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(54) Title: METHODS OF USING ANTI-LGR5 ANTIBODIES

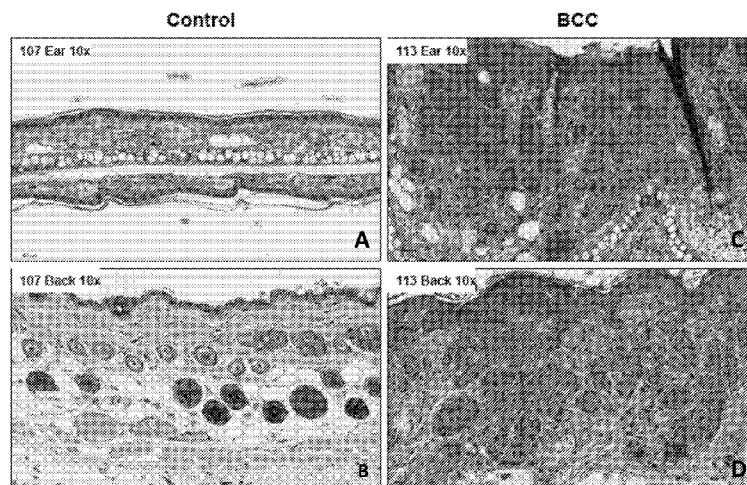


Fig. 1

(57) **Abstract:** Provided herein are methods of using anti-LGR5 antibodies, for example, for treating a hedgehog-related disease including basal cell carcinoma.



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- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))* — *with sequence listing part of description (Rule 5.2(a))*

**METHODS OF USING ANTI-LGR5 ANTIBODIES**  
**CROSS REFERENCE TO RELATED APPLICATIONS**

[001] This application claims benefit under 35 U.S.C. §119 to provisional U.S. Application No.61/879,089, filed September 17, 2013, the contents of which are hereby incorporated by reference in their entirety.

**SEQUENCE LISTING**

[002] The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on September 3, 2014, is named P5706R1-WO\_SequenceListing.txt and is 51391 bytes in size.

**FIELD OF THE INVENTION**

[003] Provided herein are methods of using anti-LGR5 antibodies, for example, for treating a hedgehog-related disease.

**BACKGROUND**

[004] One of the emerging themes in cancer biology is the dependence of cancer subtypes on certain signaling pathways for continued tumor growth. For example, mutations that activate the Hedgehog (Hh) signaling pathway drive growth of a variety of cancers including basal cell carcinomas (BCCs) and medulloblastomas, along with pancreatic, prostate, and small cell lung cancer that account for up to 25% of all human cancer deaths (Epstein, Nat. Rev. Cancer 8:743-754 (2008)). BCCs are the most prevalent cancer in the world, and nearly half of all US citizens are likely to develop this cancer before retirement (NCI 2010). Twenty years of extensive research identifying Hh pathway components and their functional roles recently culminated in the FDA approved Hh pathway antagonist vismodegib for the treatment of locally advanced or metastatic BCCs. Although vismodegib effective, not all tumor cells are sensitive to the drug and further resistance can develop.

[005] Human LGR5 is a 907 amino acid protein, of which ~540 amino acids are predicted to be in the extracellular space following cleavage of the amino-terminal signal sequence. LGR5 comprises 17 imperfect leucine-rich repeat motifs in the ectodomain, and a cysteine-rich region located between the leucine-rich repeats and the first transmembrane domain.

**SUMMARY**

[006] Provided herein are methods using anti-LGR5 antibodies. For example, provided herein are methods of treating a hedgehog-related disease in an individual comprising administering to the individual an effective amount of an anti-LGR5 antibody. For example, provided herein are methods of treating basal cell carcinoma in an individual comprising administering to the individual an effective amount of an anti-LGR5 antibody described herein.

[007] Also provided herein are methods of treating hedgehog-related disease in an individual comprising administering to the individual an effective amount of an anti-LGR5 antibody and an

effective amount of an inhibitor of the hedgehog pathway. For example, wherein the respective amounts the anti-LGR5 antibody and the inhibitor of the hedgehog pathway are effective to increase the period of response to therapy and/or delay the recurrence and/or development of resistance compared to treatment with the inhibitor of the hedgehog pathway alone.

[008] Provided herein are methods of increasing efficacy of a treatment of a hedgehog-related disease comprising an inhibitor of the hedgehog pathway in an individual, wherein the method comprises administering to the individual an effective amount of an anti-LGR5 antibody and an effective amount of the inhibitor of the hedgehog pathway.

[009] Further provided herein are methods of treating a hedgehog-related disease in an individual wherein the treatment comprises administering to the individual an effective amount of an anti-LGR5 antibody and an effective amount of an inhibitor of the hedgehog pathway, and wherein the treatment has increased efficacy compared to a standard treatment comprising administering an effective amount of the inhibitor of the hedgehog pathway without (in the absence of) the anti-LGR5 antibody.

[010] Also provided herein are methods of delaying and/or preventing development of the recurrence and/or resistance of a hedgehog-related disease to an inhibitor of the hedgehog pathway in an individual, comprising administering to the individual an effective amount of an anti-LGR5 antibody and an effective amount of the inhibitor of the hedgehog pathway. Also provided herein are methods of extending the period of sensitivity an inhibitor of the hedgehog pathway in an individual with a hedgehog-related disease comprising administering to the individual an effective amount of an anti-LGR5 antibody and an effective amount of the inhibitor of the hedgehog pathway. Provided herein are methods of extending the duration of response to an inhibitor of the hedgehog pathway in an individual with a hedgehog-related disease comprising administering to the individual an effective amount of an anti-LGR5 antibody and an effective amount of the inhibitor of the hedgehog pathway.

[011] Provided herein are methods of increasing sensitivity to an inhibitor of the hedgehog pathway in an individual with a hedgehog-related disease comprising administering to the individual an effective amount of an anti-LGR5 antibody and an effective amount of the inhibitor of the hedgehog pathway.

[012] In some embodiments of any of the methods, the anti-LGR5 antibody binds an epitope within amino acids 22-323 of SEQ ID NO: 67 or within amino acids 22-123 of SEQ ID NO: 67 and binds to LGR5 with an affinity of  $\leq 5$  nM. In some embodiments, the anti-LGR5 antibody is a monoclonal antibody. In some embodiments, the anti-LGR5 antibody is a human, humanized, or chimeric antibody. In some embodiments, the anti-LGR5 antibody is an antibody fragment that binds human LGR5 of SEQ ID NO: 67. In some embodiments, the anti-LGR5 antibody does not significantly inhibit wnt pathway signaling.

[013] In some embodiments of any of the methods, the anti-LGR5 antibody comprises: a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 30, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 31, and HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32;

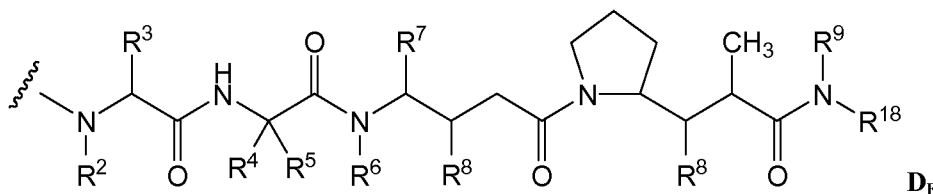
and b) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 27, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 28, and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 29.

[014] In some embodiments of any of the methods, the anti-LGR5 antibody comprises: a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 60, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 61, and HVR-H3 comprising the amino acid sequence of SEQ ID NO: 62; and b) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 57, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 58, and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 59.

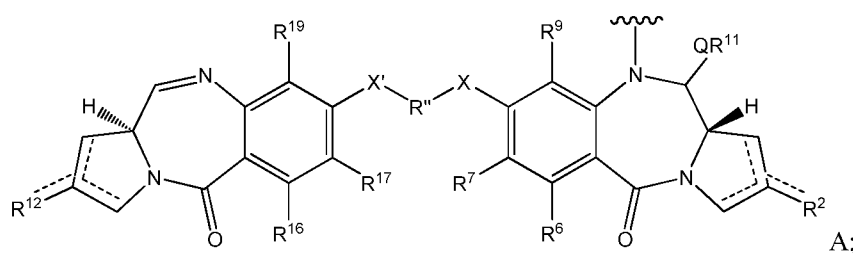
[015] In some embodiments of any of the methods, wherein the anti-LGR5 antibody comprises: a) a VH sequence of SEQ ID NO: 6 and a VL sequence of SEQ ID NO: 5; b) a VH sequence of SEQ ID NO: 8 and a VL sequence of SEQ ID NO: 7; c) a VH sequence of SEQ ID NO: 10 and a VL sequence of SEQ ID NO: 9; d) a VH sequence of SEQ ID NO: 12 and a VL sequence of SEQ ID NO: 11; e) a VH sequence of SEQ ID NO: 14 and a VL sequence of SEQ ID NO: 13; f) a VH sequence of SEQ ID NO: 16 and a VL sequence of SEQ ID NO: 15; g) a VH sequence of SEQ ID NO: 18 and a VL sequence of SEQ ID NO: 17; h) a VH sequence of SEQ ID NO: 20 and a VL sequence of SEQ ID NO: 19; or i) a VH sequence of SEQ ID NO: 26 and a VL sequence of SEQ ID NO: 25.

[016] In some embodiments of any of the methods, the anti-LGR5 antibody is conjugated to a cytotoxic agent. In some embodiments, the anti-LGR5 antibody has the formula Ab-(L-D)<sub>p</sub>, wherein: (a) Ab is the anti-LGR5 antibody; (b) L is a linker; (c) D is a drug selected from a maytansinoid, an auristatin, a calicheamicin, a pyrrolobenzodiazepine, and a nemorubicin derivative; and (d) p ranges from 1-8.

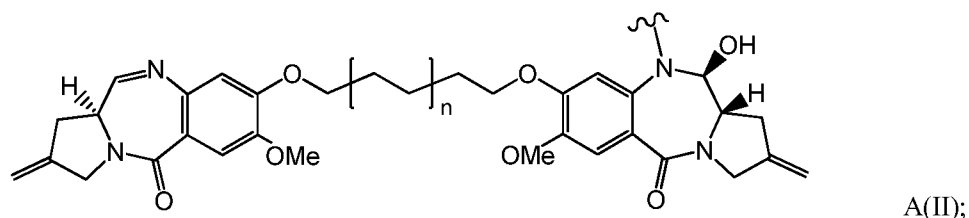
[017] In some embodiments, D is an auristatin. In some embodiments, D has formula D<sub>E</sub>



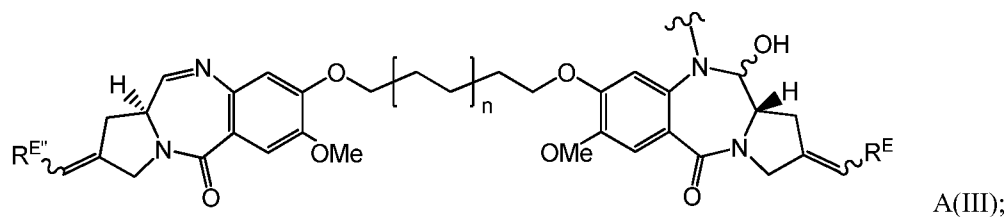
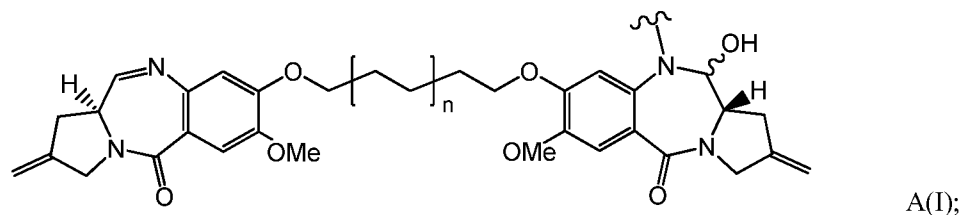
and wherein R<sup>2</sup> and R<sup>6</sup> are each methyl, R<sup>3</sup> and R<sup>4</sup> are each isopropyl, R<sup>5</sup> is H, R<sup>7</sup> is sec-butyl, each R<sup>8</sup> is independently selected from CH<sub>3</sub>, O-CH<sub>3</sub>, OH, and H; R<sup>9</sup> is H; and R<sup>18</sup> is -C(R<sup>8</sup>)<sub>2</sub>-C(R<sup>8</sup>)<sub>2</sub>-aryl. In some embodiments, the drug is MMAE. In some embodiments, D is a pyrrolobenzodiazepine of Formula A:

