule that was bound to the α-subunit with a GTP molecule. This substitution results in the releasing of the α-subunit from the β and γ subunits. The alpha subunit specifically activates the next enzyme in the cascade, adenylate cyclase. Adenylate cyclase manufactures cyclic adenosine monophosphate (cyclic AMP or cAMP), which activates protein kinase A (cAMP-dependent protein kinase). This enzyme, in turn, activates phosphorylase kinase, which, in turn, phosphorylates glycogen phosphorylase, converting into the active form called phosphorylase A.

Phosphorylase A is the enzyme responsible for the release of glucose-1-phosphate from glycogen polymers stores in hepatocytes.

There remain significant needs for better diabetis treatments, particularly T2DM. Given the important role of glucagon and GCGR in balancing blood glucose level, agents aiming at blocking their activities represent viable candidates as better therapeutics for hyperclycemia, T2DM and related disorders.

SUMMARY

The present invention is in part based on the rational generation of novel anti-GCGR antibodies with desired glucagon receptor (GCGR) binding and biological activities. In one aspect, the invention provide an anti-GCGR antibody capable of blocking GCGR binding to glucagon, wherein the antibody comprises (a) HVR L3 comprising the amino acid sequence of $QQYX_1X_2YLX_3T$ (SEQ ID NO:13); and (b) HVR H1 comprising the amino acid sequence of $GFNIYYX_4YIH$ (SEQ ID NO:14); wherein X_1 is S or G, X_2 is Y or S, X_3 is F or Y, and X_4 is S or N . In another aspect, the anti-GCGR antibody further comprises HVR H2 comprising the amino acid sequence of $AX_5X_6SPYSGSTYYADSVKG$ (SEQ ID NO:15); wherein X_5 is S or E, and X_6 is I, F or M. In another aspect, the anti-GCGR antibody also comprises HVR H3 comprising the amino acid sequence of $ARSX_7X_8IVX_9WYX_{10}YFX_{11}GFDY$ (SEQ ID NO:16); wherein X_7 is T or A, X_8 is S or A, X_9 is Y or D, X_{10} is E or D, and X_{11} is E or K. The above antibody can further comprise HVR H2 comprising the amino acid sequence of $AX_5X_6SPYSGSTYYADSVKG$ (SEQ ID NO:15); wherein X_5 is S or E, and X_6 is I, F or M. Other structural features of the anti-GCGR antibodies of the invention include, but not limited to, further comprising HVR L1 comprising the amino acid sequence of RASQSVSSAVA (SEQ ID NO:4); and further comprising HVR L2 comprising the amino acid sequence of SASSLYS (SEQ ID NO:5).

In another aspect, the invention provides an anti-GCGR antibody comprising a light chain variable domain comprising three light chain HVRs and a heavy chain variable domain comprising three heavy chain HVRs, wherein: (a) HVR-L1 comprises the amino acid sequence of RASQSVSSAVA (SEQ ID NO:4); (b) HVR-L2 comprises the amino acid sequence of SASSLYS (SEQ ID NO:5); (c) HVR-L3 comprises the amino acid sequence of $QQYX_1X_2YLX_3T$ (SEQ ID NO:13), wherein X_1 is S or G, X_2 is Y or S and X_3 is F or Y; (d) HVR-H1 comprises the amino

acid sequence of GFNIYYX$_4$YIH (SEQ ID NO:14), wherein X$_4$ is S or N; (e) HVR-H2 comprises the amino acid sequence of AX$_5$X$_6$SPYSGSTYYADSVKG (SEQ ID NO:15); wherein X$_5$ is S or E, and X$_6$ is I, F or M; and (f) HVR-H3 comprises the amino acid sequence of ARSX$_7$X$_8$IVX$_9$WYX$_{10}$YFX$_{11}$GFDY (SEQ ID NO:16); wherein X$_7$ is T or A, X$_8$ is S or A, X$_9$ is Y or D, X$_{10}$ is E or D, and X $_{11}$ is E or K. In one embodiment, the antibody comprises (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:25; (b) a VL sequence having at least 95 % sequence identity to the amino acid sequence of SEQ ID NO:26; or (c) a VH sequence as in (a) and a VL sequence as in (b). In another embodiment, the antibody comprises a VH sequence of SEQ ID NO:25. In yet another embodiment, the antibody comprises a VL sequence of SEQ ID NO:26.

In one aspect, the invention provides an anti-GCGR antibody comprising a light chain variable domain comprising the amino acid sequence of SEQ ID NO:26 and a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:25.

In another aspect, the invention provides an anti-GCGR antibody capable of blocking GCGR binding to glucagon, wherein the antibody comprises (a) HVR L1 comprising the amino acid sequence of RSSQSLVHSNGNTYLH (SEQ ID NO:30); and (b) HVR H2 comprising the amino acid sequence of GYLGFX$_{13}$GSTYYNPSLKS (SEQ ID NO:37), wherein X$_{13}$ is S or T. In one further embodiment, the antibody also comprises HVR L2 comprising the amino acid sequence of KVSNRFS (SEQ ID NO:31). In another futher embodiment, the antibody also comprises HVR L3 comprising the amino acid sequence of SQSTHX$_{14}$PWT (SEQ ID NO:50), wherein X$_{14}$ is V or S. In a preferrd embodiment, the antibody further comprises HVR H1 comprising the amino acid sequence of GDX$_{12}$ITSGFWN (SEQ ID NO:36), wherein X$_{12}$ is S or T and/or HVR H3 comprising the amino acid sequence of ASVDNSAALDY (SEQ ID NO:29).

In another aspect, the invention provides an anti-GCGR antibody comprising a light chain variable domain comprising three light chain HVRs and a heavy chain variable domain comprising three heavy chain HVRs, wherein: (a) HVR-L1 comprises the amino acid sequence of RSSQSLVHSNGNTYLH (SEQ ID NO:30); (b) HVR-L2 comprises the amino acid sequence of KVSNRFS (SEQ ID NO:31); (c) HVR-L3 comprises the amino acid sequence of SQSTHX$_{14}$PWT (SEQ ID NO:50), wherein X$_{14}$ is V or S; (d) HVR-H1 comprises the amino acid sequence of GDX$_{12}$ITSGFWN (SEQ ID NO:36), wherein X$_{12}$ is S or T; (e) HVR-H2 comprises the amino acid

sequence of GYLGFX$_{13}$GSTYYNPSLKS (SEQ ID NO:37), wherein X$_{13}$ is S or T; and (f) HVR-H3 comprises the amino acid sequence of ASVDNSAALDY (SEQ ID NO:29).

In one embodiment, the antibody comprises (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:46; (b) a VL sequence having at least 95 % sequence identity to the amino acid sequence of SEQ ID NO:47; or a VH sequence as in (a) and a VL sequence as in (b). In a preferred embodiment, the antibody comprises a VH sequence of SEQ ID NO:46; in another embodiment, the antibody comprises a VL sequence of SEQ ID NO:47.

In another aspect, the invention provides an anti-GCGR antibody comprising a light chain variable domain comprising the sequence of SEQ ID NO 47 and a heavy chain variable domain comprising the sequence of SEQ ID NO 46.

In certain aspect of the invention, the antibodies as described above are monoclonal antibodies. In one aspect, they are human, humanized, or chimeric antibodies. In another aspect, they are antibody fragments that bind GCGR. The antibodies can be of any type or subclass of the antibody, such as IgG1, IgG2, IgG3 or IgG4 antibody. The antibody can be effectorless variants. One example of effectorless antibody comprises a substitution in the constant region at, for example, N297A.

In another aspect, the inventions provides isolated nucleic acids encoding the antibodyies as described above, also provided are host cells comprising such nucleic acids. In another aspect, the invention provides an immunoconjugate comprising the antibody according to any of claims 1-28 and a cytotoxic agent. Also provided by the invention are pharmaceutical formulations that comprise the anti-GCGR antibodies as above and a pharmaceutically acceptable carrier.

In one aspect, the invention provides methods of preventing or treating glucagon-related disorders in an individual in need of such treatment comprising administering to the individual an effective amount of the anti-GCGR antibody of the invention. Preferrably, the glucagon-related disorders are selected from type1 or type 2 diabetes, hyperglycemia, impaired fasting glucose, impaired glucose tolerance, dyslipidemia, and metabolic syndrome.

In other aspects, the invention provides uses of the anti-GCGR antibodies described herein in the preparation of a medicament. In one embodiment, the medicament is for preventing or treating glucagon-related disorders.

The anti-GCGR antibodies of the invention can be used as a medicament. In one aspect the antibodies of the invention can be used in treating a glucagon-related disorder.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts the variable domain sequences of the phage anti-GCGR antibody H6.23v1 and three of its affinity matured variants v2-v4. H6.23v1-v4 have the same light chain variable domain sequences. HVRs are marked by boxes and affinity matured residue substitutions are underlined.

Figure 2 depicts the variable domain sequences of the humanized anti-CGR antibody 3C7v1 and two of its affinity matured variants v11 and v16. 3C7v1/v11/v16 have the same light chain domain sequences. HVRs are marked by boxes and affinity matured residue substitutions are underlined.

Figure 3 depicts inhibition of ^{125}I- glucagon binding to primary human hepatocytes by H6.23 series competitive antibodies.

Figures 4A-B show the inhibition of glucagon-induced G6Pase gene expression in primary human heptocytes. (A) H6.23 series antibodies. (B) 3C7 series antibodies.

Figure 5 depicts blood glucose in db/db mice 24 hours after a single dose of H6.23v2. The bar graphs show fasting blood glucose (mg/dL) versus H6.23v2 mAb (mg/kg) at various concentrations (mg/kg).

Figure 6 depicts that a single dose of H6.23.v3 provides acute reduction in blood glucose in Zucker Diabetic Fatty (ZDF) rats.

Figure 7 depicts that a single dose of H6.23.v3 improves glucose tolerance in diabetic ZDF rats.

DETAILED DESCRIPTION OF THE INVENTION

I. DEFINITIONS

The term "Glucagon Receptor" or "GCGR," as used interchangeably herein, refers to any native GCGR from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed GCGR as well as any form of GCGR that result from processing in the cell. The term also encompasses naturally occurring variants of GCGR, e.g., splice variants or allelic variants. In one embodiment, GCGR is the human GCGR according to MacNeil et al., *Biochem. Biophys. Res. Commun.* 198(1), 328-34 (1994), having the amino acid sequence of SEQ ID NO:48.

The terms "anti-GCGR antibody" and "an antibody that binds to GCGR" refer to an antibody that is capable of binding GCGR with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting GCGR. In one embodiment, the extent of binding of an anti-GCGR antibody to an unrelated, non-GCGR protein is less than about 10% of the binding of the antibody to GCGR as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to GCGR has a dissociation constant (Kd) of $\leq 1\mu M$, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M). In certain embodiments, an anti-GCGR antibody binds to an epitope of GCGR that is conserved among GCGR from different species.

An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

"Affinity" refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

An "affinity matured" antibody refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.

The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.

An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, $F(ab')_2$; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments.

An "antibody that binds to the same epitope" as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. An exemplary competition assay is provided herein.

The term "chimeric" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively.

"Effector functions" refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor); and B cell activation.

An "effective amount" of an agent, e.g., a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

"Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

The terms “full length antibody,” “intact antibody,” and “whole antibody” are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

A “human antibody” is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

A “human consensus framework” is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al., *supra.* In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al., *supra.*

A “humanized” antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region

derived from a human antibody. A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

The term "hypervariable region" or "HVR," as used herein, refers to each of the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops ("hypervariable loops"). Generally, native four-chain antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). HVRs generally comprise amino acid residues from the hypervariable loops and/or from the "complementarity determining regions" (CDRs), the latter being of highest sequence variability and/or involved in antigen recognition. Exemplary hypervariable loops occur at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3). (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987).) Exemplary CDRs (CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3) occur at amino acid residues 24-34 of L1, 50-56 of L2, 89-97 of L3, 31-35B of H1, 50-65 of H2, and 95-102 of H3. (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991).) With the exception of CDR1 in VH, CDRs generally comprise the amino acid residues that form the hypervariable loops. CDRs also comprise "specificity determining residues," or "SDRs," which are residues that contact antigen. SDRs are contained within regions of the CDRs called abbreviated-CDRs, or a-CDRs. Exemplary a-CDRs (a-CDR-L1, a-CDR-L2, a-CDR-L3, a-CDR-H1, a-CDR-H2, and a-CDR-H3) occur at amino acid residues 31-34 of L1, 50-55 of L2, 89-96 of L3, 31-35B of H1, 50-58 of H2, and 95-102 of H3. (See Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008).) Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., *supra*.

An "individual" or "subject" is a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human.

An "isolated" antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of

methods for assessment of antibody purity, see, e.g., Flatman et al., *J. Chromatogr. B* 848:79-87 (2007).