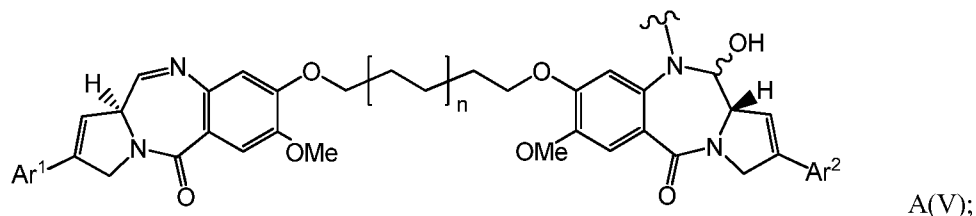
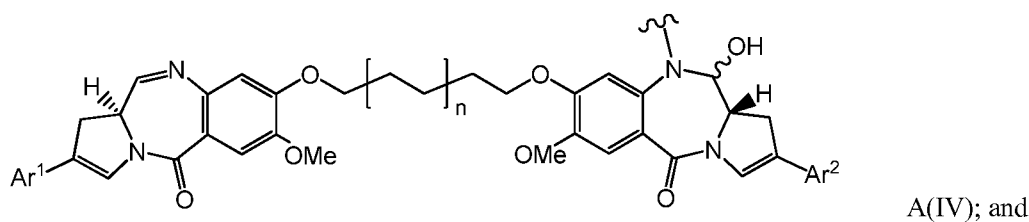


wherein the dotted lines indicate the optional presence of a double bond between C1 and C2 or C2 and C3; R<sup>2</sup> is independently selected from H, OH, =O, =CH<sub>2</sub>, CN, R, OR, =CH-R<sup>D</sup>, =C(R<sup>D</sup>)<sub>2</sub>, O-SO<sub>2</sub>-R, CO<sub>2</sub>R and COR, and optionally further selected from halo or dihalo, wherein R<sup>D</sup> is independently selected from R, CO<sub>2</sub>R, COR, CHO, CO<sub>2</sub>H, and halo; R<sup>6</sup> and R<sup>9</sup> are independently selected from H, R, OH, OR, SH, SR, NH<sub>2</sub>, NHR, NRR', NO<sub>2</sub>, Me<sub>3</sub>Sn and halo; R<sup>7</sup> is independently selected from H, R, OH, OR, SH, SR, NH<sub>2</sub>, NHR, NRR', NO<sub>2</sub>, Me<sub>3</sub>Sn and halo; Q is independently selected from O, S and NH; R<sup>11</sup> is either H, or R or, where Q is O, SO<sub>3</sub>M, where M is a metal cation; R and R' are each independently selected from optionally substituted C<sub>1-8</sub> alkyl, C<sub>3-8</sub> heterocyclyl and C<sub>5-20</sub> aryl groups, and optionally in relation to the group NRR', R and R' together with the nitrogen atom to which they are attached form an optionally substituted 4-, 5-, 6- or 7-membered heterocyclic ring; R<sup>12</sup>, R<sup>16</sup>, R<sup>19</sup> and R<sup>17</sup> are as defined for R<sup>2</sup>, R<sup>6</sup>, R<sup>9</sup> and R<sup>7</sup> respectively; R'' is a C<sub>3-12</sub> alkylene group, which chain may be interrupted by one or more heteroatoms and/or aromatic rings that are optionally substituted; and X and X' are independently selected from O, S and N(H). In some embodiments, D has the structure:

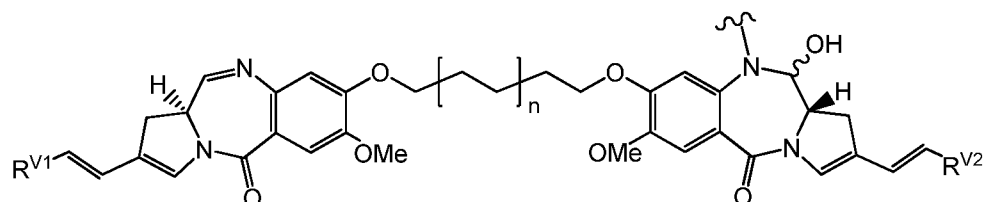


wherein n is 0 or 1. In some embodiments, D has a structure selected from:

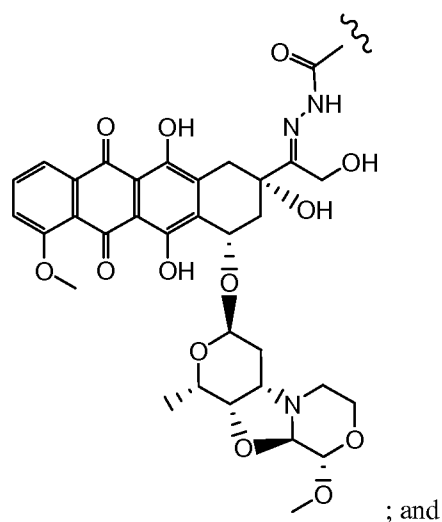


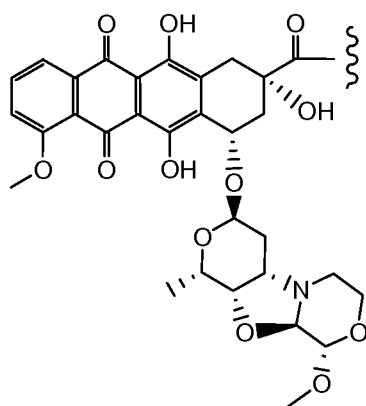


wherein  $R^E$  and  $R^{E'}$  are each independently selected from H or  $R^D$ , wherein  $R^D$  is independently selected from R,  $\text{CO}_2\text{R}$ , COR, CHO,  $\text{CO}_2\text{H}$ , and halo; wherein  $\text{Ar}^1$  and  $\text{Ar}^2$  are each independently optionally substituted  $\text{C}_{5-20}$  aryl; and wherein n is 0 or 1. In some embodiments, D is a pyrrolobenzodiazepine of Formula B:



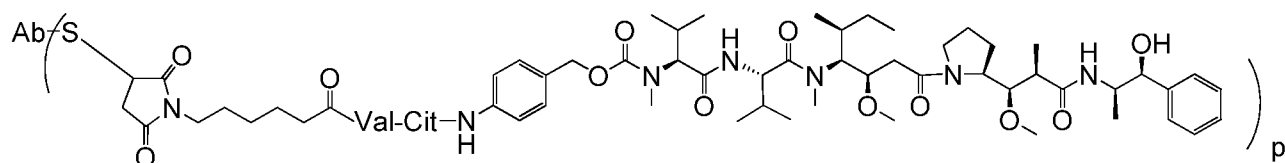
wherein the horizontal wavy line indicates the covalent attachment site to the linker;  $R^{V1}$  and  $R^{V2}$  are independently selected from H, methyl, ethyl, phenyl, fluoro-substituted phenyl, and  $\text{C}_{5-6}$  heterocyclyl; and n is 0 or 1. In some embodiments, D is a nemorubicin derivative. In some embodiments, D has a structure selected from:





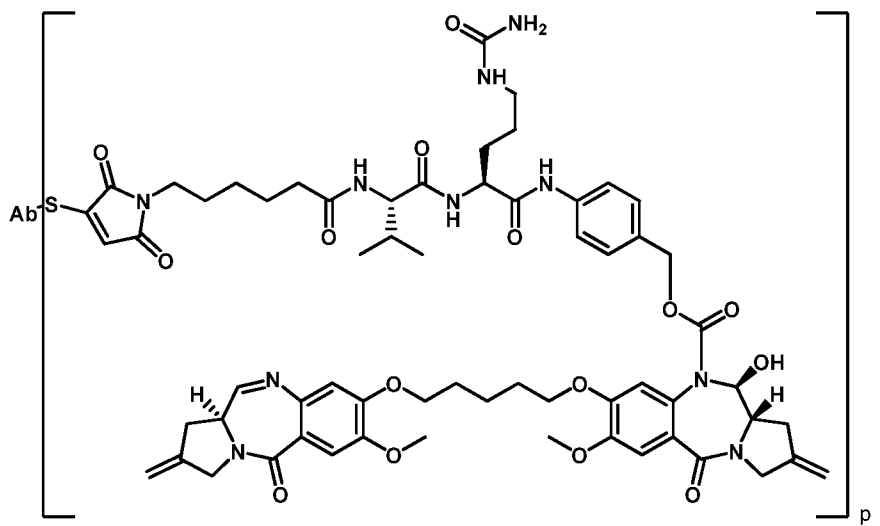
[018] In some embodiments of any of the methods, the linker is cleavable by a protease. In some embodiments, the linker comprises a val-cit dipeptide or a Phe-Lys dipeptide. In some embodiments of any of the methods, the linker is acid-labile. In some embodiments, the linker comprises hydrazone.

[019] In some embodiments of any of the methods, the anti-LGR5 antibody has the formula:



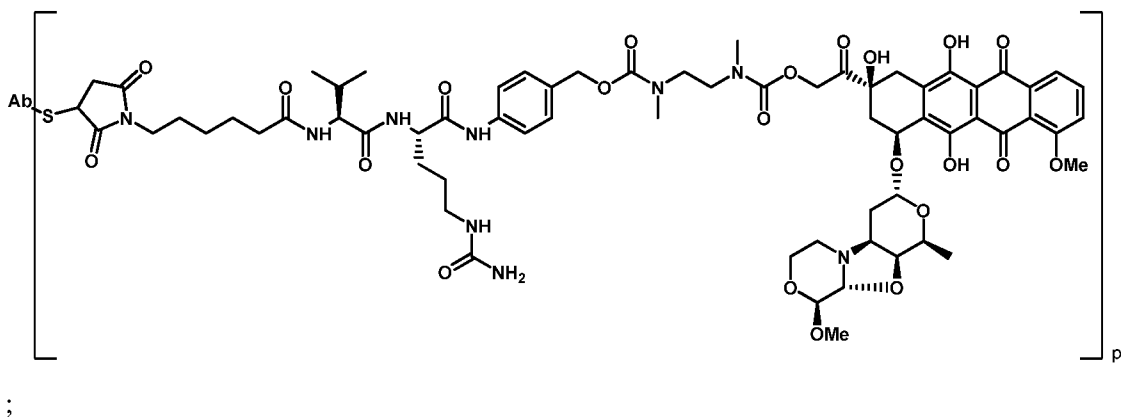
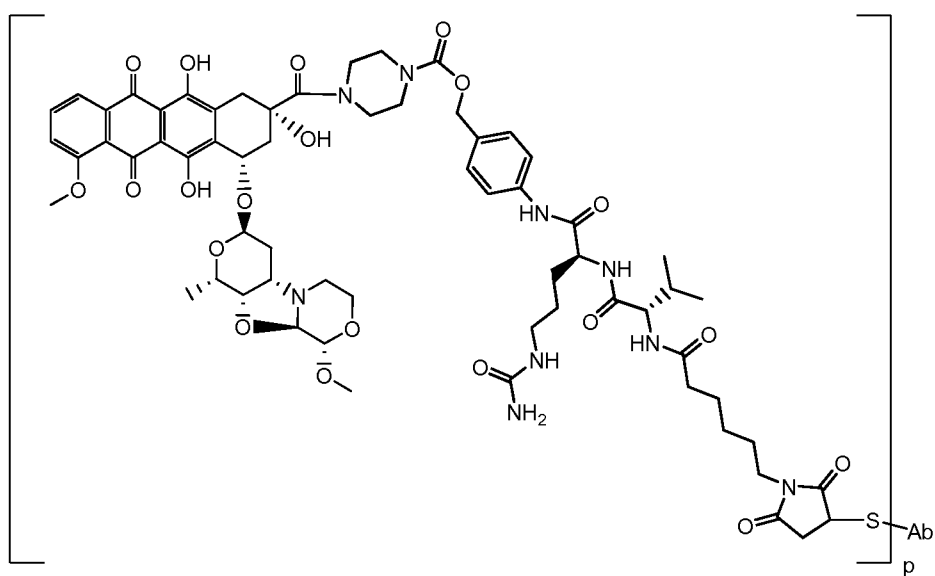
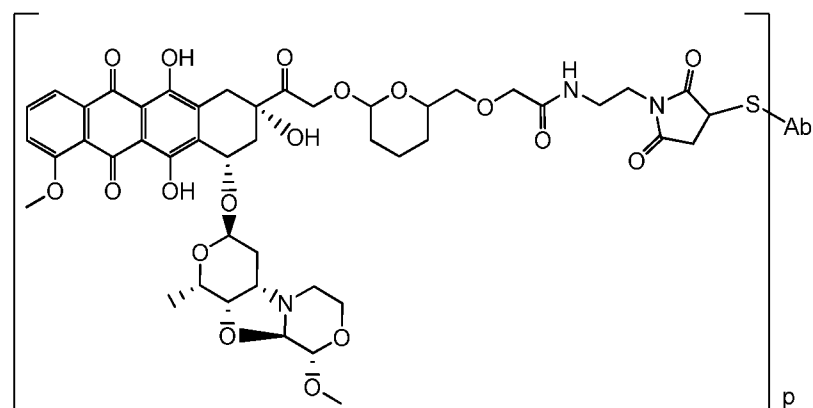
wherein S is a sulfur atom.

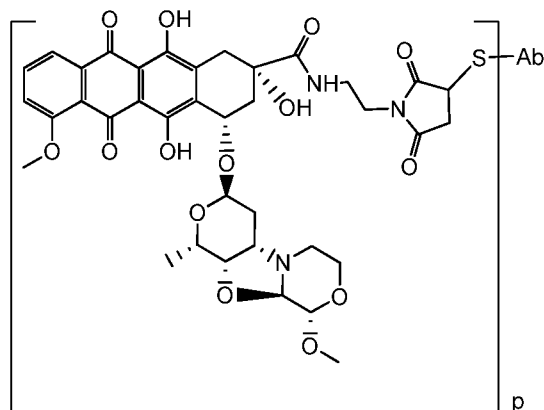
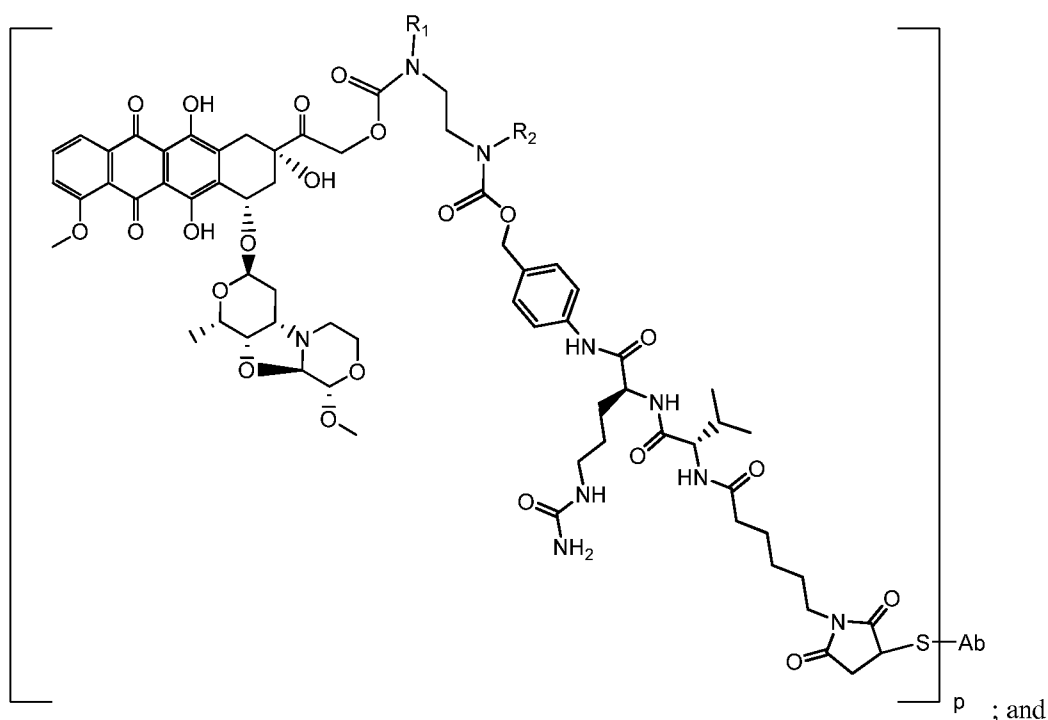
[020] In some embodiments of any of the methods, the anti-LGR5 antibody has the formula:



[021] In some embodiments of any of the methods, the anti-LGR5 antibody has the formula selected from the group consisting of:







[022] In some embodiments of any of the methods, p ranges from 2-5.

[023] In some embodiments of any of the methods, the inhibitor of the hedgehog pathway is an antagonist of smoothened. In some embodiments of any of the methods, the inhibitor of the hedgehog pathway is a cyclopamine-competitive antagonist of smoothened. In some embodiments, the antagonist of smoothened is 2-chloro-N-[4-chloro-3-(pyridin-2-yl)phenyl]-4-(methylsulfonyl)benzamide or a salt thereof. In some embodiments, the antagonist of smoothened is 2-chloro-N-[4-chloro-3-(pyridin-2-yl)phenyl]-4-(methylsulfonyl)benzamide. In some embodiments, the antagonist of smoothened is vismodegib.

[024] In some embodiments of any of the methods, the hedgehog-related disease is cancer. In some embodiments, the cancer is basal cell carcinoma. In some embodiments, the basal cell carcinoma is

locally advanced or metastatic basal cell carcinoma. In some embodiments, the cancer is medulloblastoma. In some embodiments, the hedgehog-related disease is LGR5-positive.

[025] In some embodiments of any of the methods, the inhibitor of the hedgehog pathway is administered concomitantly with the anti-LGR5 antibody. In some embodiments, the inhibitor of the hedgehog pathway is administered separately, sequentially, or simultaneously with the anti-LGR5 antibody.

### BRIEF DESCRIPTION OF THE FIGURES

[026] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[027] **Figure 1** shows the extensive basal cell carcinoma observed in K14-CreER; *Ptch1*<sup>fl/fl</sup>; *p53*<sup>fl/fl</sup> mice (C-D) compared to control mice (A-B).

[028] **Figure 2** shows extensive tumor regression in K14-CreER; *Ptch1*<sup>fl/fl</sup>; *p53*<sup>fl/fl</sup> mice upon treatment with GDC-0449 (vismodegib) (B) compared to untreated control K14-CreER; *Ptch1*<sup>fl/fl</sup>; *p53*<sup>fl/fl</sup> mice (A). Residual disease is still observed in animal K14-CreER; *Ptch1*<sup>fl/fl</sup>; *p53*<sup>fl/fl</sup> mice after treatment with 75 mg/kg GDC-0449 po BID for 56 days as shown in (C-D).

[029] **Figure 3** shows (A) expression of *Lgr5* in untreated control K14-CreER; *Ptch1*<sup>fl/fl</sup>; *p53*<sup>fl/fl</sup> mice, and (B) expression of *Lgr5* in K14-CreER; *Ptch1*<sup>fl/fl</sup>; *p53*<sup>fl/fl</sup> mice after treatment 75 mg/kg GDC-0449 po BID for 28 days. (C-D) shows effect of DTR mediated ablation in K14-CreER; *Ptch1*<sup>fl/fl</sup>; *p53*<sup>fl/fl</sup>; *Lgr5*<sup>DTR.EGFP</sup> mice in control (5d saline) (C) and DT treated (5d) (D). DAPI is in blue, GFP is in green, and the apoptosis marker, CC3, is shown in red.

[030] **Figure 4.** (A-B) show tumor regression in K14-CreER; *Ptch1*<sup>fl/fl</sup>; *p53*<sup>fl/fl</sup>; *Lgr5*<sup>DTR.EGFP</sup> mice after treatment 75 mg/kg GDC-0449 po BID for 38 days. (C-F) show enhanced tumor regression in K14-CreER; *Ptch1*<sup>fl/fl</sup>; *p53*<sup>fl/fl</sup>; *Lgr5*<sup>DTR.EGFP</sup> mice after treatment 75 mg/kg GDC-0449 po BID for 28 days followed by 75 mg/kg GDC-0449 po BID + DT for 8 days. (G-H) Shows that treatment with 75 mg/kg GDC-0449 po BID for 28 days followed by 75 mg/kg GDC-0449 po BID + DT for 8 days markedly reduces/eliminates residual superficial basal cell carcinoma in K14-CreER; *Ptch1*<sup>fl/fl</sup>; *p53*<sup>fl/fl</sup>; *Lgr5*<sup>DTR.EGFP</sup>. DAPI DNA staining is in blue. (G) Keratin 5 staining is in red. (H) The apoptosis marker, CC3, is in red.

### DETAILED DESCRIPTION

#### I. DEFINITIONS

[031] The term “hedgehog,” as used herein, refers to any member of the hedgehog family, including sonic, indian, desert and tiggy winkle. The term may be used to indicate protein or gene. The term is also used to describe homolog/ortholog sequences in different animal species.

[032] The terms “hedgehog (Hh) signaling pathway” and “hedgehog (Hh) signaling,” as used herein, are used interchangeably and refer to the chain of events normally mediated by various

members of the signaling cascade such as hedgehog, patched (Ptch), smoothened (Smo), and Gli. The hedgehog pathway can be activated even in the absence of a hedgehog protein by activating a downstream component. By way of example only, overexpression of Smo will activate the pathway in the absence of hedgehog. Hh signaling components or members of Hh signaling pathway refer to gene products that participate in the Hh signaling pathway. An Hh signaling component frequently materially or substantially affects the transmission of the Hh signal in cells/tissues, typically resulting in changes in degree of downstream gene expression level and/or phenotypic changes. Hh signaling components, depending on their biological function and effects on the final outcome of the downstream gene activation/expression, may be divided into positive and negative regulators. A positive regulator is an Hh signaling component that positively affects the transmission of the Hh signal, *i.e.*, stimulates downstream biological events when Hh is present. Examples include hedgehog, Smo, and Gli. A negative regulator is an Hh signaling component that negatively affects the transmission of the Hh signal, *i.e.*, inhibits downstream biological events when Hh is present. Examples include (but are not limited to) Ptch and SuFu.

[033] The terms “hedgehog signaling antagonist(s)”, “antagonists of Hh signaling” and “inhibitors of Hh signaling pathway,” as used herein, are used interchangeably and refer to agents that inhibit the bioactivity of a positive Hh signaling component (such as hedgehog, Ptch, or Gli) or down-regulate the expression of the Hh signaling component. They also include agents which up-regulate a negative regulator of Hh signaling component. A hedgehog signaling antagonists may be directed to a protein encoded by any of the genes in the hedgehog pathway, including (but not limited to) sonic, indian or desert hedgehog, smoothened, ptch-1, ptch-2, gli-1, gli-2, gli-3, etc. For example, an inhibitor of Hh signaling pathway may refers to any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity mediated by Hh. Examples of inhibitors include antibodies; ligand antibodies; small molecule antagonists; antisense and inhibitory RNA (*e.g.*, shRNA) molecules. In a particular embodiment, an inhibitor has a binding affinity (dissociation constant) of about 1,000 nM or less. In another embodiment, inhibitor has a binding affinity of about 100 nM or less. In another embodiment, an inhibitor has a binding affinity of about 50 nM or less. In a particular embodiment, an inhibitor inhibits Hh signaling with an IC<sub>50</sub> of 1,000 nM or less. In another embodiment, an inhibitor inhibits Hh signaling with an IC<sub>50</sub> of 500 nM or less. In another embodiment, an inhibitor inhibits Hh signaling with an IC<sub>50</sub> of 50 nM or less. In certain embodiments, the antagonist reduces or inhibits, by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more, the expression level or biological activity of one or more Hh pathway components. In some embodiments, the inhibitor of Hh signaling is an antagonist of smoothened.

[034] The terms “Hedgehog-related disorder(s), or “Hedgehog-related disease(s),” as used herein, includes diseases and disorders associated with disruption or aberrance of the Hedgehog pathway, as well as disorders associated with normal but undesired growth states relating to activation of the Hedgehog pathway. “Hedgehog-related disorder(s)” include but are not limited to tumor formation,

cancer, neoplasia, malignant hyperproliferative disorders, and non-malignant hyperproliferative disorders. “Hedgehog-related disorder(s)” also include benign prostate hyperplasia, psoriasis, wet macular degeneration, osteopetrosis and unwanted hair growth.