An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

"Isolated nucleic acid encoding an anti-GCGR antibody" refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

"Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are

disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

"Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino

acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject., A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or to slow the progression of a disease.

The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (See, e.g., Kindt et al. *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, e.g., Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

II. COMPOSITIONS AND METHODS

A. Exemplary Anti-GCGR Antibodies

H6.23 Series

In one aspect, the invention provides an anti-GCGR antibody H6.23v3 comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6.

In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:3. In one embodiment, the antibody

comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:10. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:10 and HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:3, HVR-L3 comprising the amino acid sequence of SEQ ID NO:11, and HVR-H2 comprising the amino acid sequence of SEQ ID NO:2. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:7; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:10.

In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:11.

In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:3; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:8; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:10; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:11.

In any of the above embodiments, an anti-GCGR antibody is humanized. In one embodiment, an anti-GCGR antibody comprises HVRs as in any of the above embodiments, and further comprises an acceptor human framework, e.g. a human immunoglobulin framework or a human consensus framework. In another embodiment, an anti-GCGR antibody comprises HVRs as in any of the above embodiments, and further comprises a VL comprising an FR1, FR2, FR3 and FR4 sequence of SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19 and SEQ ID NO:20 respectively. In another embodiment, an anti-GCGR antibody comprises HVRs as in any of the above embodiments, and further comprises a VH comprising an FR1, FR2, FR3 and FR4 sequence of SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23 and SEQ ID NO:24.

In another aspect, an anti-GCGR antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:25. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-GCGR antibody comprising that sequence retains the ability to bind to GCGR. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:25 In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-GCGR antibody comprises the VH sequence of SEQ ID NO:25, including post-translational modifications of that sequence.

In another aspect, an anti-GCGR antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:26. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-GCGR antibody comprising that sequence retains the ability to bind to PRO. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:26. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-

GCGR antibody comprises the VL sequence of SEQ ID NO:26, including post-translational modifications of that sequence.

In another aspect, an anti-GCGR antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:25 and SEQ ID NO:26, respectively, including post-translational modifications of those sequences.

3C7 Series:

In one aspect, the invention provides an anti-GCGR antibody 3C7v16, comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:27; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:28; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:29; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:30; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:31; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:32.

In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:34; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:33; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:29. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:29 and HVR-L3 comprising the amino acid sequence of SEQ ID NO:32. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:29, HVR-H1 comprising the amino acid sequence of SEQ ID NO:34, and HVR-H2 comprising the amino acid sequence of SEQ ID NO:35. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:34; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:33; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:29.

In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:30; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:31; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:32. In one embodiment, the

antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:30; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:31; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:32.

In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:30; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:31; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:49. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:30; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:31; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:49.

In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:34, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:35, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:29; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:30, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:31, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:32.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:34; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:28; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:29; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:30; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:31; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:32.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:34; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:28; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:29; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:30; (e) HVR-L2 comprising the amino acid

sequence of SEQ ID NO:31; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:49.

In any of the above embodiments, an anti-GCGR antibody is humanized. In one embodiment, an anti-GCGR antibody comprises HVRs as in any of the above embodiments, and further comprises an acceptor human framework, e.g. a human immunoglobulin framework or a human consensus framework. In another embodiment, an anti-GCGR antibody comprises HVRs as in any of the above embodiments, and further comprises a VL comprising an FR1, FR2, FR3 and FR4 sequence of SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40 and SEQ ID NO:41 respectively. In another embodiment, an anti-GCGR antibody comprises HVRs as in any of the above embodiments, and further comprises a VH comprising an FR1, FR2, FR3 and FR4 sequence of SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44 and SEQ ID NO:45. In SEQ ID NO:44, RVTISR*DTSKNQY*SLKLSSVTAADTAVYYC, the R* and Y* are framework mutations compared with the humanized 3C7-K2H4.

In another aspect, an anti-GCGR antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:46. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-GCGR antibody comprising that sequence retains the ability to bind to GCGR. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:46. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-GCGR antibody comprises the VH sequence in SEQ ID NO:46, including post-translational modifications of that sequence.

In another aspect, an anti-GCGR antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:47. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-GCGR antibody comprising that sequence retains

the ability to bind to PRO. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:47. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-GCGR antibody comprises the VL sequence in SEQ ID NO:47, including post-translational modifications of that sequence.

In another aspect, an anti-GCGR antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:46 and SEQ ID NO:47, respectively, including post-translational modifications of those sequences.

Amino acid sequences for various anti-GCGR antibody fragments of the present invention, including, e.g., light chain and heavy chain variable domains, HVRs and frameworks, are listed as follows:

6.23V3
HVR-H1
SEQ ID NO:1
GFNIYYNYIH

6.23V3
HVR-H2
SEQ ID NO:2
AEFSPYSGSTYYADSVKG

6.23V3
HVR-H3
SEQ ID NO:3
ARSAAIVDWYDYFKGFDY

6.23V3
HVR-L1
SEQ ID NO:4
RASQSVSSAVA

6.23V3
HVR-L2
SEQ ID NO:5
SASSLYS

6.23V3
HVR-L3
SEQ ID NO:6
QQYGSYLYT

6.23V2
HVR-H2
SEQ ID NO:7
AEISPYSGSTYYADSVKG

6.23V1
HVR-H1
SEQ ID NO:8
GFNIYYSYIH

6.23V1
HVR-H2
SEQ ID NO:9
ASISPYSGSTYYADSVKG

6.23V1
HVR-H3
SEQ ID NO:10
ARSTSIVYWYEYFEGFDY

6.23V1
HVR-L3
SEQ ID NO:11
QQYSYYLFT

6.23V4
HVR-H2
SEQ ID NO:12
AEMSPYSGSTYYADSVKG

6.23VL VARIATION
HVR-L3
SEQ ID NO:13
QQYX1X2YLX3T (X1 = S or G; X2 = Y or S; X3 = F or Y).

6.23VH VARIATION
HVR-H1
SEQ ID NO:14
GFNIYYX4YIH (X4 =S or N)

6.23VH VARIATION
HVR-H2
SEQ ID NO:15
AX5X6SPYSGSTYYADSVKG (X5 = S or E; X6 = I, F or M)

6.23VH VARIATION
HVR-H3
SEQ ID NO:16
ARSX7X8IVX9WYX10YFX11GFDY (X7 = T or A; X8 = S or A; X9 = Y or D; X10 = E or D; X11 = E or K)

6.23VL framework
FRL1
SEQ ID NO:17
DIQMTQSPSSLSASVGDRVTITC

6.23VL framework
FRL2
SEQ ID NO:18
WYQQKPGKAPKLLIY

6.23VL framework
FRL3
SEQ ID NO:19
GVPSRFSGSRSGTDFTLTISSLQPEDFATYYC

6.23VL framework

FRL4
SEQ ID NO:20
FGQGTKVEIK

6.23VH framework
FRH1
SEQ ID NO:21
EVQLVESGGGLVQPGGSLRLSCAAS

6.23VH framework
FRH2
SEQ ID NO:22
WVRQAPGKGLEWV

6.23VH framework
FRH3
SEQ ID NO:23
RFTISADTSKNTAYLQMNSLRAEDTAVYYC

6.23VH framework
FRH4
SEQ ID NO:24
WGQGTLVTVSS

6.23V3 VH
VH
SEQ ID NO:25
EVQLVESGGGLVQPGGSLRLSCAASGFNIYYNYIHWVRQAPGKGLEWVAEFSPYSGSTYYADSVKGRFTISADTSKNTA
YLQMNSLRAEDTAVYYCARSAAIVDWYDYFKGFDYWGQGTLVTVSS

6.23V3 VL
VL
SEQ ID NO:26
DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFTLTISSLQ
PEDFATYYCQQYGSYLYTFGQGTKVEIK

3C7v16
HVR H1
SEQ ID NO:27
GDTITSGFWN

3C7v16
HVR H2
SEQ ID NO:28
GYIGFTGSTYYNPSLKS

3C7v16
HVR H3
SEQ ID NO:29
ASVDNSAALDY

3C7v16
HVR L1
SEQ ID NO:30
RSSQSLVHSNGNTYLH

3C7v16
HVR L2
SEQ ID NO:31
KVSNRFS

3C7v16
HVR L3
SEQ ID NO:32
SQSTHVPWT

3c7V1
HVR H2
SEQ ID NO:33
GYIGFSGSTYYNPSLKS

3C7V1
HVR H1
SEQ ID NO:34
GDSITSGFWN

3C7V11
HVR H2
SEQ ID NO:35
GYIGFTGSTYYNPSLKS

3C7 VH VARIATION
HVR H1
SEQ ID No:36
GDX12ITSGFWN (X12 = S or T)

3C7 VH VARIATION
HVR H2
SEQ ID NO:37
GYIGFX13GSTYYNPSLKS (X13 = S or T)

3C7 VLframework
FRL1
SEQ ID NO:38
DIVMTQTPLSLPVTPGQPASISC

3C7 VLframework
FRL2
SEQ ID NO:39
WYLQKPGQSPQLLIY

3C7 VLframework
FRL3
SEQ ID NO:40
GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC

3C7 VLframework
FRL4
SEQ ID NO:41
FGQGTKVEIK

3C7 VH framework
FRH1
SEQ ID NO:42
EVQLVESGPGLVKPSETLSLTCTVS

3C7 VH framework
FRH2
SEQ ID NO:43
WIRQPPGKGLEWI

3C7 VH framework

FRH3
SEQ ID NO:44
RVTISR*DTSKNQY*SLKLSSVTAADTAVYYC
R* AND Y* are framework mutations compared with 3C7-K2H4 reference

3C7 VH framework
FRH4
SEQ ID NO:45
WGQGTLVTVTVSS

3C7 VH
SEQ ID NO:46
EVQLVESGPGLVKPSETLSLTCTVSGDTITSGFWNWIRQPPGKGLEWIGYIGFTGSTYYNPSLKSRVTISRDTSKNQYS
LKLSSVTAADTAVYYCASVDNSAALDYWGQGTLVTVSS

3C7 VL
SEQ ID NO:47
DIVMTQTPLSLPVTPGQPASISCRSSQSLVHSNGNTYLHWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCSQSTHVPWTFGQGTKVEIK

3C7 V50
HVR L3
SEQ ID No:49
SQSTHSPWT

3C7 VL VARIATION
HVR L3
SEQ ID No:50
SQSTHX14PWT (X14 = S or V)

In a further aspect, the invention provides an antibody that binds to the same epitope as an anti-GCGR antibody provided herein. For example, in certain embodiments, an antibody is provided that binds to the same epitope as an anti-GCGR antibody H6.23v2 and/or 3C7. In certain embodiments, an antibody is provided that binds to an epitope within GCGR consisting of amino acids L50, F62, Y65, L85, W87, K90 and R94 of SEQ ID NO:48. In another aspect, an antibody is provided that binds to an epitope within GCGR consisting of amino acids F33, K35 W36 , K37, Y39, G40 , N41, P72, N74, T75, P82, W83, Y84, L85, P86, W87 of SEQ ID NO:48. Epitope for each mAb is mapped using alanine scanning of the ECD of GCGR expressed on the surface of phage particles.

In a further aspect of the invention, an anti-GCGR antibody according to any of the above embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody. In one embodiment, an anti-GCGR antibody is an antibody fragment, e.g., a Fv, Fab, Fab', scFv, diabody, or $F(ab')_2$ fragment. In another embodiment, the antibody is a full length antibody, e.g., an intact Phage-derived (e.g., from a YSGX library), Effectorless (N297A), IgG1/kappa LC antibody or other antibody class or isotype as defined herein. In a further aspect, an anti-GCGR

antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 1-7 below:

1. Antibody Affinity

In certain embodiments, an antibody provided herein has a dissociation constant (Kd) of ≤ 1μM, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M).

In one embodiment, Kd is measured by a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen as described by the following assay. Solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, e.g., Chen et al., *J. Mol. Biol.* 293:865-881(1999)). To establish conditions for the assay, MICROTITER® multi-well plates (Thermo Scientific) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (e.g., consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., *Cancer Res.* 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, 150 μl/well of scintillant (MICROSCINT-20 ™; Packard) is added, and the plates are counted on a TOPCOUNT ™ gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

According to another embodiment, Kd is measured using surface plasmon resonance assays using a BIACORE®-2000 or a BIACORE®-3000 (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran

biosensor chips (CM5, BIAcore, Inc.) are activated with *N*-ethyl-*N'*- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 μg/ml (~0.2 μM) before injection at a flow rate of 5 μl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25°C at a flow rate of approximately 25 μl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio k_{off}/k_{on}. See, e.g., Chen et al., *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds 10^6 M^{-1} s^{-1} by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

Kinetic binding measurements can also be performed on an Octet Red instrument (ForteBio, Menlo Park, CA, USA). For example, all washes, dilutions and measurements are performed in Octet buffer (0.2% dodecylmaltoside, or DDM, – PBS) with the plate shaking at 1000 rpm. Streptavidin biosensors are equilibrated in Octet buffer for 10 min and then loaded with biotinylated GCGR (from viral lysate in 1% DDM, diluted 1:8 in Octet Buffer) for 5 min and washed for 10 min. For the association phase, the ligand-coated streptavidin tips are immersed in anti-GCGR antibody fragments for 10 min (eight serial two-fold dilutions, starting at 500 or 50 nM). Dissociation of the Ab-GCGR complex can be measured in wells containing Octet buffer alone for 600 s. KD, Ka and Kd are determined with Octet evaluation software v6.3 using a 1:1 binding model with global fitting.