[035] The term “substantially the same,” as used herein, denotes a sufficiently high degree of similarity between two numeric values, such that one of skill in the art would consider the difference between the two values to be of little or no biological and/or statistical significance within the context of the biological characteristic measured by said values (*e.g.*, Kd values or expression). The difference between said two values is, for example, less than about 50%, less than about 40%, less than about 30%, less than about 20%, and/or less than about 10% as a function of the reference/comparator value.

[036] The phrase “substantially different,” as used herein, denotes a sufficiently high degree of difference between two numeric values such that one of skill in the art would consider the difference between the two values to be of statistical significance within the context of the biological characteristic measured by said values (*e.g.*, Kd values). The difference between said two values is, for example, greater than about 10%, greater than about 20%, greater than about 30%, greater than about 40%, and/or greater than about 50% as a function of the value for the reference/comparator molecule.

[037] 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.

[038] “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.

[039] 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.

[040] The terms “anti-LGR5 antibody” and “an antibody that binds to LGR5” refer to an antibody that is capable of binding LGR5 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting LGR5. In one embodiment, the extent of binding of an anti-LGR5 antibody to an unrelated, non-LGR5 protein is less than about 10% of the binding of the antibody to LGR5 as measured, *e.g.*, by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to LGR5 has a dissociation constant ( $K_d$ ) of  $\leq 1\mu\text{M}$ ,  $\leq 100\text{ nM}$ ,  $\leq 10\text{ nM}$ ,  $\leq 5\text{ nM}$ ,  $\leq 4\text{ nM}$ ,  $\leq 3\text{ nM}$ ,  $\leq 2\text{ nM}$ ,  $\leq 1\text{ nM}$ ,  $\leq 0.1\text{ nM}$ ,  $\leq 0.01\text{ nM}$ , or  $\leq 0.001\text{ nM}$  (*e.g.*,  $10^{-8}\text{ M}$  or less, *e.g.* from  $10^{-8}\text{ M}$  to  $10^{-13}\text{ M}$ , *e.g.*, from  $10^{-9}\text{ M}$  to  $10^{-13}\text{ M}$ ). In certain embodiments, an anti-LGR5 antibody binds to an epitope of LGR5 that is conserved among LGR5 from different species.

[041] The term “antibody” is used herein 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.

[042] An “antibody fragment” refers to a molecule other than an intact antibody that comprises a portion of an intact antibody and 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')<sub>2</sub>; diabodies; linear antibodies; single-chain antibody molecules (*e.g.* scFv); and multispecific antibodies formed from antibody fragments.

[043] 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.

[044] The terms “cancer” and “cancerous” refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth/proliferation. Examples of cancer include, but are not limited to, carcinoma, lymphoma (*e.g.*, Hodgkin's and non-Hodgkin's lymphoma), blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, small intestine cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, leukemia and other lymphoproliferative disorders, and various types of head and neck cancer.

[045] 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.

[046] 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<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, IgG<sub>4</sub>, IgA<sub>1</sub>, and IgA<sub>2</sub>. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called  $\alpha$ ,  $\delta$ ,  $\epsilon$ ,  $\gamma$ , and  $\mu$ , respectively.

[047] The term “cytotoxic agent” as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to, radioactive isotopes (*e.g.*, At<sup>211</sup>, I<sup>131</sup>, I<sup>125</sup>, Y<sup>90</sup>, Re<sup>186</sup>, Re<sup>188</sup>, Sm<sup>153</sup>, Bi<sup>212</sup>, P<sup>32</sup>, Pb<sup>212</sup> and radioactive isotopes of Lu); chemotherapeutic agents or drugs (*e.g.*, methotrexate, adriamycin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof; and the various antitumor or anticancer agents disclosed below.

[048] “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.

[049] 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.

[050] The term “epitope” refers to the particular site on an antigen molecule to which an antibody binds.

[051] 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.

[052] “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.

[053] 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.

[054] The term “glycosylated forms of LGR5” refers to naturally occurring forms of LGR5 that are post-translationally modified by the addition of carbohydrate residues.

[055] 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.

[056] 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.

[057] 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*.

[058] 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.

[059] 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*.

[060] An “immunoconjugate” is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

[061] 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.

[062] 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).

[063] 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.

[064] “Isolated nucleic acid encoding an anti-LGR5 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.

[065] The term “LGR5,” as used herein, refers to any native, mature LGR5 which results from processing of an LGR5 precursor protein in a cell. The term includes LGR5 from any vertebrate source, including mammals such as primates (*e.g.* humans and cynomolgus monkeys) and rodents (*e.g.*, mice and rats), unless otherwise indicated. The term also includes naturally occurring variants of LGR5, *e.g.*, splice variants or allelic variants. The amino acid sequence of an exemplary human LGR5 precursor protein, with signal sequence (amino acids 1-21) is shown in SEQ ID NO: 67. The amino acid sequence of an exemplary mature human LGR5 .

[066] The term “LGR5-positive cancer” refers to a cancer comprising cells that express LGR5 on their surface. For the purposes of determining whether a cell expresses LGR5 on the surface, LGR5 mRNA expression is considered to correlate to LGR5 expression on the cell surface. In some embodiments, expression of LGR5 mRNA is determined by a method selected from in situ hybridization and RT-PCR (including quantitative RT-PCR). Alternatively, expression of LGR5 on the cell surface can be determined, for example, using antibodies to LGR5 in a method such as immunohistochemistry, FACS, etc.

[067] The term “LGR5-positive cell” refers to a cell that expresses LGR5 on its surface.

[068] 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.

[069] A “naked antibody” refers to an antibody that is not conjugated to a heterologous moiety (*e.g.*, a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation.

[070] “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 ( $\kappa$ ) and lambda ( $\lambda$ ), based on the amino acid sequence of its constant domain.

[071] 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.

[072] “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.

[073] 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:

$$100 \text{ times the fraction } X/Y$$

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.

[074] 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.

[075] 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.

[076] 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.

[077] The term “concomitantly” is used herein to refer to administration of two or more therapeutic agents, give in close enough temporal proximity where their individual therapeutic effects overlap in time. Accordingly, concurrent administration includes a dosing regimen when the administration of one or more agent(s) continues after discontinuing the administration of one or more other agent(s). In some embodiments, the concomitantly administration is concurrently, sequentially, and/or simultaneously.

[078] By “reduce or inhibit” is meant the ability to cause an overall decrease of 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or greater. Reduce or inhibit can refer to the symptoms of the disorder being treated, the presence or size of metastases, or the size of the primary tumor.

[079] 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*, 6<sup>th</sup> 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).

[080] 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."

[081] "Alkyl" is C<sub>1</sub>-C<sub>18</sub> hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms. Examples are methyl (Me, -CH<sub>3</sub>), ethyl (Et, -CH<sub>2</sub>CH<sub>3</sub>), 1-propyl (n-Pr, n-propyl, -CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 2-propyl (i-Pr, i-propyl, -CH(CH<sub>3</sub>)<sub>2</sub>), 1-butyl (n-Bu, n-butyl, -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 2-methyl-1-propyl (i-Bu, i-butyl, -CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 2-butyl (s-Bu, s-butyl, -CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub>), 2-methyl-2-propyl (t-Bu, t-butyl, -C(CH<sub>3</sub>)<sub>3</sub>), 1-pentyl (n-pentyl, -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 2-pentyl (-CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 3-pentyl (-CH(CH<sub>2</sub>CH<sub>3</sub>)<sub>2</sub>), 2-methyl-2-butyl (-C(CH<sub>3</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 3-methyl-2-butyl (-CH(CH<sub>3</sub>)CH(CH<sub>3</sub>)<sub>2</sub>), 3-methyl-1-butyl (-CH<sub>2</sub>CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 2-methyl-1-butyl (-CH<sub>2</sub>CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub>), 1-hexyl (-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 2-hexyl (-CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 3-hexyl (-CH(CH<sub>2</sub>CH<sub>3</sub>)(CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>)), 2-methyl-2-pentyl (-C(CH<sub>3</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 3-methyl-2-pentyl (-CH(CH<sub>3</sub>)CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub>), 4-methyl-2-pentyl (-CH(CH<sub>3</sub>)CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 3-methyl-3-pentyl (-C(CH<sub>3</sub>)(CH<sub>2</sub>CH<sub>3</sub>)<sub>2</sub>), 2-methyl-3-pentyl (-CH(CH<sub>2</sub>CH<sub>3</sub>)CH(CH<sub>3</sub>)<sub>2</sub>), 2,3-dimethyl-2-butyl (-C(CH<sub>3</sub>)<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 3,3-dimethyl-2-butyl (-CH(CH<sub>3</sub>)C(CH<sub>3</sub>)<sub>3</sub>).

[082] The term "C<sub>1</sub>-C<sub>8</sub> alkyl," as used herein refers to a straight chain or branched, saturated or unsaturated hydrocarbon having from 1 to 8 carbon atoms. Representative "C<sub>1</sub>-C<sub>8</sub> alkyl" groups include, but are not limited to, -methyl, -ethyl, -n-propyl, -n-butyl, -n-pentyl, -n-hexyl, -n-heptyl, -n-octyl, -n-nonyl and -n-decyl; while branched C<sub>1</sub>-C<sub>8</sub> alkyls include, but are not limited to, -isopropyl, -sec-butyl, -isobutyl, -tert-butyl, -isopentyl, 2-methylbutyl, unsaturated C<sub>1</sub>-C<sub>8</sub> alkyls include, but are not limited to, -vinyl, -allyl, -1-butenyl, -2-butenyl, -isobutylenyl, -1-pentenyl, -2-pentenyl, -3-methyl-1-butenyl, -2-methyl-2-butenyl, -2,3-dimethyl-2-butenyl, 1-hexyl, 2-hexyl, 3-hexyl, -acetylenyl, -propynyl, -1-butylnyl, -2-butylnyl, -1-pentynyl, -2-pentynyl, -3-methyl-1 butynyl. A C<sub>1</sub>-C<sub>8</sub> alkyl group can be unsubstituted or substituted with one or more groups including, but not limited to, -C<sub>1</sub>-C<sub>8</sub> alkyl, -O-(C<sub>1</sub>-C<sub>8</sub> alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH<sub>2</sub>, -C(O)NHR', -C(O)N(R')<sub>2</sub>, -NHC(O)R', -SO<sub>3</sub>R', -S(O)<sub>2</sub>R', -S(O)R', -OH, -halogen, -N<sub>3</sub>, -NH<sub>2</sub>, -NH(R'), -N(R')<sub>2</sub> and -CN; where each R' is independently selected from H, -C<sub>1</sub>-C<sub>8</sub> alkyl and aryl.

[083] The term "C<sub>1</sub>-C<sub>12</sub> alkyl," as used herein refers to a straight chain or branched, saturated or unsaturated hydrocarbon having from 1 to 12 carbon atoms. A C<sub>1</sub>-C<sub>12</sub> alkyl group can be unsubstituted or substituted with one or more groups including, but not limited to, -C<sub>1</sub>-C<sub>8</sub> alkyl, -O-

(C<sub>1</sub>-C<sub>8</sub> alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH<sub>2</sub>, -C(O)NHR', -C(O)N(R')<sub>2</sub> - NHC(O)R', -SO<sub>3</sub>R', -S(O)<sub>2</sub>R', -S(O)R', -OH, -halogen, -N<sub>3</sub>, -NH<sub>2</sub>, -NH(R'), -N(R')<sub>2</sub> and -CN; where each R' is independently selected from H, -C<sub>1</sub>-C<sub>8</sub> alkyl and aryl.

[084] The term "C<sub>1</sub>-C<sub>6</sub> alkyl," as used herein refers to a straight chain or branched, saturated or unsaturated hydrocarbon having from 1 to 6 carbon atoms. Representative "C<sub>1</sub>-C<sub>6</sub> alkyl" groups include, but are not limited to, -methyl, -ethyl, -n-propyl, -n-butyl, -n-pentyl, and -n-hexyl; while branched C<sub>1</sub>-C<sub>6</sub> alkyls include, but are not limited to, -isopropyl, -*sec*-butyl, -isobutyl, -*tert*-butyl, -isopentyl, and 2-methylbutyl; unsaturated C<sub>1</sub>-C<sub>6</sub> alkyls include, but are not limited to, -vinyl, -allyl, -1-butenyl, -2-butenyl, and -isobutyl-1-enyl, -1-pentenyl, -2-pentenyl, -3-methyl-1-butenyl, -2-methyl-2-butenyl, -2,3-dimethyl-2-butenyl, 1-hexyl, 2-hexyl, and 3-hexyl. A C<sub>1</sub>-C<sub>6</sub> alkyl group can be unsubstituted or substituted with one or more groups, as described above for C<sub>1</sub>-C<sub>8</sub> alkyl group.

[085] The term "C<sub>1</sub>-C<sub>4</sub> alkyl," as used herein refers to a straight chain or branched, saturated or unsaturated hydrocarbon having from 1 to 4 carbon atoms. Representative "C<sub>1</sub>-C<sub>4</sub> alkyl" groups include, but are not limited to, -methyl, -ethyl, -n-propyl, -n-butyl; while branched C<sub>1</sub>-C<sub>4</sub> alkyls include, but are not limited to, -isopropyl, -*sec*-butyl, -isobutyl, -*tert*-butyl; unsaturated C<sub>1</sub>-C<sub>4</sub> alkyls include, but are not limited to, -vinyl, -allyl, -1-butenyl, -2-butenyl, and -isobutyl-1-enyl. A C<sub>1</sub>-C<sub>4</sub> alkyl group can be unsubstituted or substituted with one or more groups, as described above for C<sub>1</sub>-C<sub>8</sub> alkyl group.

[086] "Alkoxy" is an alkyl group singly bonded to an oxygen. Exemplary alkoxy groups include, but are not limited to, methoxy (-OCH<sub>3</sub>) and ethoxy (-OCH<sub>2</sub>CH<sub>3</sub>). A "C<sub>1</sub>-C<sub>5</sub> alkoxy" is an alkoxy group with 1 to 5 carbon atoms. Alkoxy groups may be unsubstituted or substituted with one or more groups, as described above for alkyl groups.

[087] "Alkenyl" is C<sub>2</sub>-C<sub>18</sub> hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, i.e. a carbon-carbon, *sp*<sup>2</sup> double bond. Examples include, but are not limited to: ethylene or vinyl (-CH=CH<sub>2</sub>), allyl (-CH<sub>2</sub>CH=CH<sub>2</sub>), cyclopentenyl (-C<sub>5</sub>H<sub>7</sub>), and 5-hexenyl (-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH=CH<sub>2</sub>). A "C<sub>2</sub>-C<sub>8</sub> alkenyl" is a hydrocarbon containing 2 to 8 normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, i.e. a carbon-carbon, *sp*<sup>2</sup> double bond.

[088] "Alkynyl" is C<sub>2</sub>-C<sub>18</sub> hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, i.e. a carbon-carbon, *sp* triple bond. Examples include, but are not limited to: acetylenic (-C≡CH) and propargyl (-CH<sub>2</sub>C≡CH). A "C<sub>2</sub>-C<sub>8</sub> alkynyl" is a hydrocarbon containing 2 to 8 normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, i.e. a carbon-carbon, *sp* triple bond.

[089] "Alkylene" refers to a saturated, branched or straight chain or cyclic hydrocarbon radical of 1-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkane. Typical alkylene radicals include, but are

not limited to: methylene ( $-\text{CH}_2-$ ), 1,2-ethyl ( $-\text{CH}_2\text{CH}_2-$ ), 1,3-propyl ( $-\text{CH}_2\text{CH}_2\text{CH}_2-$ ), 1,4-butyl ( $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$ ), and the like.

[090] A “ $\text{C}_1$ - $\text{C}_{10}$  alkylene” is a straight chain, saturated hydrocarbon group of the formula  $-(\text{CH}_2)_{1-10}-$ . Examples of a  $\text{C}_1$ - $\text{C}_{10}$  alkylene include methylene, ethylene, propylene, butylene, pentylene, hexylene, heptylene, octylene, nonylene and decalene.

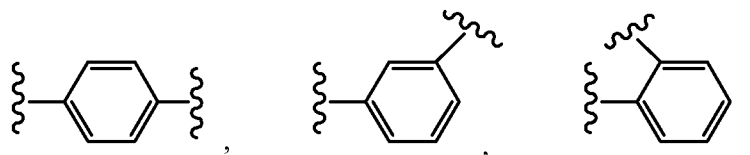
[091] “Alkenylene” refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkene. Typical alkenylene radicals include, but are not limited to: 1,2-ethylene ( $-\text{CH}=\text{CH}-$ ).

[092] “Alkynylene” refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkyne. Typical alkynylene radicals include, but are not limited to: acetylene ( $-\text{C}\equiv\text{C}-$ ), propargyl ( $-\text{CH}_2\text{C}\equiv\text{C}-$ ), and 4-pentynyl ( $-\text{CH}_2\text{CH}_2\text{CH}_2\text{C}\equiv\text{C}-$ ).

[093] “Aryl” refers to a carbocyclic aromatic group. Examples of aryl groups include, but are not limited to, phenyl, naphthyl and anthracenyl. A carbocyclic aromatic group or a heterocyclic aromatic group can be unsubstituted or substituted with one or more groups including, but not limited to,  $-\text{C}_1$ - $\text{C}_8$  alkyl,  $-\text{O}-(\text{C}_1$ - $\text{C}_8$  alkyl), -aryl,  $-\text{C}(\text{O})\text{R}'$ ,  $-\text{OC}(\text{O})\text{R}'$ ,  $-\text{C}(\text{O})\text{OR}'$ ,  $-\text{C}(\text{O})\text{NH}_2$ ,  $-\text{C}(\text{O})\text{NHR}'$ ,  $-\text{C}(\text{O})\text{N}(\text{R}')_2$ ,  $-\text{NHC}(\text{O})\text{R}'$ ,  $-\text{S}(\text{O})_2\text{R}'$ ,  $-\text{S}(\text{O})\text{R}'$ ,  $-\text{OH}$ , -halogen,  $-\text{N}_3$ ,  $-\text{NH}_2$ ,  $-\text{NH}(\text{R}')$ ,  $-\text{N}(\text{R}')_2$  and  $-\text{CN}$ ; wherein each  $\text{R}'$  is independently selected from H,  $-\text{C}_1$ - $\text{C}_8$  alkyl and aryl.

[094] A “ $\text{C}_5$ - $\text{C}_{20}$  aryl” is an aryl group with 5 to 20 carbon atoms in the carbocyclic aromatic rings. Examples of  $\text{C}_5$ - $\text{C}_{20}$  aryl groups include, but are not limited to, phenyl, naphthyl and anthracenyl. A  $\text{C}_5$ - $\text{C}_{20}$  aryl group can be substituted or unsubstituted as described above for aryl groups. A “ $\text{C}_5$ - $\text{C}_{14}$  aryl” is an aryl group with 5 to 14 carbon atoms in the carbocyclic aromatic rings. Examples of  $\text{C}_5$ - $\text{C}_{14}$  aryl groups include, but are not limited to, phenyl, naphthyl and anthracenyl. A  $\text{C}_5$ - $\text{C}_{14}$  aryl group can be substituted or unsubstituted as described above for aryl groups.

[095] An “arylene” is an aryl group which has two covalent bonds and can be in the ortho, meta, or para configurations as shown in the following structures:



in which the phenyl group can be unsubstituted or substituted with up to four groups including, but not limited to,  $-\text{C}_1$ - $\text{C}_8$  alkyl,  $-\text{O}-(\text{C}_1$ - $\text{C}_8$  alkyl), -aryl,  $-\text{C}(\text{O})\text{R}'$ ,  $-\text{OC}(\text{O})\text{R}'$ ,  $-\text{C}(\text{O})\text{OR}'$ ,  $-\text{C}(\text{O})\text{NH}_2$ ,  $-\text{C}(\text{O})\text{NHR}'$ ,  $-\text{C}(\text{O})\text{N}(\text{R}')_2$ ,  $-\text{NHC}(\text{O})\text{R}'$ ,  $-\text{S}(\text{O})_2\text{R}'$ ,  $-\text{S}(\text{O})\text{R}'$ ,  $-\text{OH}$ , -halogen,  $-\text{N}_3$ ,  $-\text{NH}_2$ ,  $-\text{NH}(\text{R}')$ ,  $-\text{N}(\text{R}')_2$  and  $-\text{CN}$ ; wherein each  $\text{R}'$  is independently selected from H,  $-\text{C}_1$ - $\text{C}_8$  alkyl and aryl.

[096] "Arylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or  $sp^3$  carbon atom, is replaced with an aryl radical. Typical arylalkyl groups include, but are not limited to, benzyl, 2-phenylethan-1-yl, 2-phenylethen-1-yl, naphthylmethyl, 2-naphthylethan-1-yl, 2-naphthylethen-1-yl, naphthobenzyl, 2-naphthophenylethan-1-yl and the like. The arylalkyl group comprises 6 to 20 carbon atoms, *e.g.* the alkyl moiety, including alkanyl, alkenyl or alkynyl groups, of the arylalkyl group is 1 to 6 carbon atoms and the aryl moiety is 5 to 14 carbon atoms.

[097] "Heteroarylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or  $sp^3$  carbon atom, is replaced with a heteroaryl radical. Typical heteroarylalkyl groups include, but are not limited to, 2-benzimidazolylmethyl, 2-furylethyl, and the like. The heteroarylalkyl group comprises 6 to 20 carbon atoms, *e.g.* the alkyl moiety, including alkanyl, alkenyl or alkynyl groups, of the heteroarylalkyl group is 1 to 6 carbon atoms and the heteroaryl moiety is 5 to 14 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S. The heteroaryl moiety of the heteroarylalkyl group may be a monocycle having 3 to 7 ring members (2 to 6 carbon atoms or a bicycle having 7 to 10 ring members (4 to 9 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S), for example: a bicyclo [4,5], [5,5], [5,6], or [6,6] system.

[098] "Substituted alkyl," "substituted aryl," and "substituted arylalkyl" mean alkyl, aryl, and arylalkyl respectively, in which one or more hydrogen atoms are each independently replaced with a substituent. Typical substituents include, but are not limited to, -X, -R, -O<sup>-</sup>, -OR, -SR, -S<sup>-</sup>, -NR<sub>2</sub>, -NR<sub>3</sub>, =NR, -CX<sub>3</sub>, -CN, -OCN, -SCN, -N=C=O, -NCS, -NO, -NO<sub>2</sub>, =N<sub>2</sub>, -N<sub>3</sub>, NC(=O)R, -C(=O)R, -C(=O)NR<sub>2</sub>, -SO<sub>3</sub><sup>-</sup>, -SO<sub>3</sub>H, -S(=O)<sub>2</sub>R, -OS(=O)<sub>2</sub>OR, -S(=O)<sub>2</sub>NR, -S(=O)R, -OP(=O)(OR)<sub>2</sub>, -P(=O)(OR)<sub>2</sub>, -PO<sub>3</sub>, -PO<sub>3</sub>H<sub>2</sub>, -C(=O)R, -C(=O)X, -C(=S)R, -CO<sub>2</sub>R, -CO<sub>2</sub>, -C(=S)OR, -C(=O)SR, -C(=S)SR, -C(=O)NR<sub>2</sub>, -C(=S)NR<sub>2</sub>, -C(=NR)NR<sub>2</sub>, where each X is independently a halogen: F, Cl, Br, or I; and each R is independently -H, C<sub>2</sub>-C<sub>18</sub> alkyl, C<sub>6</sub>-C<sub>20</sub> aryl, C<sub>3</sub>-C<sub>14</sub> heterocycle, protecting group or prodrug moiety. Alkylene, alkenylene, and alkynylene groups as described above may also be similarly substituted.

[099] "Heteroaryl" and "heterocycle" refer to a ring system in which one or more ring atoms is a heteroatom, *e.g.* nitrogen, oxygen, and sulfur. The heterocycle radical comprises 3 to 20 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S. A heterocycle may be a monocycle having 3 to 7 ring members (2 to 6 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S) or a bicycle having 7 to 10 ring members (4 to 9 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S), for example: a bicyclo [4,5], [5,5], [5,6], or [6,6] system.