2. *Antibody Fragments*

In certain embodiments, an antibody provided herein is an antibody fragment. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, $F(ab')_2$, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, see Hudson et al. *Nat. Med.* 9:129-134 (2003). For a review of scFv fragments, see, e.g., Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and $F(ab')_2$ fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046.

Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., *Nat. Med.* 9:129-134 (2003); and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., *Nat. Med.* 9:129-134 (2003).

Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; *see,* e.g., U.S. Patent No. 6,248,516 B1).

Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. *E. coli* or phage), as described herein.

3. *Chimeric and Humanized Antibodies*

In certain embodiments, an antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, e.g., in U.S. Patent No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (e.g., a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, e.g., CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the HVR residues are derived), e.g., to restore or improve antibody specificity or affinity.

Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, e.g., in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, *Methods* 36:25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer*, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, e.g., Sims et al. *J. Immunol.* 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter et al. *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); and Presta et al. *J. Immunol.*, 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (see, e.g., Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, e.g., Baca et al., *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok et al., *J. Biol. Chem.* 271:22611-22618 (1996)).

4. Human Antibodies

In certain embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are

described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008).

Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat. Biotech.* 23:1117-1125 (2005). See also, e.g., U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HUMAB® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE® technology). Human variable regions from intact antibodies generated by such animals may be further modified, e.g., by combining with a different human constant region.

Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, e.g., Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., *J. Immunol.*, 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, *Xiandai Mianyixue*, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then

be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

5. Library-Derived Antibodies

Antibodies of the invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, e.g., in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132(2004).

In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al., *EMBO J,* 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

Antibodies of the invention can also be obtained essentially as described by Hötzel et al. (Protein Engineering Design and Selection 24:679-689, 2011) using biotinylated GCGR expressed in baculovirus particles and solubilizing the biotinylated GCGR to select phage display libraries.

Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

6. Multispecific Antibodies

In certain embodiments, an antibody provided herein is a multispecific antibody, e.g. a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding specificities is for GCGR and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of GCGR. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express GCGR. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, *Nature* 305: 537 (1983)), WO 93/08829, and Traunecker et al., *EMBO J.* 10: 3655 (1991)), and "knob-in-hole" engineering (see, e.g., U.S. Patent No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (see, e.g., US Patent No. 4,676,980, and Brennan et al., *Science*, 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (see, e.g., Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992)); using "diabody" technology for making bispecific antibody fragments (see, e.g., Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (see,e.g. Gruber et al., *J. Immunol.*, 152:5368 (1994)); and preparing trispecific antibodies as described, e.g., in Tutt et al. *J. Immunol.* 147: 60 (1991).

Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (see, e.g. US 2006/0025576A1).

The antibody or fragment herein also includes a "Dual Acting FAb" or "DAF" comprising an antigen binding site that binds to GCGR as well as another, different antigen (see, US 2008/0069820, for example).

*7. **Antibody Variants***

In certain embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding.

a) Substitution, Insertion, and Deletion Variants

In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table 1 under the heading of "conservative substitutions." More substantial changes are provided in Table 1 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

Table 1

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val

Original Residue	Exemplary Substitutions	Preferred Substitutions
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr

Original Residue	Exemplary Substitutions	Preferred Substitutions
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

Amino acids may be grouped according to common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.* a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g. binding affinity).

Alterations (e.g., substitutions) may be made in HVRs, e.g., to improve antibody affinity. Such alterations may be made in HVR "hotspots," i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (e.g., 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR "hotspots" or SDRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or

eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g. for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

b) Glycosylation variants

In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at

about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, e.g., US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8,* knockout CHO cells (see, e.g., Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

Antibodies variants are further provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, e.g., in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana *et al.*). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

c) **Fc region variants**

In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (*e.g.*, a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (*e.g.* a substitution) at one or more amino acid positions.

In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express Fc(RIII only, whereas monocytes express Fc(RI, Fc(RII and Fc(RIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (see, e.g. Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (see Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in a animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996); Cragg, M.S. et al., *Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half life determinations can also be performed using methods known in the art (see, e.g., Petkova, S.B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006)).

Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269,

270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

Certain antibody variants with improved or diminished binding to FcRs are described. (See, e.g., U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.* 9(2): 6591-6604 (2001).)

In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, e.g., substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

In some embodiments, alterations are made in the Fc region that result in altered (*i.e.*, either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie et al. *J. Immunol.* 164: 4178-4184 (2000).

Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (US Patent No. 7,371,826).

See also Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

d) <u>Cysteine engineered antibody variants</u>

In certain embodiments, it may be desirable to create cysteine engineered antibodies, e.g., "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible

sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described, e.g., in U.S. Patent No. 7,521,541.

e) **Antibody Derivatives**

In certain embodiments, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

In another embodiment, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam et al., *Proc. Natl. Acad. Sci. USA* 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

B. Recombinant Methods and Compositions

Antibodies may be produced using recombinant methods and compositions, e.g., as described in U.S. Patent No. 4,816,567. In one embodiment, isolated nucleic acid encoding an anti-GCGR antibody described herein is provided. Such nucleic acid may encode an amino acid sequence comprising the VL and/or an amino acid sequence comprising the VH of the antibody (e.g., the light and/or heavy chains of the antibody). In a further embodiment, one or more vectors (e.g., expression vectors) comprising such nucleic acid are provided. In a further embodiment, a host cell comprising such nucleic acid is provided. In one such embodiment, a host cell comprises (e.g., has been transformed with): (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and an amino acid sequence comprising the VH of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the VH of the antibody. In one embodiment, the host cell is eukaryotic, e.g. a Chinese Hamster Ovary (CHO) cell or lymphoid cell (e.g., Y0, NS0, Sp20 cell). In one embodiment, a method of making an anti-GCGR antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

For recombinant production of an anti-GCGR antibody, nucleic acid encoding an antibody, e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, e.g., U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523. (See also Charlton, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody

fragments in *E. coli*.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. See Gerngross, *Nat. Biotech.* 22:1409-1414 (2004), and Li et al., *Nat. Biotech.* 24:210-215 (2006).

Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures can also be utilized as hosts. *See,* e.g., US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants).

Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, e.g., in Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR$^-$ CHO cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki and Wu, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003).

C. Assays

Anti-GCGR antibodies provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

1. Binding assays and other assays

In one aspect, an antibody of the invention is tested for its antigen binding activity, e.g., by known methods such as ELISA, Western blot, etc.

In another aspect, competition assays may be used to identify an antibody that competes with the anti-GCGR antibody 6.23 for binding to GCGR. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by 6.23. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, NJ).

In an exemplary competition assay, immobilized GCGR is incubated in a solution comprising a first labeled antibody that binds to GCGR (e.g., 6.23) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to GCGR. The second antibody may be present in a hybridoma supernatant. As a control, immobilized GCGR is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to GCGR, excess unbound antibody is removed, and the amount of label associated with immobilized GCGR is measured. If the amount of label associated with immobilized GCGR is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to GCGR. See Harlow and Lane (1988) *Antibodies: A Laboratory Manual* ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

2. Activity assays

In certain embodiments , assays are provided for identifying anti-GCGR antibodies thereof having biological activity. Biological activities of the antibodies of the invention may include, but not limited to, binding extracellular domains of GCGR expressed in cells, inhibiting glucagon binding to cells expressing GCGR, inhibiting glucagon-induced cAMP production in cells expressing GCGR, inhibiting glucagon-induced cAMP response element luciferase reporter activity in cells expressing GCGR, inhibiting glucagon-induced gene expression in primary hepatocytes, inhibiting glucagon-induced glucose production in mice, reducing fed and fasted blood glucose in diabetic mice and rats, improving glucose tolerance in diabetic mice and rats, increasing serum amino acids, glucagon and glp-1 levels in mice, rats and cynomolgous monkeys, promoting alpha cell hyperplasia in mice and rats. Antibodies having such biological activity in vivo and/or in vitro are also provided.

Various assays for testing the biological activities of the antibodies of the invention are known and readily available. For example, Fluorescent Assisted Cell Sorting (FACS) can be used to measure antibody binding to target receptors expressed on cell surface. Scatchard analysis of radio-labeled glucagon, such as I^{125}-glucagon, can be used to measure antibody's inhibitory activities on glucagon's binding to cells expressing GCGR. Cell-based reporter assay, such as a cAMP response element (CRE)-luciferase construct, can be used to test antibody's inhibition of glucagon-induced cAMP activity in the presence of GCGR. Glucagon-induced gene expression in primary hepatocytes and inhibition of such glucagon activity by the antibody of the invention can be measured using a branched chain sandwich nucleic acid hybridization assay to measure the amount of mRNA of known glucagon target genes such as PEPCK, G6Pase and Pgc1alpha.

Glucagon-induced hepatic glucose output in mice and inhibition of glucagon activity in vivo after pre-administration of antibody to the mice by injection can be measured. For example, blood glucose rises rapidly after injection of a given concentration of glucagon and blood glucose levels are measured in fasted mice before antibody administration, 1 hour after antibody administration, and 5, 10, 15, 20, 30, 60, 90 and 120 minutes after glucagon administration. Blood glucose can be measured in fasting or fed mice or rats or cynomolgous monkeys 1, 3, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, 312 and 336 hours after administration of a single dose of antibody. Serum amino acid levels (all 20 protein encoding amino acids and ornithine) are

measured using HPLC of derivatized amino acids in fasting or fed mice or rats or cynomolgous monkeys 1, 3, 8, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, 312 and 336 hours after administration of a single dose of antibody. Serum glucagon and total glucagon-like peptide 1 levels are measured using ELISA in fasting or fed mice or rats or cynomolgous monkeys 1, 3, 8, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, 312 and 336 hours after administration of a single dose of antibody. Glucose tolerance is measured in fasting mice or rats 1, 3, 4, or 8 days after administration of a single or multiple doses of antibody. Alpha cell area is calculated in pancreatic sections isolated from mice or rats 1, 3, 4, or 8 days after administration of a single or multiple doses of antibody; alpha cells are stained for glucagon using standard IHC methods.

D. Methods and Compositions for Diagnostics and Detection

In certain embodiments, any of the anti-GCGR antibodies provided herein is useful for detecting the presence of GCGR in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample comprises a cell or tissue.

In one embodiment, an anti-GCGR antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of GCGR in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with an anti-GCGR antibody as described herein under conditions permissive for binding of the anti-GCGR antibody to GCGR, and detecting whether a complex is formed between the anti-GCGR antibody and GCGR. Such method may be an *in vitro* or *in vivo* method. In one embodiment, an anti-GCGR antibody is used to select subjects eligible for therapy with an anti-GCGR antibody, e.g. where GCGR is a biomarker for selection of patients.

In certain embodiments, labeled anti-GCGR antibodies are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes ^{32}P, ^{14}C, ^{125}I, ^{3}H, and ^{131}I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, e.g., firefly luciferase and bacterial luciferase

(U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

E. Pharmaceutical Formulations

Pharmaceutical formulations of an anti-GCGR antibody as described herein are prepared by mixing such antibody having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include insterstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958. Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

The formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules.

The formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

F. Therapeutic Methods and Compositions

Any of the anti-GCGR antibodies provided herein may be used in therapeutic methods.

In one aspect, an anti-GCGR antibody for use as a medicament is provided. In further aspects, an anti-GCGR antibody for use in treating diabetes is provided. In certain embodiments, an anti-GCGR antibody for use in a method of treatment is provided. In certain embodiments, the invention provides an anti-GCGR antibody for use in a method of treating an individual having diabetes comprising administering to the individual an effective amount of the anti-GCGR antibody. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below.

In a further aspect, the invention provides for the use of an anti-GCGR antibody in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of diabetes. In a further embodiment, the medicament is for use in a method of treating diabetes comprising administering to an individual having diabetes an effective amount of the medicament. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. In a further embodiment, the medicament is for An "individual" according to any of the above embodiments may be a human.

In a further aspect, the invention provides pharmaceutical formulations comprising any of the anti-GCGR antibodies provided herein, e.g., for use in any of the above therapeutic methods. In one embodiment, a pharmaceutical formulation comprises any of the anti-GCGR antibodies provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises any of the anti-GCGR antibodies provided herein and at least one additional therapeutic agent, e.g., as described below.