[0100] Exemplary heterocycles are described, *e.g.*, in Paquette, Leo A., "Principles of Modern Heterocyclic Chemistry" (W.A. Benjamin, New York, 1968), particularly Chapters 1, 3, 4, 6, 7, and 9; "The Chemistry of Heterocyclic Compounds, A series of Monographs" (John Wiley & Sons, New York, 1950 to present), in particular Volumes 13, 14, 16, 19, and 28; and *J. Am. Chem. Soc.* (1960) 82:5566.



[0101] Examples of heterocycles include by way of example and not limitation pyridyl, dihydropyridyl, tetrahydropyridyl (piperidyl), thiazolyl, tetrahydrothiophenyl, sulfur oxidized tetrahydrothiophenyl, pyrimidinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, imidazolyl, tetrazolyl, benzofuranyl, thianaphthalenyl, indolyl, indolenyl, quinolinyl, isoquinolinyl, benzimidazolyl, piperidinyl, 4-piperidonyl, pyrrolidinyl, 2-pyrrolidonyl, pyrrolinyl, tetrahydrofuranyl, bis-tetrahydrofuranyl, tetrahydropyranyl, bis-tetrahydropyranyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl, octahydroisoquinolinyl, azocinyl, triazinyl, 6H-1,2,5-thiadiazinyl, 2H,6H-1,5,2-dithiazinyl, thienyl, thianthrenyl, pyranyl, isobenzofuranyl, chromenyl, xanthenyl, phenoxathinyl, 2H-pyrrolyl, isothiazolyl, isoxazolyl, pyrazinyl, pyridazinyl, indoliziny, isoindolyl, 3H-indolyl, 1H-indazolyl, purinyl, 4H-quinoliziny, phthalazinyl, naphthyridinyl, quinoxaliny, quinazolinyl, cinnolinyl, pteridinyl, 4aH-carbazolyl, carbazolyl,  $\beta$ -carbolinyl, phenanthridinyl, acridinyl, pyrimidinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, furazanyl, phenoxazinyl, isochromanyl, chromanyl, imidazolidinyl, imidazoliny, pyrazolidinyl, pyrazoliny, piperazinyl, indoliny, isoindoliny, quinuclidinyl, morpholinyl, oxazolidinyl, benzotriazolyl, benzisoxazolyl, oxindolyl, benzoxazoliny, and isatinoyl.

[0102] By way of example and not limitation, carbon bonded heterocycles are bonded at position 2, 3, 4, 5, or 6 of a pyridine, position 3, 4, 5, or 6 of a pyridazine, position 2, 4, 5, or 6 of a pyrimidine, position 2, 3, 5, or 6 of a pyrazine, position 2, 3, 4, or 5 of a furan, tetrahydrofuran, thiofuran, thiophene, pyrrole or tetrahydropyrrole, position 2, 4, or 5 of an oxazole, imidazole or thiazole, position 3, 4, or 5 of an isoxazole, pyrazole, or isothiazole, position 2 or 3 of an aziridine, position 2, 3, or 4 of an azetidine, position 2, 3, 4, 5, 6, 7, or 8 of a quinoline or position 1, 3, 4, 5, 6, 7, or 8 of an isoquinoline. Still more typically, carbon bonded heterocycles include 2-pyridyl, 3-pyridyl, 4-pyridyl, 5-pyridyl, 6-pyridyl, 3-pyridazinyl, 4-pyridazinyl, 5-pyridazinyl, 6-pyridazinyl, 2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, 6-pyrimidinyl, 2-pyrazinyl, 3-pyrazinyl, 5-pyrazinyl, 6-pyrazinyl, 2-thiazolyl, 4-thiazolyl, or 5-thiazolyl.

[0103] By way of example and not limitation, nitrogen bonded heterocycles are bonded at position 1 of an aziridine, azetidine, pyrrole, pyrrolidine, 2-pyrroline, 3-pyrroline, imidazole, imidazolidine, 2-imidazoline, 3-imidazoline, pyrazole, pyrazoline, 2-pyrazoline, 3-pyrazoline, piperidine, piperazine, indole, indoline, 1H-indazole, position 2 of a isoindole, or isoindoline, position 4 of a morpholine, and position 9 of a carbazole, or  $\beta$ -carboline. Still more typically, nitrogen bonded heterocycles include 1-aziridyl, 1-azetetyl, 1-pyrrolyl, 1-imidazolyl, 1-pyrazolyl, and 1-piperidinyl.

[0104] A "C<sub>3</sub>-C<sub>8</sub> heterocycle" refers to an aromatic or non-aromatic C<sub>3</sub>-C<sub>8</sub> carbocycle in which one to four of the ring carbon atoms are independently replaced with a heteroatom from the group consisting of O, S and N. Representative examples of a C<sub>3</sub>-C<sub>8</sub> heterocycle include, but are not limited to, benzofuranyl, benzothiophene, indolyl, benzopyrazolyl, coumarinyl, isoquinolinyl, pyrrolyl, thiophenyl, furanyl, thiazolyl, imidazolyl, pyrazolyl, triazolyl, quinolinyl, pyrimidinyl, pyridinyl, pyridonyl, pyrazinyl, pyridazinyl, isothiazolyl, isoxazolyl and tetrazolyl. A C<sub>3</sub>-C<sub>8</sub> heterocycle can be

unsubstituted or substituted with up to seven groups including, but not limited to, -C<sub>1</sub>-C<sub>8</sub> alkyl, -O-(C<sub>1</sub>-C<sub>8</sub> alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH<sub>2</sub>, -C(O)NHR', -C(O)N(R')<sub>2</sub>, -NHC(O)R', -S(O)<sub>2</sub>R', -S(O)R', -OH, -halogen, -N<sub>3</sub>, -NH<sub>2</sub>, -NH(R'), -N(R')<sub>2</sub> and -CN; wherein each R' is independently selected from H, -C<sub>1</sub>-C<sub>8</sub> alkyl and aryl.

[0105] "C<sub>3</sub>-C<sub>8</sub> heterocyclo" refers to a C<sub>3</sub>-C<sub>8</sub> heterocycle group defined above wherein one of the heterocycle group's hydrogen atoms is replaced with a bond. A C<sub>3</sub>-C<sub>8</sub> heterocyclo can be unsubstituted or substituted with up to six groups including, but not limited to, -C<sub>1</sub>-C<sub>8</sub> alkyl, -O-(C<sub>1</sub>-C<sub>8</sub> alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH<sub>2</sub>, -C(O)NHR', -C(O)N(R')<sub>2</sub>, -NHC(O)R', -S(O)<sub>2</sub>R', -S(O)R', -OH, -halogen, -N<sub>3</sub>, -NH<sub>2</sub>, -NH(R'), -N(R')<sub>2</sub> and -CN; wherein each R' is independently selected from H, -C<sub>1</sub>-C<sub>8</sub> alkyl and aryl.

[0106] A "C<sub>3</sub>-C<sub>20</sub> heterocycle" refers to an aromatic or non-aromatic C<sub>3</sub>-C<sub>8</sub> carbocycle in which one to four of the ring carbon atoms are independently replaced with a heteroatom from the group consisting of O, S and N. A C<sub>3</sub>-C<sub>20</sub> heterocycle can be unsubstituted or substituted with up to seven groups including, but not limited to, -C<sub>1</sub>-C<sub>8</sub> alkyl, -O-(C<sub>1</sub>-C<sub>8</sub> alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH<sub>2</sub>, -C(O)NHR', -C(O)N(R')<sub>2</sub>, -NHC(O)R', -S(O)<sub>2</sub>R', -S(O)R', -OH, -halogen, -N<sub>3</sub>, -NH<sub>2</sub>, -NH(R'), -N(R')<sub>2</sub> and -CN; wherein each R' is independently selected from H, -C<sub>1</sub>-C<sub>8</sub> alkyl and aryl.

[0107] "C<sub>3</sub>-C<sub>20</sub> heterocyclo" refers to a C<sub>3</sub>-C<sub>20</sub> heterocycle group defined above wherein one of the heterocycle group's hydrogen atoms is replaced with a bond.

[0108] "Carbocycle" means a saturated or unsaturated ring having 3 to 7 carbon atoms as a monocycle or 7 to 12 carbon atoms as a bicycle. Monocyclic carbocycles have 3 to 6 ring atoms, still more typically 5 or 6 ring atoms. Bicyclic carbocycles have 7 to 12 ring atoms, *e.g.* arranged as a bicyclo [4,5], [5,5], [5,6] or [6,6] system, or 9 or 10 ring atoms arranged as a bicyclo [5,6] or [6,6] system. Examples of monocyclic carbocycles include cyclopropyl, cyclobutyl, cyclopentyl, 1-cyclopent-1-enyl, 1-cyclopent-2-enyl, 1-cyclopent-3-enyl, cyclohexyl, 1-cyclohex-1-enyl, 1-cyclohex-2-enyl, 1-cyclohex-3-enyl, cycloheptyl, and cyclooctyl.

[0109] A "C<sub>3</sub>-C<sub>8</sub> carbocycle" is a 3-, 4-, 5-, 6-, 7- or 8-membered saturated or unsaturated non-aromatic carbocyclic ring. Representative C<sub>3</sub>-C<sub>8</sub> carbocycles include, but are not limited to, -cyclopropyl, -cyclobutyl, -cyclopentyl, -cyclopentadienyl, -cyclohexyl, -cyclohexenyl, -1,3-cyclohexadienyl, -1,4-cyclohexadienyl, -cycloheptyl, -1,3-cycloheptadienyl, -1,3,5-cycloheptatrienyl, -cyclooctyl, and -cyclooctadienyl. A C<sub>3</sub>-C<sub>8</sub> carbocycle group can be unsubstituted or substituted with one or more groups including, but not limited to, -C<sub>1</sub>-C<sub>8</sub> alkyl, -O-(C<sub>1</sub>-C<sub>8</sub> alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH<sub>2</sub>, -C(O)NHR', -C(O)N(R')<sub>2</sub>, -NHC(O)R', -S(O)<sub>2</sub>R', -S(O)R', -OH, -halogen, -N<sub>3</sub>, -NH<sub>2</sub>, -NH(R'), -N(R')<sub>2</sub> and -CN; where each R' is independently selected from H, -C<sub>1</sub>-C<sub>8</sub> alkyl and aryl.

[0110] A "C<sub>3</sub>-C<sub>8</sub> carbocyclo" refers to a C<sub>3</sub>-C<sub>8</sub> carbocycle group defined above wherein one of the carbocycle groups' hydrogen atoms is replaced with a bond.

[0111] "Linker" refers to a chemical moiety comprising a covalent bond or a chain of atoms that covalently attaches an antibody to a drug moiety. In various embodiments, linkers include a divalent radical such as an alkylidyl, an arylidyl, a heteroarylidyl, moieties such as:  $-(CR_2)_nO(CR_2)_n-$ , repeating units of alkyloxy (e.g. polyethylenoxy, PEG, polymethyleneoxy) and alkylamino (e.g. polyethyleneamino, Jeffamine™); and diacid ester and amides including succinate, succinamide, diglycolate, malonate, and caproamide. In various embodiments, linkers can comprise one or more amino acid residues, such as valine, phenylalanine, lysine, and homolysine.

[0112] The term "chiral" refers to molecules which have the property of non-superimposability of the mirror image partner, while the term "achiral" refers to molecules which are superimposable on their mirror image partner.

[0113] The term "stereoisomers" refers to compounds which have identical chemical constitution, but differ with regard to the arrangement of the atoms or groups in space.

[0114] "Diastereomer" refers to a stereoisomer with two or more centers of chirality and whose molecules are not mirror images of one another. Diastereomers have different physical properties, e.g. melting points, boiling points, spectral properties, and reactivities. Mixtures of diastereomers may separate under high resolution analytical procedures such as electrophoresis and chromatography.

[0115] "Enantiomers" refer to two stereoisomers of a compound which are non-superimposable mirror images of one another.

[0116] Stereochemical definitions and conventions used herein generally follow S. P. Parker, Ed., *McGraw-Hill Dictionary of Chemical Terms* (1984) McGraw-Hill Book Company, New York; and Eliel, E. and Wilen, S., *Stereochemistry of Organic Compounds* (1994) John Wiley & Sons, Inc., New York. Many organic compounds exist in optically active forms, i.e., they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L, or R and S, are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l or (+) and (-) are employed to designate the sign of rotation of plane-polarized light by the compound, with (-) or l meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. For a given chemical structure, these stereoisomers are identical except that they are mirror images of one another. A specific stereoisomer may also be referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture or a racemate, which may occur where there has been no stereoselection or stereospecificity in a chemical reaction or process. The terms "racemic mixture" and "racemate" refer to an equimolar mixture of two enantiomeric species, devoid of optical activity.

[0117] "Leaving group" refers to a functional group that can be substituted by another functional group. Certain leaving groups are well known in the art, and examples include, but are not limited to,

a halide (*e.g.*, chloride, bromide, iodide), methanesulfonyl (mesyl), *p*-toluenesulfonyl (tosyl), trifluoromethylsulfonyl (triflate), and trifluoromethylsulfonate.

[0118] The term “protecting group” refers to a substituent that is commonly employed to block or protect a particular functionality while reacting other functional groups on the compound. For example, an “amino-protecting group” is a substituent attached to an amino group that blocks or protects the amino functionality in the compound. Suitable amino-protecting groups include, but are not limited to, acetyl, trifluoroacetyl, *t*-butoxycarbonyl (BOC), benzyloxycarbonyl (CBZ) and 9-fluorenylmethylenoxycarbonyl (Fmoc). For a general description of protecting groups and their use, *see* T. W. Greene, *Protective Groups in Organic Synthesis*, John Wiley & Sons, New York, 1991, or a later edition.

## II. THERAPEUTIC METHODS

### A. Therapeutic Methods

[0119] Provided herein are methods using anti-LGR5 antibodies. For example, provided herein are methods of treating a hedgehog-related disease in an individual comprising administering to the individual an effective amount of an anti-LGR5 antibody. For example, provided herein are methods of treating basal cell carcinoma in an individual comprising administering to the individual an effective amount of an anti-LGR5 antibody described herein. In some embodiments, the anti-LGR5 antibody is an anti-LGR5 antibody conjugated to a cytotoxic agent (also referred to herein as immunoconjugate). In some embodiments, the anti-LGR5 antibody does not significantly inhibit *wnt* pathway signaling. In some embodiments, the hedgehog-related disease is basal cell carcinoma (“BCC”). In some embodiments, the basal cell carcinoma is locally advanced BCC or metastatic BCC. In some embodiments, the hedgehog-related disease is medulloblastoma.

[0120] Also provided herein are methods of treating hedgehog-related disease in an individual comprising administering to the individual an effective amount of an anti-LGR5 antibody and an effective amount of an inhibitor of the hedgehog pathway. For example, wherein the respective amounts the anti-LGR5 antibody and the inhibitor of the hedgehog pathway are effective to increase the period of response to therapy and/or delay the recurrence and/or development of resistance compared to treatment with the inhibitor of the hedgehog pathway alone. In some embodiments, the inhibitor of the hedgehog pathway is an antagonist of smoothened. In some embodiments, the inhibitor of the hedgehog pathway is a cyclopamine-competitive antagonist of smoothened. In some embodiments, the antagonist of smoothened is 2-chloro-N-[4-chloro-3-(pyridin-2-yl)phenyl]-4-(methylsulfonyl)benzamide or a salt thereof. In some embodiments, the antagonist of smoothened is 2-chloro-N-[4-chloro-3-(pyridin-2-yl)phenyl]-4-(methylsulfonyl)benzamide. In some embodiments, the antagonist of smoothened is vismodegib. In some embodiments, the anti-LGR5 antibody is an anti-LGR5 antibody conjugated to a cytotoxic agent (also referred to herein as immunoconjugate). In some embodiments, the anti-LGR5 antibody does not significantly inhibit *wnt* pathway signaling. In some embodiments, the hedgehog-related disease is basal cell carcinoma (“BCC”). In some embodiments,

the basal cell carcinoma is locally advanced BCC or metastatic BCC. In some embodiments, the hedgehog-related disease is medulloblastoma.

[0121] Provided herein are methods of increasing efficacy of a treatment of a hedgehog-related disease comprising an inhibitor of the hedgehog pathway in an individual, wherein the method comprises administering to the individual an effective amount of an anti-LGR5 antibody and an effective amount of the inhibitor of the hedgehog pathway. In some embodiments, the inhibitor of the hedgehog pathway is an antagonist of smoothened. In some embodiments, the inhibitor of the hedgehog pathway is a cyclopamine-competitive antagonist of smoothened. In some embodiments, the antagonist of smoothened is 2-chloro-N-[4-chloro-3-(pyridin-2-yl)phenyl]-4-(methylsulfonyl)benzamide or a salt thereof. In some embodiments, the antagonist of smoothened is 2-chloro-N-[4-chloro-3-(pyridin-2-yl)phenyl]-4-(methylsulfonyl)benzamide. In some embodiments, the antagonist of smoothened is vismodegib. In some embodiments, the anti-LGR5 antibody is an anti-LGR5 antibody conjugated to a cytotoxic agent (also referred to herein as immunoconjugate). In some embodiments, the anti-LGR5 antibody does not significantly inhibit wnt pathway signaling. In some embodiments, the hedgehog-related disease is basal cell carcinoma ("BCC"). In some embodiments, the basal cell carcinoma is locally advanced BCC or metastatic BCC. In some embodiments, the hedgehog-related disease is medulloblastoma.

[0122] Further provided herein are methods of treating a hedgehog-related disease in an individual wherein the treatment comprises administering to the individual an effective amount of an anti-LGR5 antibody and an effective amount of an inhibitor of the hedgehog pathway, and wherein the treatment has increased efficacy compared to a standard treatment comprising administering an effective amount of the inhibitor of the hedgehog pathway without (in the absence of) the anti-LGR5 antibody. In some embodiments, the inhibitor of the hedgehog pathway is an antagonist of smoothened. In some embodiments, the inhibitor of the hedgehog pathway is a cyclopamine-competitive antagonist of smoothened. In some embodiments, the antagonist of smoothened is 2-chloro-N-[4-chloro-3-(pyridin-2-yl)phenyl]-4-(methylsulfonyl)benzamide or a salt thereof. In some embodiments, the antagonist of smoothened is 2-chloro-N-[4-chloro-3-(pyridin-2-yl)phenyl]-4-(methylsulfonyl)benzamide. In some embodiments, the antagonist of smoothened is vismodegib. In some embodiments, the anti-LGR5 antibody is an anti-LGR5 antibody conjugated to a cytotoxic agent (also referred to herein as immunoconjugate). In some embodiments, the anti-LGR5 antibody does not significantly inhibit wnt pathway signaling. In some embodiments, the hedgehog-related disease is basal cell carcinoma ("BCC"). In some embodiments, the basal cell carcinoma is locally advanced BCC or metastatic BCC. In some embodiments, the hedgehog-related disease is medulloblastoma.

[0123] Also provided herein are methods of delaying and/or preventing development of the recurrence and/or resistance of a hedgehog-related disease to an inhibitor of the hedgehog pathway in an individual, comprising administering to the individual an effective amount of an anti-LGR5 antibody and an effective amount of the inhibitor of the hedgehog pathway. Also provided herein are

methods of extending the period of sensitivity an inhibitor of the hedgehog pathway in an individual with a hedgehog-related disease comprising administering to the individual an effective amount of an anti-LGR5 antibody and an effective amount of the inhibitor of the hedgehog pathway. Provided herein are methods of extending the duration of response to an inhibitor of the hedgehog pathway in an individual with a hedgehog-related disease comprising administering to the individual an effective amount of an anti-LGR5 antibody and an effective amount of the inhibitor of the hedgehog pathway. In some embodiments, the inhibitor of the hedgehog pathway is an antagonist of smoothened. In some embodiments, the inhibitor of the hedgehog pathway is a cyclopamine-competitive antagonist of smoothened. In some embodiments, the antagonist of smoothened is 2-chloro-N-[4-chloro-3-(pyridin-2-yl)phenyl]-4-(methylsulfonyl)benzamide or a salt thereof. In some embodiments, the antagonist of smoothened is 2-chloro-N-[4-chloro-3-(pyridin-2-yl)phenyl]-4-(methylsulfonyl)benzamide. In some embodiments, the antagonist of smoothened is vismodegib. In some embodiments, the anti-LGR5 antibody is an anti-LGR5 antibody conjugated to a cytotoxic agent (also referred to herein as immunoconjugate). In some embodiments, the anti-LGR5 antibody does not significantly inhibit wnt pathway signaling. In some embodiments, the hedgehog-related disease is basal cell carcinoma ("BCC"). In some embodiments, the basal cell carcinoma is locally advanced BCC or metastatic BCC. In some embodiments, the hedgehog-related disease is medulloblastoma.

[0124] Provided herein are methods of increasing sensitivity to an inhibitor of the hedgehog pathway in an individual with a hedgehog-related disease comprising administering to the individual an effective amount of an anti-LGR5 antibody and an effective amount of the inhibitor of the hedgehog pathway. In some embodiments, the inhibitor of the hedgehog pathway is an antagonist of smoothened. In some embodiments, the inhibitor of the hedgehog pathway is a cyclopamine-competitive antagonist of smoothened. In some embodiments, the antagonist of smoothened is 2-chloro-N-[4-chloro-3-(pyridin-2-yl)phenyl]-4-(methylsulfonyl)benzamide or a salt thereof. In some embodiments, the antagonist of smoothened is 2-chloro-N-[4-chloro-3-(pyridin-2-yl)phenyl]-4-(methylsulfonyl)benzamide. In some embodiments, the antagonist of smoothened is vismodegib. In some embodiments, the anti-LGR5 antibody is an anti-LGR5 antibody conjugated to a cytotoxic agent (also referred to herein as immunoconjugate). In some embodiments, the anti-LGR5 antibody does not significantly inhibit wnt pathway signaling. In some embodiments, the hedgehog-related disease is basal cell carcinoma ("BCC"). In some embodiments, the basal cell carcinoma is locally advanced BCC or metastatic BCC. In some embodiments, the hedgehog-related disease is medulloblastoma.

[0125] Cancer having resistance to a therapy as used herein includes a cancer which is not responsive and/or reduced ability of producing a significant response (*e.g.*, partial response and/or complete response) to the therapy. Resistance may be acquired resistance which arises in the course of a treatment method. In some embodiments, the acquired drug resistance is transient and/or reversible drug tolerance. Transient and/or reversible drug resistance to a therapy includes wherein the drug resistance is capable of regaining sensitivity to the therapy after a break in the treatment method. In

some embodiments, the acquired resistance is permanent resistance. Permanent resistance to a therapy includes a genetic change conferring drug resistance.

[0126] Cancer having sensitivity to a therapy as used herein includes cancer which is responsive and/or capable of producing a significant response (*e.g.*, partial response and/or complete response).

[0127] Methods of determining of assessing acquisition of resistance and/or maintenance of sensitivity to a therapy are known in the art. In some embodiments, resistance may be indicated by a change in IC<sub>50</sub>, EC<sub>50</sub> or decrease in tumor growth in drug tolerant persisters and/or drug tolerant expanded persisters. In some embodiments, the change is greater than about any of 50%, 100%, and/or 200%. In addition, changes in acquisition of resistance and/or maintenance of sensitivity may be assessed *in vivo* for examples by assessing response, duration of response, and/or time to progression to a therapy, *e.g.*, partial response and complete response. Changes in acquisition of resistance and/or maintenance of sensitivity may be based on changes in response, duration of response, and/or time to progression to a therapy in a population of individuals, *e.g.*, number of partial responses and complete responses.

[0128] In some embodiments, the cancer is LGR5-positive. Presence of various biomarkers in a sample can be analyzed by a number of methodologies, many of which are known in the art and understood by the skilled artisan, including, but not limited to, immunohistochemistry (“IHC”), Western blot analysis, immunoprecipitation, molecular binding assays, ELISA, ELIFA, fluorescence activated cell sorting (“FACS”), MassARRAY, proteomics, quantitative blood based assays (as for example Serum ELISA), biochemical enzymatic activity assays, *in situ* hybridization, Southern analysis, Northern analysis, whole genome sequencing, polymerase chain reaction (“PCR”) including quantitative real time PCR (“qRT-PCR”) and other amplification type detection methods, such as, for example, branched DNA, SISBA, TMA and the like, RNA-Seq, FISH, microarray analysis, gene expression profiling, and/or serial analysis of gene expression (“SAGE”), as well as any one of the wide variety of assays that can be performed by protein, gene, and/or tissue array analysis. Typical protocols for evaluating the status of genes and gene products are found, for example in Ausubel *et al.*, eds., 1995, Current Protocols In Molecular Biology, Units 2 (Northern Blotting), 4 (Southern Blotting), 15 (Immunoblotting) and 18 (PCR Analysis). Multiplexed immunoassays such as those available from Rules Based Medicine or Meso Scale Discovery (“MSD”) may also be used.