Antibodies of the invention can be used either alone or in combination with other agents in a therapy. For instance, an antibody of the invention may be co-administered with at least one additional therapeutic agent. In certain embodiments, an additional therapeutic agent is a metformin or a DPP-IV inhibitor. Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant.

An antibody of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

Antibodies of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

For the prevention or treatment of disease, the appropriate dosage of an antibody of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 μg/kg to 15 mg/kg (e.g. 0.1mg/kg-10mg/kg) of antibody can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (*e.g.* such that the patient receives from about two to about twenty, or *e.g.* about six doses of the antibody). An initial higher loading dose, followed by one or more lower doses may be administered. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

It is understood that any of the above formulations or therapeutic methods may be carried out using an immunoconjugate of the invention in place of or in addition to an anti-GCGR antibody.

G. Articles of Manufacture

In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibody of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

It is understood that any of the above articles of manufacture may include an immunoconjugate of the invention in place of or in addition to an anti-GCGR antibody.

III. EXAMPLES

The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

Example 1. Generation and Selection of Anti-GCGR Antibodies

This example describes the generation and selection of exemplary anti-GCGR antibodies. Specifically, anti-GCGR antibodies based on a mouse hybridoma 3C7 and anti-GCGR antibodies based on a phage-derived clone 6.23 are described herein.

Production of 3C7 Series anti-GCGR Antibodies

Balb/c mice were immunized with 293 cells stably expressing human GCGR and tested for titers against GCGR by flow cytometry with cells expressing and not expressing GCGR. B cells from mice responding to GCGR were fused to myeloma cells and positive clones were identified by flow cytometry of cells expressing GCGR with supernatants of hybridomas. The variable regions of 3C7 were obtained by RT-PCR using the 5' RACE method, using a 3' primer derived from the constant region, and cloned in pCR-TOPO® vector (Invitrogen). Clones with inserts were identified by sequencing.

The consensus sequence for the human heavy chain subgroup IV and the light chain subgroup k II were used as the framework for the humanization of 3C7. This framework has been successfully used in the humanization of other murine antibodies (Werther et al., supra; Carter et al., supra; Presta et al. *J. Immunol.* 151:2623–2632 (1993); and Eigenbrot et al. *Proteins* 18:49–62 (1994)). The HVRs were according to Kabat et al., supra. All humanized variants were initially made and screened for binding as F(ab)s expressed in *E. coli*. Typical yields from 500 ml shake flasks were 0.1–0.4 mg F(ab).

3C7v1 is a humanized mouse mAb, effectorless (N297A), IgG1. 3C7v1 has two framework mutations in the heavy chain variable region subgroup IV consensus compared with the reference 3C7-K2H4. There are three HVRs in the heavy chain -- HVR H1, HVR H2 and HVR H3. The 3C7.v1 light chain has a kappa variable region subgroup II consensus framework sequence

that matches reference 3C7-K2H4 and also has three HVRs -- HVR L1, HVR L2 and HVR L3. Affinity maturation of 3C7v1 resulted in variants 3C7v11 and 3C7v16. Figure 2 shows alignment of the 3C7v1, 3C7v11 and 3C7v16 of the V_L and V_H, respectively.

The Table 2 below shows the mutations in humanized 3C7.

Table 2. Mutations in Humanized 3C7

Heavy chain mutations (Kabat number)	Effect
S28T	Removes potential deamidation site
S54T	Increases affinity for human GCGR. Increases affinity for mouse GCGR with N97G mutation.
S54E	Increases on-rate (Ka) of binding for human GCGR
N97G	Allows binding of 3C7 to both human and mouse GCGR
A100S	Selected when sorting with mouse GCGR
Light chain mutations	
S27eW	Selected when sorting with mouse GCGR
V94S	Selected when sorting with hman and mouse GCGR
V94W	Selected when sorting with mouse GCGR

Production of the 6.23 Series Anti-GCGR Antibodies

Phage display method was used to obtain the 6.23 series anti-GCGR antibodies. The GCGR membrane protein was biotinylated and incorporated in the envelope membrane of baculovirus particles. Virus particles were grown in insect cells and then concentrated and semi-purified. Detergent was added to virus preparation to solubilize GCGR and supernatant containing

solubilized protein was used for phage library sorting. Biotinylated, solubilized GCGR proteins were used for sorting a minimalist synthetic antibody library with restricted chemical diversity at HVRs, designated as YSGX library (Fellhouse et al (2007) *J Mol Biol.* 373:924-40). After the initial sorting, 33 unique potential binders were identified in 96 sequenced clones. All clones were reformatted as human IgG1 and 31 of the the 33 unique clones bound surface epitopes of GCGR as determined by flow cytometry with live GCGR-expressing 293 cells.

Example 2. Binding Assays of Anti-GCGR Antibodies

Binding of Anti-CGCR Antibodies to Soluble GCGR

Binding of anti-GCGR antibody variants were investigated using a BIACORE® biosensor (Karlsson et al. Methods: A Comparison to Methods in Enzymology 6:97 108 (1994)). Concentrations of antibodies were determined by quantitative amino acid analysis. GCGR molecule was coupled to a CM-5 biosensor chip through primary amine groups according to manufacturer's instructions (Pharmacia). Off-rate kinetics were measured by saturating the chip with antibody (35 uL of 2 .uM antibody at a flow rate of 20 ul/min) and then switching to buffer (PBS-0.05% polysorbate 20). Data points from 0 to 4500 sec were used for off-rate kinetic analysis. The dissociation rate constant (k_{off}) was obtained from the slope of the plot of ln(RO/R) versus time, where RO is the signal at t=0 and R is the signal at each time point.

On-rate kinetics were measured using two-fold serial dilutions of antibody (0.0625 2 mM). The slope, K_s, was obtained from the plot of ln (-dR/dt) versus time for each antibody concentration using the BIAcore kinetics evaluation software as described in the Pharmacia Biosensor manual. R is the signal at time t. Data between 80 and 168, 148, 128, 114, 102, and 92 sec were used for 0.0625, 0.125, 0.25, 0.5, 1, and 2 mM antibody, respectively. The association rate constant (k_{on}) was obtained from the slope of the plot of K_s versus antibody concentration. At the end of each cycle, bound antibody was removed by injecting 5 uL of 50 mM HCl at a flow rate of 20 uL/min to regenerate the chip.

Bindings of anti-GCGR antibody variants to soluble GCGR were also investigated using Octet™ biosensors. GCGR was obtained in baculovirus particles, using a modification of the procedures described by Loisel et al. (Nat. Biotechnol. 1997;15:1300-1304). A baculovirus transfer vector based on the pFastBac™ Dual vector (Invitrogen) was constructed for expression

of biotinylated membrane protein. The vector includes a cassette with the baculovirus gp64 promoter followed by a multiple cloning site and a baculovirus vp39 promoter driving transcription of the Escherichia coli birA gene in opposite direction from the gp64 promoter. A cytomegalovirus promoter driving low-level expression of the green fluorescent protein (GFP) was located after the birA sequence to facilitate virus titration. The GCGR genes were cloned in the multiple cloning site fused to a sequence encoding a linker and AviTag™ sequence at the 3' end. Baculovirus was produced and propagated up to second passage following the protocols used for the bac-to-bac system (Invitrogen). Viruses incorporating GCGR were produced by infecting $1.8x10^9$ Sf9 cells in 600 ml of ESF media (Expression Systems) with enough baculovirus to infect .95% of cells by 24h as assessed by GFP expression. Cultures were harvested 40 h post-infection, cells were pelleted by centrifugation at 5000 xg for 10 min, and virus in the supernatant was pelleted by centrifugation at 25 000 x g for 4 h at 48C. Pellets with virus were resuspended in 8 ml of PBS (150 mM NaCl, 10 mM sodium phosphate, pH 7.4), layered on top of a 4 ml 35% (w/v) sucrose cushion and centrifuged in an SW40Ti rotor (Beckman) at 30000 rpm for 1 h at 48°C. The supernatant with debris was discarded, the virus pellet gently rinsed once with PBS, resuspended in 1.2 ml of PBS with protease inhibitor cocktail (Roche) and stored at 48C for up to 2 weeks. Expression of protein was monitored by western blotting of whole baculovirus particles with a streptavidin-horseradish peroxidase conjugate (Pierce).

Baculovirus lysates containing GCGR were produced by mixing the baculovirus suspension containing GCGR with 200 µl of PBS containing 0.5% BSA (PBS-B) and 100 µl of 5% dodecylmaltoside (DDM, Anatrace) in PBS. The baculovirus lysates were incubated on ice for 30 min and centrifuged at 16 000 × g for 30 min at 4°C to remove virus capsids and other insoluble debris. The clear supernatants containing solubilized protein were transferred to a new tube.

Kinetic binding measurements were performed on an Octet Red instrument (ForteBio, Menlo Park, CA, USA). All washes, dilutions and measurements were performed in Octet buffer (0.2% dodecylmaltoside, or DDM, – PBS) with the plate shaking at 1000 rpm. Streptavidin biosensors were equilibrated in Octet buffer for 10 min and then loaded with biotinylated GCGR (from viral lysate in 1% DDM, diluted 1:8 in Octet Buffer) for 5 min and washed for 10 min. For the association phase, the ligand-coated streptavidin tips were immersed in anti-GCGR Fab

fragments for 10 min (eight serial two-fold dilutions, starting at 500 or 50 nM). Dissociation of the Fab-GCGR complex was measured in wells containing Octet buffer alone for 600 s. KD, Ka and Kd were determined with Octet evaluation software v6.3 using a 1:1 binding model with global fitting.

A kinetic analysis of the 6.23 series anti-GCGR variants binding to solubilized, full length GCGR was performed. This compared the antibodies in the 6.23 series – v1-v4 with each other. Table 3 shows that H6.23v3 has a 3-fold increase in affinity (K_d) over H6.23v2 due to a decreased off rate.

Table 3. Affinity of H6.23 Variants for Soluble GCGR

Fab variant	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_d (nM)
H6.23v1	4.9×10^{4}	3.3×10^{-4}	6.76
H6.23v2	2.2×10^{5}	2.1×10^{-4}	0.95
H6.23v3	3.3×10^{5}	4.8×10^{-5}	0.14
H6.23v4	1.8×10^{5}	5.0×10^{-5}	0.28

The affinity of 3C7 variants for soluble N-terminal domain GCGR was determined using Biacore assay. The results are shown in Table 4 below.

Table 4. Affinity of 3C7 Variants for Soluble GCGR

Fab Variants	Kon (M-1s-1)	Koff (s-1)	KD
3C7.v1	4.3 × 105	9.7 × 10-4	2.2 nM
3C7.v11	5.3 × 105	6.8 × 10-4	1.3 nM
3C7.v16	5.4 × 105	8.4 × 10-4	1.6 nM

Binding epitope for each mAb is mapped using alanine scanning of the ECD of GCGR expressed on the surface of phage particles. Epitopes for H6.23v3 and 3C7 are conformational. Specifically, the following amino acids of the ECD of GCGR are important for anti-GCGR binding: L50, F62, Y65, L85, W87, K90 and R94 for H6.23v3; and F33, K35 W36 , K37, Y39, G40 , N41' P72, N74, T75, P82, W83, Y84, L85, P86, W87 for 3C7.

Binding of Anti-GCGR Antibodies to Cell-bound GCGR

Binding of GCGR antibodies to cell surface GCGR was measured by flow cytometry. HEK293 cells stably expressing Human GCGR or Human GLP1R were detached from tissue culture dishes using 10mM EDTA. $5x10^5$ cells were added to tubes and centrifuged at 350xg for 5 minutes at 4°C. The cells were washed twice with FACS Buffer (PBS, 0.1% Bovine Serum Albumin), resuspended in 100µL of the appropriate anti-GCGR antibody (10µg/mL in FACS Buffer) or FACS Buffer alone (no primary control) and for 1 hour on ice. Cells were centrifuged (350xg, 5 min, 4°C) and washed twice with FACS Buffer, resuspended in 100µL of Goat anti-Human IgG, Alexa Fluor 488 (1:500 in FACS Buffer) and incubated 30 minutes on ice. Cells were centrifuged (350xg, 5 min, 4°C), washed twice in FACS Buffer and resuspended in 500µL of FACS Buffer. Cells were passed through a mesh filter into FACS reading tubes and fluorescence at 488nm was measured by flow cytometry on a FACScan Calibur4 instrument (Becton Dickenson). Data was analyzed using FlowJo flow cytometry analysis software.

Example 3. Inhibition Assays of Anti-GCGR Antibodies

Inhibition of Cell-bound GCGR's Binding to Glucagon

Binding of anti-GCGR antibodies H6.23 series in primary human hepatocytes was measured by a competitive binding assay using ^{125}I-glucagon in a 96-well Costar round bottom plate (Corning; Lowell, MA). Primary human hepatocytes (5×10^4/well) from Celsis (Chicago, IL) were incubated with 3 nM (final concentration) of ^{125}I -glucagon (PerkinElmer; Waltham, MA) for 2 hours at room temperature, in the presence of anti-GCGR antibodies at various concentrations ranging from 0 – 1000 nM. After separation of bound and free ligands by centrifugation, the cell-bound ^{125}I -glucagon was quantified in a gamma counter. The mean CPM (count per minute) values from triplicate wells were plotted as a function of antibody concentration. Unrelated human IgG_1 was used as isotype control for anti-GCGR. As shown in

Figure 3, the antibody's ability to inhibit radio-labeled glucagon binding to cell-bound GCGR is comparable among antibody variants H6.23v2, H6.23v3 and H6.23 v4..

Inhibition of Glucagon-induced cAMP Production

This example describes a cell-based reporter assay of glucagon-induced activation of a cAMP response element (CRE)-luciferase construct co-expressed in 293-cells expressing human or mouse or rat or cynomolgous GCGR and inhibition of glucagon activity in these cells after incubation by antibody. Sub-confluent Human GCGR stable cells were transfected in solution under the following conditions (per well): $5x10^4$ cells, 0.1μg pGL4.29 luciferase plasmid, 2ng pRL-SV40 renilla plasmid, 0.25μL Lipofectamine™ 2000 in optimem media as per manufacturer's instructions. The transfection mixture was plated into 96-well plates (100μL/well final volume) and incubated at 37°C in a humidified atmosphere of 5% CO_2 for 3 hours. Media (DMEM, 10% FBS, 2mM l-glutamine) was added to the plate (150μL/well) and cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 overnight (16-20 hours). For glucagon dose response: the media was replaced with 95μL/well of fresh assay media (DMEM, 5% FBS, 2mM l-glutamine). For antibodies the media was replaced with 95μL/well of antibody dilution series (in assay media). Cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 for 1 hour, glucagon was spiked in (5μL/well of 20X stock) and cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 for 5 hours. Luciferase activity (luminescence) was measured using the Dual-Go Luciferase Assay System (as per manufacturer's instructions). Luciferase values were normalized to renilla values for each well and data was calculated as % of maximum signal. EC_{50} and IC_{50} values were determined from non-linear regression models using Prism Graphpad v5.0 software.

Inhitibion of Glucagon-induced Gene Expression

In this example, glucagon-induced gene expression in primary hepatocytes and inhibition of glucagon activity in these cells after incubation with anti-GCGR antibody were studied. The amount of mRNA of known glucagon target genes such as PEPCK, G6Pase and Pgc1alpha was measured using a branched chain sandwich nucleic acid hybridization assay (Quantigene 2.0 - Panomics), Primary human hepatocytes were plated onto collagen-coated polypropylene plates at 2.19×10^5 cells/cm^2 in InvitroGRO CP Medium + Torpedo Antibiotic Mix (Celsis-In Vitro

Technologies, Baltimore MD). Plates were incubated at 37°C overnight to allow for cell attachment. Medium was changed to assay medium (125mM NaCl, 5mM KCl, 1.8mM $CaCl_2$, 2.6mM $MgSO_4$, 25mM Hepes, 2mM Pyruvate, pH 7.4) and cells were incubated for 2 hours. Blank vehicle or 50nM glucagon was then added, and the hepatocyte cultures were incubated for 2 hours. When the effects of anti-GCGR antibodies were to be examined, they were diluted in assay buffer added to the cultures 2 hours prior to the addition of glucagon. At the end of the experiment, 3X Quantigene 2.0 lysis buffer was added to the cells (for 1X final concentration), the cells were mixed up and down 20 times, incubated at 50°C in a convection oven, mixed 20 additional times and stored at -80°C until ready for analysis.

Quantigene 2.0 assay was run on the primary human hepatocyte lysates as per manufacturer (Affymetrix) instructions under the following conditions: A probe set for human G6Pase RNA (Accession No. NM_000151) was used. A probe set for human RPLP0 RNA (Accession No. NM_ 001002) was used as a control. Lysates were diluted in 1X Quantigene 2.0 lysis buffer for use in the assay (1:8 dilutions for Human G6pase and 1:40 dilution for Human RPLP0). 1X Quantigene 2.0 lysis buffer only was added to several wells as a blank (BL) control. For analysis of assay data, the average BL value for each gene/probeset was subtracted from the experimental values. For each well of the plate, a ratio of Human G6pase/Human RPLP0 background subtracted values was calculated. Fold induction of human G6pase was then calculated for each well treated with glucagon compared to the average of the sample treated with blank vehicle.

As shown in Figure 4, the exposure of primary hepatocytes to glucagon increased the expression of G6Pase by about 7x fold compared with the basal levels, which confirms the glucagon's role in inducing G6Pase mRNA expression. Figure 4 also shows that H6.23 variants (v2-v4) as well as 3C7 varaints (v1, v11 and v16) are capable of interfering with glucagon-induced G6Pase expression.

Inhititory Activities in Animal Diabetes Models

A. db/db mice

To evaluate whether anti-GCGR therapy would improve T2DM, diabetic (*db/db*) mice were used as an in vivo model of insulin resistance and T2DM. *db/db* mice between 8-10 weeks of age are characterized by hyperinsulinemia and insulin resistance, thus representing earlier stages

of the disease, while mice 12-14 weeks of age are characterized by elevated glucose levels in addition to hyperinsulinemia, thus representing advanced stages of T2DM. Four groups of six mice at age of 12 weeks were used for the study: one control group and three treated with anti-GCGR antibody H6.23v2 The *db/db* mice were fasted for 24 hours four days prior to treatment, then bled next day for measuring pre-dose fasting glucose levels using a glucometer). Three days later (day 0), each group was administered with corresponding antibody at following single dose: control mAb at 5 mg/kg; H6.23v2 at 1 mg/kg, 3 mg/kg and 5 mg/kg respectively. The *db/db mice* were then fasted for 24 hours before being bled for measuring fasting glucose levels.

As shown in Figure 5, H6.23v2 treatment at 5 mg/kg dose leads to reduced blood glucose in 12 week old *db/db* mice.

B. ZDF Rats

An animal model of Type II diabetes was also chosen to test the glycemic activity of the anti-GCGR antibody. The animal chosen, the Zucker rat, is a well known model of obesity associated with diabetes in the rat. Stern et al., Proc. Soc. Exp. Biol. Med., 139: 66-69 (1972). A substrain of these rats, the Zucker Diabetic Fatty strain (ZDF), is a good model of Type II diabetes, since they become obese and insulin resistant at an early age, with progressive b-cell failure and then frank diabetes. Johnson et al., Science, 250: 546-549 (1990). In the present example intravenous bolus injections of anti-GCGR antibodies were given and effects on the blood glucose and insulin concentrations were studied.

10-12 week old diabetic rats (7/group) were divided into three treatment groups and given IV of: 1) Vehicle; 2) anti-GCGR H6.23v3 (1 mg/kg); or 3) anti-GCGR H6.23v3 (5 mg/kg). The dose of antibody was chosen as a dose that might cause a fall in blood glucose levels.

Two blood samples were collected from each rat via the tail vein prior to IV dosing with the anti-GCGR antibody and then blood samples were collected on day 1, 2, 3, 6, 7 and 9 post-dose. Blood glucose concentration was measured using a glucometer.

Statistical comparisons for each time point were made by t-test. A p value of <0.05 was considered as being statistically significant.

The results from the study are shown in Figure 6. The panel shows the fed blood glucose (mg/dL) levels over the course of nine days post-treatment with the anti-GCGR antibody. The IV injection of H6.23v3 antbody caused an immediate, large, maintained and statistically significant ($p<001$ vs. control, excipient-treated rats) fall in blood glucose. In comparison, there was no change in blood glucose in the vehicle treated rats. After 6 days blood glucose returned toward baseline pre-treated levels in the animals treated with H6.23v3 at 1 mg/kg while blood glucose levels remained close to normal levels for 9 days in animals treated with 5 mg/kg of antibody. . This is suggestive of a long-term effect of the anti-GCGR antibody on blood glucose.

In the above experiment it was shown that the administration of one injection of an glucagon antagonist reduced blood glucose levels. Since one injection of the anti-GCGR antibody showed efficacy on glycemic parameters, the following glucose tolerance test was performed. Obese male Zucker Diabetic Fatty (ZDF) rats (12 weeks of age, 275-350 g, Genetic Models Inc., Indianapolis, Ind. 46268) were group housed (3/cage) in a room controlled for temperature and lighting and fed the pelleted rat diet specified by the breeders (Purina 5008, 6% fat breeder chow) and tap water ad libitum. Animals were randomized into three treatment groups and into cages to give groups balance so as to have equivalent initial blood glucose levels, insulin levels, and body weights.

The experiment consisted of three groups of rats with five rats per group receiving 100 uL injections as follows: 1) Vehicle; 2) H6.23v3 at 1 mg/kg; 3) H6.23v3 at 5 mg/kg. A glucose tolerance test was performed on day 8 of the study. Rat food was withdrawn. After a 15-hour fast, a blood sample was taken via the tail vein. Rats then received 2 g/kg dextrose (50%) by intraperitoneal (ip) injection. A blood sample (10 .mu.l) was taken via tail vein at 20, 40, 60, 90 and 120 minutes after dextrose administration. Blood glucose concentration was measured using a glucometer.

After the glucose tolerance test was completed, animals were re-fed. Statistical comparisons for each time point were made by t-test. A p value of <0.05 was considered as being statistically significant.

Figure 7 shows the effect of a glucose tolerance test in these diabetic rats on blood glucose concentrations. A glucose tolerance test can be viewed as a simulated meal. The rats were given an

intraperitoneal injection of glucose at 2 g/kg of body weight and then bled again following the injection. Figure 7 shows the change in blood glucose two hours following the glucose challenge. In the diabetic rats treated with injections of excipient, blood glucose rose substantially at the 20 minute time point. There was no difference in the rise in blood glucose 8 days after treatment with 1 mg/kg anti-GCGR H6.23v3 and the vehicle control while treatment with the 5 mg/kg anti-GCGR H6.23v3 blunted this rise ($p<0.1$ vs. control).

This study administering anti-GCGR antibody to diabetic rats shows that blood glucose can be controlled by manipulating the endogenous glucagon system. The direct measures of blood glucose control show the efficacy of this class of anti-GCGR antibody in improving the diabetic state.

C. SZT-treated Rats

A syndrome similar to diabetes can be induced in animals by administration of streptozotocin (STZ). Portha B. et al., Diabete Metab. 15: 61-75 (1989). By killing pancreatic cells which produce insulin, STZ decreases the amount of serum insulin in treated animals. diabetes was induced in Male Sprague Dawley rats (Charles River, UK) with an intraperitoneal injection (80 mg/kg) of streptozotocin (STZ; Sigma) given after an overnight fast. The drug was freshly dissolved in sterile citrate buffer pH 4.5 immediately before injection.2 days after STZ injection, diabetic rats, as determined by fed blood glucose levels greater than 385 mg/dl, were divided into two groups of twelve rats each.

Rats in group were treated with vehicle and rats in group 2 were treated with anti-GCGR antibody H6.23v3 (5 mg/kg) (day 0) by IV administration. One week after this initial treatment (day 7), the mice in group 2, were given a second dose of anti-GCGR antibody H6.23v3 (5 mg/kg). All animals not receiving antibody were injected with vehicle.

After one further week (day 14) the pancreata were harvested, sectioned and immunohistochemistry was used to determine the alpha-cell area (μ^2). Anti-GCGR antibody H6.23v3 treatment increased the alpha cell area 5-fold from 200 μ^2 for the untreated group to 1000 μ^2 for the STZ-treated rats that were also treated with anti-GCGR H6.23v3 antibody.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

WHAT IS CLAIMED IS:

1. An anti-glucagon receptor (GCGR) antibody capable of blocking GCGR binding to glucagon, wherein the antibody comprises (a) HVR L3 comprising the amino acid sequence of $QQYX_1X_2YLX_3T$ (SEQ ID NO:13); and (b) HVR H1 comprising the amino acid sequence of $GFNIYYX_4YIH$ (SEQ ID NO:14); wherein X_1 is S or G, X_2 is Y or S, X_3 is F or Y, and X_4 is S or N.

2. The antibody of claim 1, further comprising HVR H2 comprising the amino acid sequence of $AX_5X_6SPYSGSTYYADSVKG$ (SEQ ID NO:15); wherein X_5 is S or E, and X_6 is I, F or M.

3. The antibody of claim 1, further comprising HVR H3 comprising the amino acid sequence of $ARSX_7X_8IVX_9WYX_{10}YFX_{11}GFDY$ (SEQ ID NO:16); wherein X_7 is T or A, X_8 is S or A, X_9 is Y or D, X_{10} is E or D, and X_{11} is E or K.

4. The antibody of claim 3, further comprising HVR H2 comprising the amino acid sequence of $AX_5X_6SPYSGSTYYADSVKG$ (SEQ ID NO:15); wherein X_5 is S or E, and X_6 is I, F or M.

5. The antibody of claim 1, further comprising HVR L1 comprising the amino acid sequence of RASQSVSSAVA (SEQ ID NO:4).

6. The antibody of claim 1, further comprising HVR L2 comprising the amino acid sequence of SASSLYS (SEQ ID NO:5).

7. The antibody of claim 6, further comprising HVR L1 comprising the amino acid sequence of RASQSVSSAVA (SEQ ID NO:4).

8. An anti-GCGR antibody comprising a light chain variable domain comprising three light chain HVRs and a heavy chain variable domain comprising three heavy chain HVRs, wherein:

(a) HVR-L1 comprises the amino acid sequence of RASQSVSSAVA (SEQ ID NO:4);

(b) HVR-L2 comprises the amino acid sequence of SASSLYS (SEQ ID NO:5);

(c) HVR-L3 comprises the amino acid sequence of $QQYX_1X_2YLX_3T$ (SEQ ID NO:13), wherein X_1 is S or G, X_2 is Y or S and X_3 is F or Y;

(d) HVR-H1 comprises the amino acid sequence of $GFNIYYX_4YIH$ (SEQ ID NO:14), wherein X_4 is S or N;

(e) HVR-H2 comprises the amino acid sequence of $AX_5X_6SPYSGSTYYADSVKG$ (SEQ ID NO:15); wherein X_5 is S or E, and X_6 is I, F or M; and

(f) HVR-H3 comprises the amino acid sequence of $ARSX_7X_8IVX_9WYX_{10}YFX_{11}GFDY$ (SEQ ID NO:16); wherein X_7 is T or A, X_8 is S or A, X_9 is Y or D, X_{10} is E or D, and X_{11} is E or K.

9. The antibody of claim 8, comprising (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:25; (b) a VL sequence having at least 95 % sequence identity to the amino acid sequence of SEQ ID NO:26; or (c) a VH sequence as in (a) and a VL sequence as in (b).

10. The antibody of claim 8, comprising a VH sequence of SEQ ID NO:25.

11. The antibody of claim 8, comprising a VL sequence of SEQ ID NO:26.

12. An anti-GCGR antibody comprising a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:25 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:26.

13. An anti-GCGR antibody capable of blocking GCGR binding to glucagon, wherein the antibody comprises (a) HVR L1 comprising the amino acid sequence of RSSQSLVHSNGNTYLH (SEQ ID NO:30); and (b) HVR H2 comprising the amino acid sequence of $GYLGFX_{13}GSTYYNPSLKS$ (SEQ ID NO:37), wherein X_{13} is S or T.

14. The antibody of claim 13, further comprising HVR L2 comprising the amino acid sequence of: KVSNRFS (SEQ ID NO:31).

15. The antibody of claim 13, further comprising HVR L3 comprising the amino acid sequence of $SQSTH\ X_{14}PWT$ (SEQ ID NO:50), wherein X_{14} is V or S.

16. The antibody of claim 14, further comprising HVR L3 comprising the amino acid sequence of SQSTH X_{14}PWT (SEQ ID NO:50), wherein X_{14} is V or S.

17. The antibody of claim 13, further comprising HVR H1 comprising the amino acid sequence of GDX_{12}ITSGFWN (SEQ ID NO:36), wherein X_{12} is S or T.

18. The antibody of claim 13, further comprising HVR H3 comprising the amino acid sequence of ASVDNSAALDY (SEQ ID NO:29).

19. The antibody of claim 17, further comprising HVR H3 comprising the amino acid sequence of ASVDNSAALDY (SEQ ID NO:29).

20. An anti-GCGR antibody comprising a light chain variable domain comprising three light chain HVRs and a heavy chain variable domain comprising three heavy chain HVRs, wherein:

(a) HVR-L1 comprises the amino acid sequence of RSSQSLVHSNGNTYLH (SEQ ID NO:30);

(b) HVR-L2 comprises the amino acid sequence of KVSNRFS (SEQ ID NO:31);

(c) HVR-L3 comprises the amino acid sequence of SQSTHVPWT (SEQ ID NO:32);

(d) HVR-H1 comprises the amino acid sequence of GDX_{12}ITSGFWN (SEQ ID NO:36), wherein X_{12} is S or T;

(e) HVR-H2 comprises the amino acid sequence of GYLGFX_{13}GSTYYNPSLKS (SEQ ID NO:37), wherein X_{13} is S or T; and

(f) HVR-H3 comprises the amino acid sequence of ASVDNSAALDY (SEQ ID NO:29).

21. The antibody of claim 20, comprising (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:46; (b) a VL sequence having at least 95 % sequence identity to the amino acid sequence of SEQ ID NO:47; or a VH sequence as in (a) and a VL sequence as in (b).

22. The antibody of claim 20, comprising a VH sequence of SEQ ID NO:46.

23. The antibody of claim 20, comprising a VL sequence of SEQ ID NO:47.

24. An anti-GCGR antibody comprising a heavy chain variable domain comprising the sequence of SEQ ID NO 46 and a light chain variable domain comprising the sequence of SEQ ID NO 47.

25. The antibody of claim 1, 8, 12, 13, 20 or 24, which is a monoclonal antibody.

26. The antibody of claim 1, 8, 12, 13, 20 or 24, which is a human, humanized, or chimeric antibody.

27. The antibody of claim 1, 8, 12, 13, 20 or 24, which is an antibody fragment that binds GCGR.

28. The antibody of claim 1, 8, 12, 13, 20 or 24, which is an IgG1, IgG2, IgG3 or IgG4 antibody.

29. Isolated nucleic acid encoding the antibody according to any of claims 1-28.

30. A host cell comprising the nucleic acid of claim 29.

31. An immunoconjugate comprising the antibody according to any of claims 1-28 and a cytotoxic agent.

32. A pharmaceutical formulation comprising the anti-GCGR antibody according to any of claims 1-28 and a pharmaceutically acceptable carrier.

33. A method of preventing or treating glucagon-related disorders in an individual in need of such treatment comprising administering to the individual an effective amount of the anti-GCGR antibody of claim 1, 8, 12, 13, 20 or 24.

34. The method of claim 33 wherein the disorders are selected from type1 or type 2 diabetes, hyperglycemia, impaired fasting glucose, impaired glucose tolerance, dyslipidemia, and metabolic syndrome.

35. The use of the anti-GCGR antibody of claim 1, 8, 12, 13, 20 or 24 in the preparation of a medicament.

36. The use of claim 35, wherein the medicament is for preventing or treating glucagon-related disorders.

37. The antibody of claim 1, 8, 12, 13, 20 or 24 for use as a medicament.

38. The antibody of claim 1, 8, 12, 13, 20 or 24 for use in treating a glucagon-related disorder.

39. The antibody of claim 1, 8, 12, 13, 20 or 24, which is an effectorless antibody.

40. The antibody of claim 39, which comprises an amino acid substitution of N297A.

Light chains

```
6.23v1      DIQMTQSPSSLSASVGDRVTITC RASQSVSSAVA WYQ
6.23v2-v4   DIQMTQSPSSLSASVGDRVTITC RASQSVSSAVA WYQ

6.23v1      QKPGKAPKLLIY SASSLYS  GVPSRFSGSRSGTDFTLTIS
6.23v2-v4   QKPGKAPKLLIY SASSLYS  GVPSRFSGSRSGTDFTLTIS

6.23v1      SLQPEDFATYYC QQYSYYLFT FGQGTKVEIK
6.23v2-v4   SLQPEDFATYYC QQYGSYLYT FGQGTKVEIK
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Heavy Chains

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6.23v1      EVQLVESGGGLVQPGGSLRLSCAAS GFNIYYSYIH  WVRQAPGKG
6.23v2      EVQLVESGGGLVQPGGSLRLSCAAS GFNIYYNYIH  WVRQAPGKG
6.23v3      EVQLVESGGGLVQPGGSLRLSCAAS GFNIYYNYIH  WVRQAPGKG
6.23v4      EVQLVESGGGLVQPGGSLRLSCAAS GFNIYYNYIH  WVRQAPGKG

6.23v1      LEWV ASI SPYSGSTYYADSVKG RFTISADTSKNTAYLQMNSLR
6.23v2      LEWV AEI SPYSGSTYYADSVKG RFTISADTSKNTAYLQMNSLR
6.23v3      LEWV AEFSPYSGSTYYADSVKG RFTISADTSKNTAYLQMNSLR
6.23v4      LEWV ASMSPYSGSTYYADSVKG RFTISADTSKNTAYLQMNSLR

6.23v1      AEDTAVYYC ARSTS IVYWYEYFEGF DY WGQGTLVTVSS
6.23v2      AEDTAVYYC ARSAAIVDWYDYFKGFDY WGQGTLVTVSS
6.23v3      AEDTAVYYC ARSAAIVDWYDYFKGFDY WGQGTLVTVSS
6.23v4      AEDTAVYYC ARSAAIVDWYDYFKGFDY WGQGTLVTVSS
```

Figure 1

Light chain

3C7.v1/v11/v16	DIVMTQTPLSLPVTPGQPASISCRSSQSLLHSSGNTYLHWYLQKPGQ
3C7.v50	DIVMTQTPLSLPVTPGQPASISCRSSQSLLHSSGNTYLHWYLQKPGQ
3C7.v1/v11/v16	SPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYY
3C7.v50	SPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYY
3C7.v1/v11/v16	CSQSTHVPWTFGQGTKVEIK
3C7.v50	CSQSTHSPWTFGQGTKVEIK

Heavy Chain

3C7.v1	EVQLVESGPGLVKPSETLSLTCTVSGDSITSGFWNWIRQPPGKG
3C7.v11	EVQLVESGPGLVKPSETLSLTCTVSGDSITSGFWNWIRQPPGKG
3C7.v16/v50	EVQLVESGPGLVKPSETLSLTCTVSGDTITSGFWNWIRQPPGKG
3C7.v1	LEWIGYIGFSGSTYYNPSLKSRVTISRDTSKNQYSLKLSSVTAA
3C7.v11	LEWIGYIGFTGSTYYNPSLKSRVTISRDTSKNQYSLKLSSVTAA
3C7.v16/v50	LEWIGYIGFTGSTYYNPSLKSRVTISRDTSKNQYSLKLSSVTAA
3C7.v1	DTAVYYCASVDNSAALDYWGQGTLVTVSS
3C7.v11	DTAVYYCASVDNSAALDYWGQGTLVTVSS
3C7.v16/v50	DTAVYYCASVDNSAALDYWGQGTLVTVSS

Figure 2

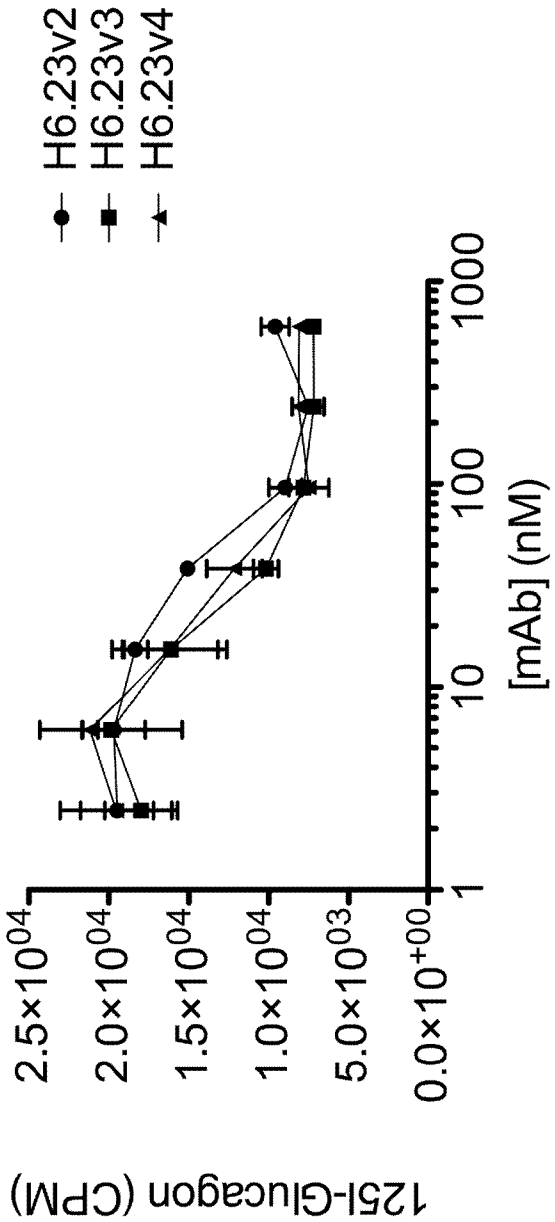
H6.23v2
H6.23v3
H6.23v4
125I-Glucagon (CPM)
2.5×10^04
2.0×10^04
1.5×10^04
1.0×10^04
5.0×10^03
0.0×10^+00
1
10
100
1000
[mAb] (nM)

Figure 3

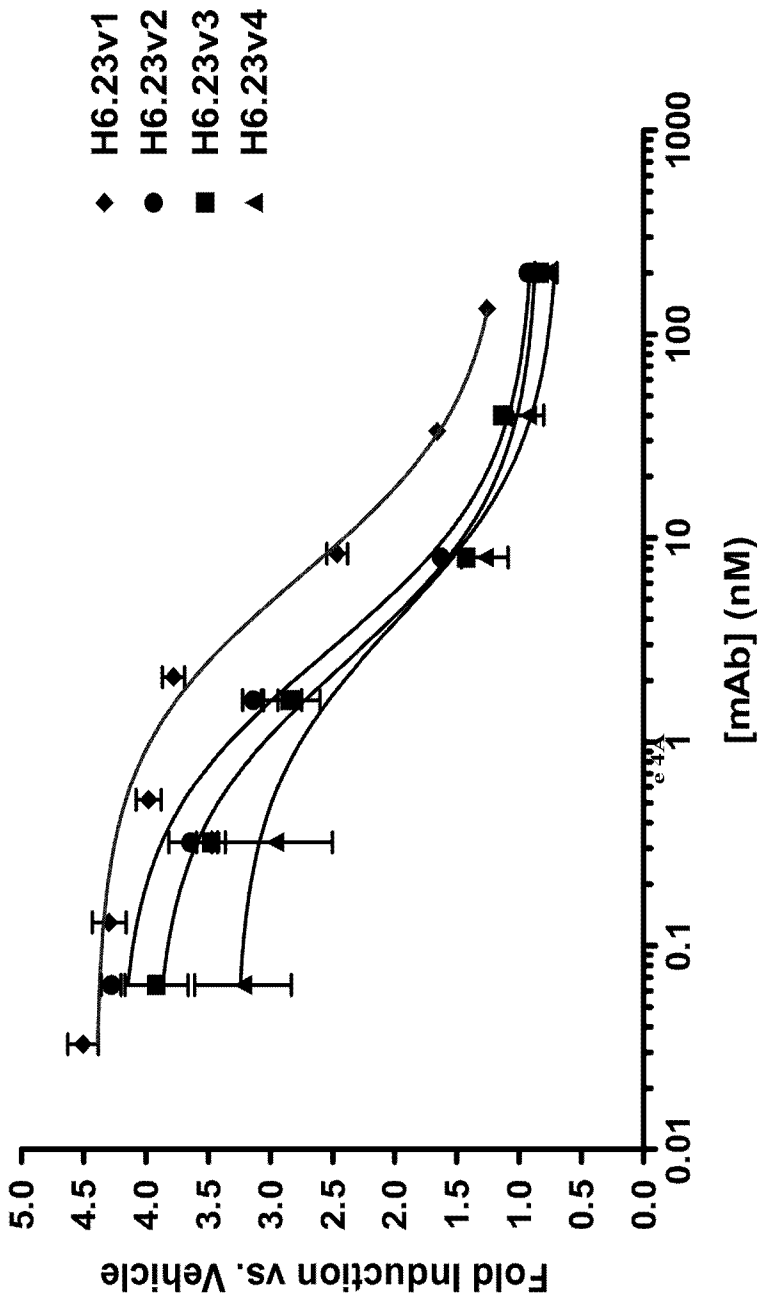
H6.23v1
H6.23v2
H6.23v3
H6.23v4
Fold Induction vs. Vehicle
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.01
0.1
1
10
100
1000
[mAb] (nM)

Figure 4A

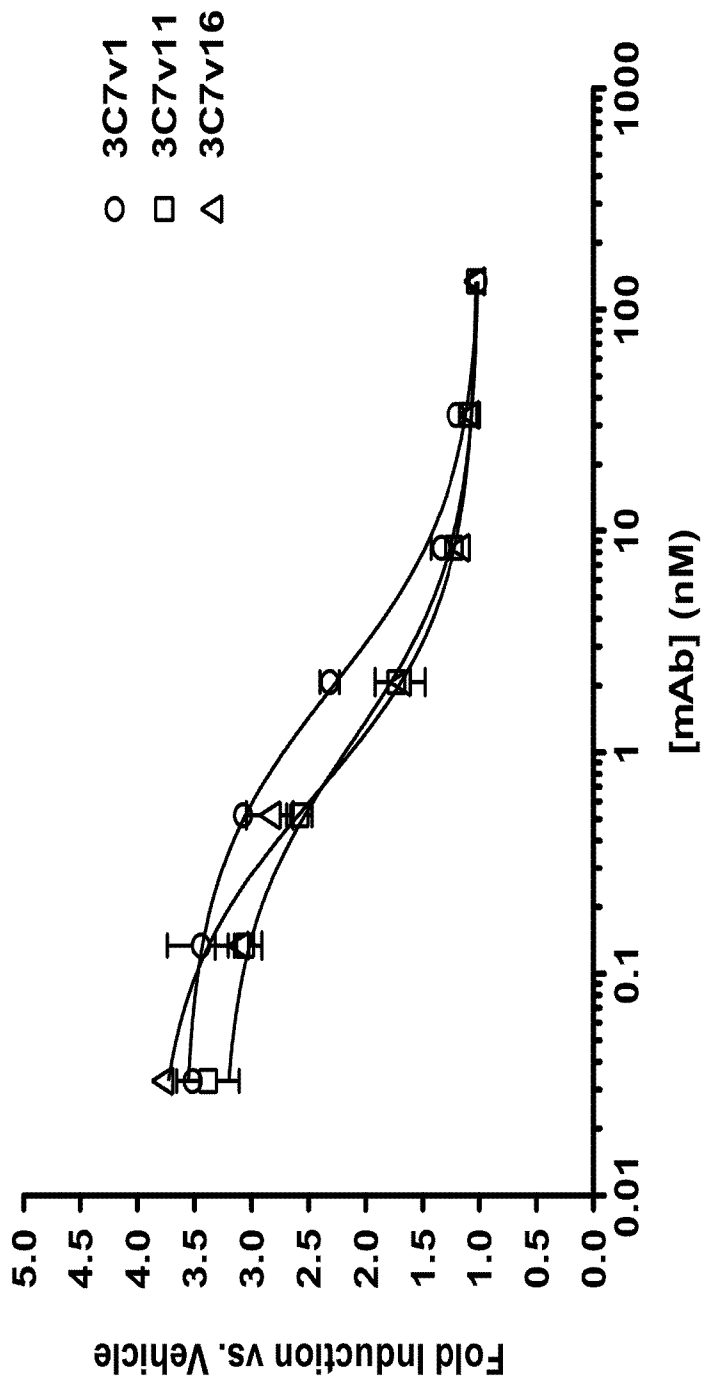
3C7v1
3C7v11
3C7v16
Fold Induction vs. Vehicle
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.01
0.1
1
10
100
1000
[mAb] (nM)

Figure 4B

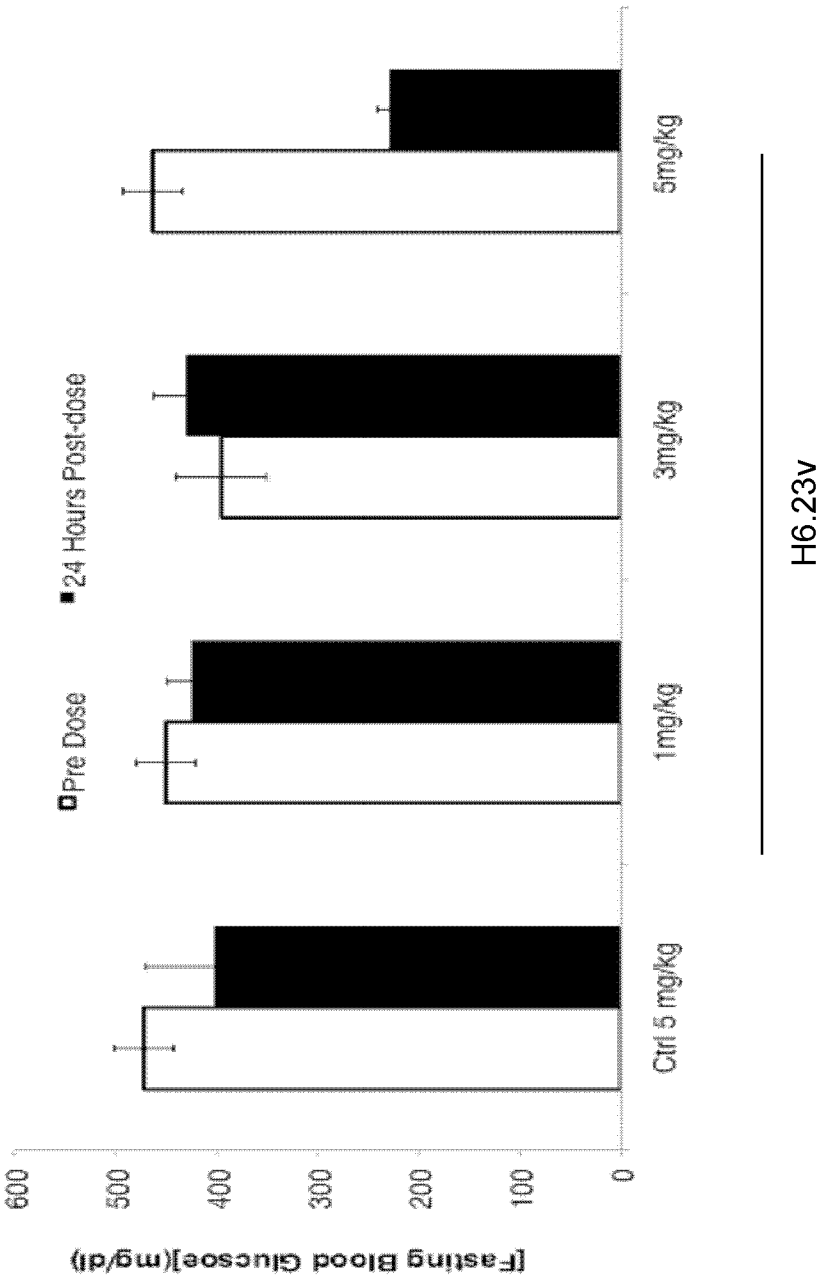
[Fasting Blood Glucose](mg/dl)
600
500
400
300
200
100
0
Pre Dose
24 Hours Post-dose
Ctrl 5 mg/kg
1mg/kg
3mg/kg
5mg/kg
H6.23v

Figure 5

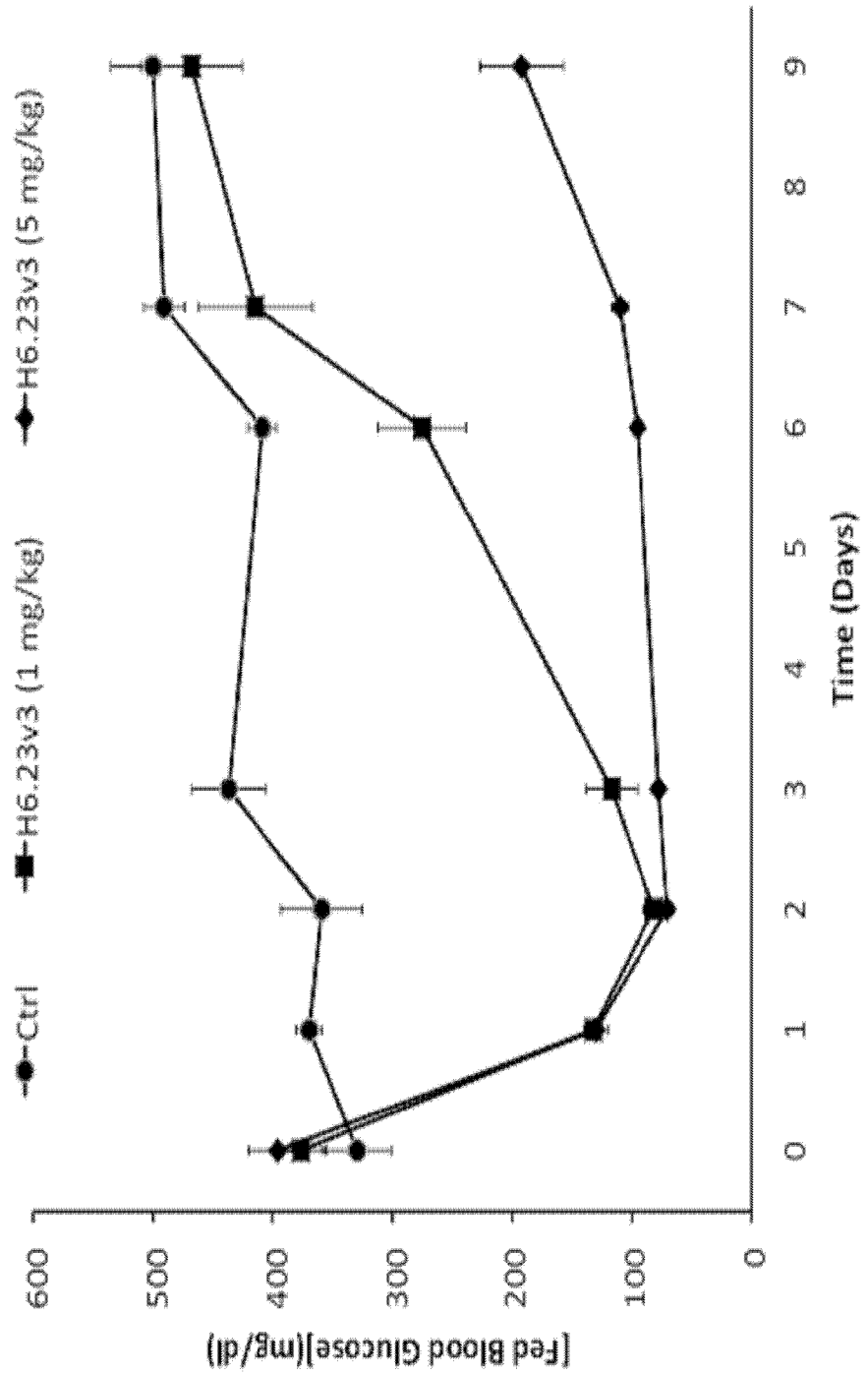
Ctrl
H6.23v3 (1 mg/kg)
H6.23v3 (5 mg/kg)
[Fed Blood Glucose](mg/dl)
600
500
400
300
200
100
0
0
1
2
3
4
5
6
7
8
9
Time (Days)

Figure 6

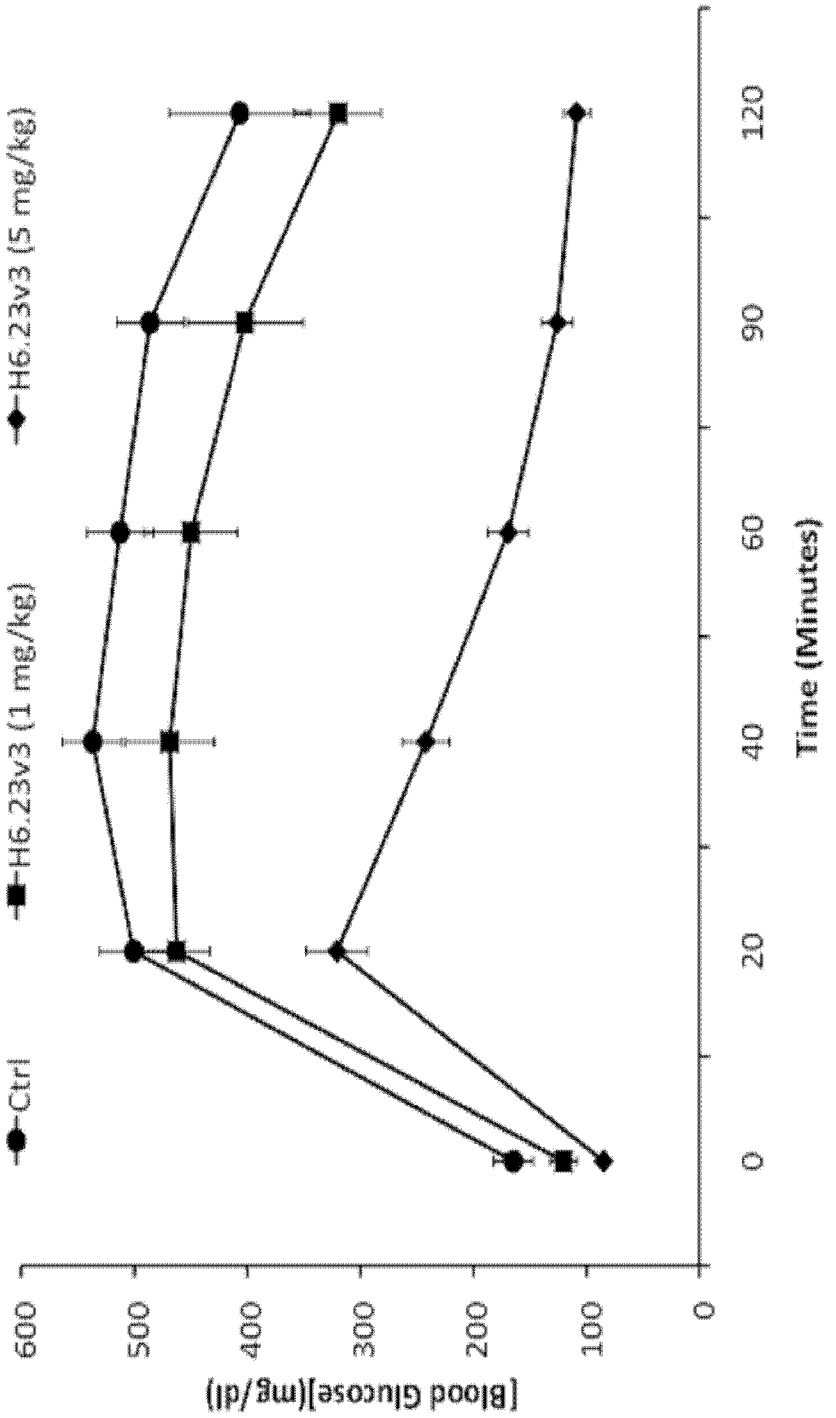
Ctrl
H6.23v3 (1 mg/kg)
H6.23v3 (5 mg/kg)
[Blood Glucose](mg/dl)
600
500
400
300
200
100
0
0
20
40
60
90
120
Time (Minutes)

Figure 7

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/060923

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/395 C07K16/28
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/036341 A2 (AMGEN INC [US]; YAN HAI [US]; HU SHAW-FEN SYLVIA [US]; BOONE THOMAS C) 27 March 2008 (2008-03-27) the whole document	1-40
X	WO 2009/120530 A1 (LILLY CO ELI [US]; MILLICAN ROHN LEE JR [US]; KORYTKO ANDREW IHOR [US]) 1 October 2009 (2009-10-01) the whole document	1-40
X	WO 2011/030935 A1 (NEOPHARM CO LTD [KR]; LEE EUNKYUNG [KR]; SEO SEONG-KYUNG [KR]; KIM TAE) 17 March 2011 (2011-03-17) the whole document	1-40

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 March 2013	25/03/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Merlos, Ana Maria

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/060923

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YAN HAI ET AL: "Fully human monoclonal antibodies antagonizing the glucagon receptor improve glucose homeostasis in mice and monkeys", JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, US, vol. 329, no. 1, 1 April 2009 (2009-04-01) , pages 102-111, XP009119374, ISSN: 0022-3565, DOI: 10.1124/JPET.108.147009 the whole document	1-40
X	WEI GU ET AL: "Long-Term Inhibition of the Glucagon Receptor with a Monoclonal Antibody in Mice Causes Sustained Improvement in Glycemic Control, with Reversible â-Cell Hyperplasia and Hyperglucagonemia", JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, US, vol. 331, no. 3, 1 December 2009 (2009-12-01), pages 871-881, XP008151861, ISSN: 0022-3565, DOI: 10.1124/JPET.109.157685 [retrieved on 2009-08-31] the whole document	1-40
X	GU WEI ET AL: "Glucagon receptor antagonist-mediated improvements in glycemic control are dependent on functional pancreatic GLP-1 receptor", AMERICAN JOURNAL OF PHYSIOLOGY - ENDOCRINOLOGY AND METABOLISM, vol. 299, no. 4, October 2010 (2010-10), pages E624-E632, XP002693276, the whole document	1-40
X	LAU YVONNE Y ET AL: "Pharmacokinetic and pharmacodynamic modeling of a monoclonal antibody antagonist of glucagon receptor in male ob/ob mice.", THE AAPS JOURNAL DEC 2009, vol. 11, no. 4, December 2009 (2009-12), pages 700-709, XP002693277, ISSN: 1550-7416 the whole document	1-40

-/--

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/060923

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WRIGHT L M ET AL: "Structure of Fab hGR-2 F6, a competitive antagonist of the glucagon receptor", ACTA CRYSTALLOGRAPHICA SECTION D: BIOLOGICAL CRYSTALLOGRAPHY, MUNKSGAARD PUBLISHERS LTD. COPENHAGEN, DK, vol. 56, no. Pt 5, 1 May 2000 (2000-05-01) , pages 573-580, XP002475612, ISSN: 0907-4449, DOI: 10.1107/S090744490000233X the whole document --- C. G. UNSON ET AL: "Antibodies against specific extracellular epitopes of the glucagon receptor block glucagon binding.", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 93, no. 1, 9 January 1996 (1996-01-09), pages 310-315, XP055042066, ISSN: 0027-8424, DOI: 10.1073/pnas.93.1.310 the whole document --- BUGGY J ET AL: "Human glucagon receptor monoclonal antibodies: Antagonism of glucagon actionand use in receptor characterization", HORMONE AND METABOLIC RESEARCH, THIEME-STRATTON, STUTTGART, DE, vol. 28, no. 5, 1 January 1996 (1996-01-01), pages 215-219, XP009098472, ISSN: 0018-5043 the whole document --- KOTH CHRISTOPHER M ET AL: "Molecular basis for negative regulation of the glucagon receptor.", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA 4 SEP 2012, vol. 109, no. 36, 4 September 2012 (2012-09-04), pages 14393-14398, XP002693278, ISSN: 1091-6490 the whole document --- -/--	1-40 1-40 1-40 1-40

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/060923

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HOETZEL ISIDRO ET AL: "Efficient production of antibodies against a mammalian integral membrane protein by phage display", PROTEIN ENGINEERING DESIGN & SELECTION, vol. 24, no. 9, Sp. Iss. SI, September 2011 (2011-09), pages 679-689, XP002693279, the whole document	1-40

A	BOSTROM JENNY ET AL: "Improving antibody binding affinity and specificity for therapeutic development", METHODS IN MOLECULAR BIOLOGY, HUMANA PRESS INC, NJ, US, vol. 525, 1 January 2009 (2009-01-01), pages 353-376, XP009135953, ISSN: 1064-3745 the whole document	1-40

T	KIM WOOK-DONG ET AL: "Human Monoclonal Antibodies against Glucagon Receptor Improve Glucose Homeostasis by Suppression of Hepatic Glucose Output in Diet-Induced Obese Mice", PLOS ONE, vol. 7, no. 12, December 2012 (2012-12), XP002693281, the whole document	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/060923

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

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

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

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

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

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

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

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

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2012/060923

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008036341 A2	27-03-2008	AR 062895 A1	10-12-2008
		AU 2007297652 A1	27-03-2008
		CA 2662613 A1	27-03-2008
		CL 26682007 A1	09-05-2008
		CR 10736 A	30-06-2009
		EA 200970262 A1	30-10-2009
		EP 2074149 A2	01-07-2009
		JP 2010504331 A	12-02-2010
		KR 20090059161 A	10-06-2009
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		US 2011223160 A1	15-09-2011
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		AU 2009228866 A1	01-10-2009
		CA 2719761 A1	01-10-2009
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		EA 201071126 A1	29-04-2011
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		PE 16742009 A1	04-11-2009
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		KR 20120056800 A	04-06-2012
		WO 2011030935 A1	17-03-2011

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-12(completely); 25-40(partially)

An anti-glucagon receptor (GCGR) antibody capable of blocking GCGR binding to glucagon comprising in different combinations HVL1, HVL2, HVL3 (hyper-variable region light) according to the sequence of SEQ ID NOS 4, 5, 13 and HVH1, HVH2, HVH3 (hyper-variable region heavy) according to the sequence of SEQ ID NOS 14, 15 and 16 and/or comprising a VH and a VL according to the sequence of SEQ ID NOS 25 and 26 (H6.23v3); corresponding nucleic acid, host cell comprising it; pharmaceutical composition comprising the antibody and therapeutic use of the antibody

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2. claims: 13-24(completely); 25-40(partially)

An anti-glucagon receptor (GCGR) antibody capable of blocking GCGR binding to glucagon comprising in different combinations HVL1, HVL2, HVL3 (hyper-variable region light) according to the sequence of SEQ ID NOS 30, 31, 50 and HVH1, HVH2, HVH3 (hyper-variable region heavy) according to the sequence of SEQ ID NOS 36, 37 and 29 and/or comprising a VH and a VL according to the sequence of SEQ ID NOS 46 and 47 (3C7); corresponding nucleic acid, host cell comprising it; pharmaceutical composition comprising the antibody and therapeutic use of the antibody

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