[0129] Inhibition of cell proliferation *in vitro* may be assayed using the CellTiter-Glo™ Luminescent Cell Viability Assay, which is commercially available from Promega (Madison, WI). That assay determines the number of viable cells in culture based on quantitation of ATP present, which is an indication of metabolically active cells. *See* Crouch *et al.* (1993) *J. Immunol. Meth.* 160:81-88, US Pat. No. 6602677. The assay may be conducted in 96- or 384-well format, making it amenable to automated high-throughput screening (HTS). *See* Cree *et al.* (1995) *AntiCancer Drugs* 6:398-404. The assay procedure involves adding a single reagent (CellTiter-Glo® Reagent) directly to cultured cells. This results in cell lysis and generation of a luminescent signal produced by a luciferase reaction. The

luminescent signal is proportional to the amount of ATP present, which is directly proportional to the number of viable cells present in culture. Data can be recorded by luminometer or CCD camera imaging device. The luminescence output is expressed as relative light units (RLU).

[0130] In a further aspect, the invention provides a method for treating LGR5-positive cancer. In one embodiment, the method comprises administering to an individual having such LGR5-positive cancer an effective amount of an anti-LGR5 antibody including immunoconjugate. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below.

[0131] In some embodiments, an LGR5-positive cancer is a cancer that receives an anti-LGR5 immunohistochemistry (IHC) or in situ hybridization (ISH) score greater than “0,” which corresponds to very weak or no staining in >90% of tumor cells. In another embodiment, an LGR5-positive cancer expresses LGR5 at a 1+, 2+ or 3+ level. In some embodiments, an LGR5-positive cancer is a cancer that expresses LGR5 according to a reverse-transcriptase PCR (RT-PCR) assay that detects LGR5 mRNA. In some embodiments, the RT-PCR is quantitative RT-PCR.

[0132] An “individual” according to any of the above embodiments may be a human.

[0133] In a further aspect, the invention provides pharmaceutical formulations comprising any of the anti-LGR5 antibodies including immunoconjugate provided herein, *e.g.*, for use in any of the above therapeutic methods. In one embodiment, a pharmaceutical formulation comprises any of the anti-LGR5 antibodies including immunoconjugates provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises any of the anti-LGR5 antibodies including immunoconjugates provided herein and at least one additional therapeutic agent, *e.g.*, as described below.

[0134] Antibodies including immunoconjugates can be used either alone or in combination with other agents in a therapy (*e.g.*, inhibitors of hedgehog signaling). For instance, an antibody including immunoconjugate may be co-administered with at least one additional therapeutic agent (*e.g.*, inhibitors of hedgehog signaling).

[0135] 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 including immunoconjugate can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant. Antibodies including immunoconjugates can also be used in combination with radiation therapy.

[0136] An antibody including immunoconjugates (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.

[0137] Antibodies including immunoconjugates 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 including immunoconjugates 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 including immunoconjugates 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.

[0138] For the prevention or treatment of disease, the appropriate dosage of an antibody including immunoconjugates (when used alone or in combination with one or more other additional therapeutic agents (*e.g.*, inhibitor of hedgehog pathway) will depend on the type of disease to be treated, the type of antibody including immunoconjugate, the severity and course of the disease, whether the antibody including immunoconjugate is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody including immunoconjugate, and the discretion of the attending physician. The antibody including immunoconjugate 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  $\mu\text{g/kg}$  to 15  $\text{mg/kg}$  (*e.g.* 0.1 $\text{mg/kg}$ -10 $\text{mg/kg}$ ) of antibody including immunoconjugate 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  $\mu\text{g/kg}$  to 100  $\text{mg/kg}$  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 including immunoconjugate would be in the range from about 0.05  $\text{mg/kg}$  to about 10  $\text{mg/kg}$ . Thus, one or more doses of about 0.5  $\text{mg/kg}$ , 2.0  $\text{mg/kg}$ , 4.0  $\text{mg/kg}$  or 10  $\text{mg/kg}$  (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.

[0139] It is understood that any of the above formulations or therapeutic methods may be carried out using both an immunoconjugate and an anti-LGR5 antibody.

**B. Anti-LGR5 Antibodies for Use in the Methods Described Herein**

[0140] Provided herein are anti-LGR5 antibodies for use in the methods described herein. In some embodiments, the anti-LGR5 antibodies that bind to LGR5. LGR5 is a seven-transmembrane protein found, for example, on the surface of actively cycling intestinal stem cells.

[0141] Anti-LGR5 antibodies useful in the methods described herein include, but are not limited to, the anti-LGR5 antibodies described in US 2010/0275280, US 2011/017699, US 8158757, US 8158758, WO 2013/067054, WO 2013/067055, WO 2013/067057, WO 2013/067060, and PCT/US2013/034629, which are hereby incorporated by reference in their entirety.

[0142] An exemplary naturally occurring human LGR5 precursor protein sequence, with signal sequence (amino acids 1-21) is provided in SEQ ID NO: 67, and the corresponding mature LGR5 protein sequence (corresponding to amino acids 22-907 of SEQ ID NO: 67).

[0143] In certain embodiments, an anti-LGR5 antibody has at least one or more of the following characteristics, in any combination: (a) binds to an epitope within amino acids 22-555 of SEQ ID NO: 67; (b) binds LGR5 with an affinity of  $\leq 5$  nM, or  $\leq 4$  nM, or  $\leq 3$  nM, or  $\leq 2$  nM, or  $\leq 1$  nM, and optionally  $\geq 0.0001$  nM, or  $\geq 0.001$  nM, or  $\geq 0.01$  nM; (c) does not significantly disrupt the binding of R-spondin (RSPO) to LGR5; (d) does not significantly disrupt beta-catenin signaling; (e) does not significantly disrupt RSPO activation of LGR5 signaling; (f) activates caspase 3 cleavage; (g) recognizes both human and rodent LGR5; (h) recognizes human LGR5 but not rodent LGR5; (i) does not significantly inhibit tumor growth in its unconjugated form; and (j) does not induce stem cell differentiation. In some embodiments, the anti-LGR5 antibody is 8E11 and humanized variants thereof, such as hu8E11.v2; YW353; 2H6; and 3G12. In some embodiments, LGR5 is human LGR5. In some embodiments, LGR5 is selected from human, cynomolgus monkey, mouse, and rat LGR5.

*(a) binds to an epitope within amino acids 22-555 of SEQ ID NO: 67*

[0144] Methods of determining whether an anti-LGR5 antibody binds to an epitope of LGR5 are known in the art. In some embodiments, binding of an anti-LGR5 antibody to an epitope of LGR5 (e.g., within amino acids 22-555 of SEQ ID NO: 67) may be determined by expressing LGR5 polypeptides with N- and C-terminal deletions in 293 cells and testing by FACS binding of the antibody to the truncated polypeptides. In some embodiments, a substantial reduction ( $\geq 70\%$  reduction) or elimination of binding of the antibody to a truncated polypeptide relative to binding to full-length LGR5 expressed in 293 cells indicates that the antibody does not bind to that truncated polypeptide. In some embodiments, LGR5 is human LGR5. In some embodiments, LGR5 is human LGR5 or cynomolgus monkey LGR5.

[0145] In some embodiments, the epitope of LGR5 comprises the lucine rich N-terminal domain of LGR5 (e.g., amino acid residues 25-66 of SEQ ID NO:67). In some embodiments, the epitope of LGR5 comprises one or more lucine rich repeats (LRR) of LGR5 (e.g., amino acid residues 67-446 of

SEQ ID NO:67; LRRs 1-16 of LGR5). ). In some embodiments, the epitope of LGR5 comprises LRR 1 of LGR5 (e.g., amino acid residues 67-90 of SEQ ID NO:67). In some embodiments, the epitope of LGR5 comprises LRR 2 of LGR5 (e.g., amino acid residues 91-112 of SEQ ID NO:67). In some embodiments, the epitope of LGR5 comprises LRR 3 of LGR5 (e.g., amino acid residues 115-136 of SEQ ID NO:67). In some embodiments, the epitope of LGR5 comprises LRR 4 of LGR5 (e.g., amino acid residues 139-160 of SEQ ID NO:67). In some embodiments, the epitope of LGR5 comprises LRR 5 of LGR5 (e.g., amino acid residues 163-184 of SEQ ID NO:67). In some embodiments, the epitope of LGR5 comprises LRR 6 of LGR5 (e.g., amino acid residues 187-208 of SEQ ID NO:67). In some embodiments, the epitope of LGR5 comprises LRR 7 of LGR5 (e.g., amino acid residues 211-232 of SEQ ID NO:67). In some embodiments, the epitope of LGR5 comprises LRR 8 of LGR5 (e.g., amino acid residues 235-256 of SEQ ID NO:67). In some embodiments, the epitope of LGR5 comprises LRR 9 of LGR5 (e.g., amino acid residues 258-279 of SEQ ID NO:67). In some embodiments, the epitope of LGR5 comprises LRR 10 of LGR5 (e.g., amino acid residues 282-303 of SEQ ID NO:67). In some embodiments, the epitope of LGR5 comprises LRR 11 of LGR5 (e.g., amino acid residues 306-328 of SEQ ID NO:67). In some embodiments, the epitope of LGR5 comprises LRR 12 of LGR5 (e.g., amino acid residues 329-350 of SEQ ID NO:67). In some embodiments, the epitope of LGR5 comprises LRR 13 of LGR5 (e.g., amino acid residues 353-374 of SEQ ID NO:67). In some embodiments, the epitope of LGR5 comprises LRR 14 of LGR5 (e.g., amino acid residues 375-396 of SEQ ID NO:67). In some embodiments, the epitope of LGR5 comprises LRR 15 of LGR5 (e.g., amino acid residues 399-420 of SEQ ID NO:67). In some embodiments, the epitope of LGR5 comprises LRR 16 of LGR5 (e.g., amino acid residues 423-446 of SEQ ID NO:67). In some embodiments, the epitope of LGR5 comprises any of LRR1 to LRR11, LRR2 to LRR11, LRR3 to LRR11, LLR1 to LLR3, LLR2 to LLR3, LLR2 to LLR8, LLR3 to LL7, or LLR4 to LLR6.

[0146] In some embodiments, the epitope of LGR5 comprises an epitope within amino acids 22-555 of SEQ ID NO: 67. In some embodiments, the epitope of LGR5 comprises an epitope within amino acids 22-424 of SEQ ID NO: 67. In some embodiments, the epitope of LGR5 comprises an epitope within amino acids 22-123 of SEQ ID NO: 67. In some embodiments, the epitope of LGR5 comprises an epitope within amino acids 22-323 of SEQ ID NO: 67. In some embodiments, the epitope of LGR5 comprises an epitope within amino acids 324-555 of SEQ ID NO: 67. In some embodiments, the epitope of LGR5 comprises an epitope within amino acids 324-424 of SEQ ID NO: 67.

[0147] It is understood that aspect and embodiments described herein include “consisting” and/or “consisting effectually of” aspects and embodiments.

*(b) binds LGR5 with an affinity of  $\leq 5$  nM, or  $\leq 4$  nM, or  $\leq 3$  nM, or  $\leq 2$  nM, or  $\leq 1$  nM, and optionally  $\geq 0.0001$  nM, or  $\geq 0.001$  nM, or  $\geq 0.01$  nM*

[0148] Methods of determining binding affinity are known in the art. In some embodiments, the binding affinity may be determined according to a BIAcore<sup>®</sup> assay. Specifically, in some

embodiments,  $K_d$  may be measured using surface plasmon resonance assays using a BIAcore®-3000 (BIAcore, Inc., Piscataway, NJ). BIAcore™ research grade CM5 chips may be activated with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) reagents according to the supplier's instructions. Goat anti-human Fc IgGs may be coupled to the chips to achieve approximately 10,000 response units (RU) in each flow cell. Unreacted coupling groups may be blocked with 1M ethanolamine. For kinetics measurements, anti-LGR5 antibodies may be captured to achieve approximately 300 RU. Two-fold serial dilutions of human LGR5 ECD (for example, amino acids 22-557 (or a similar fragment, such as 22-555) fused to His-Fc expressed in a baculovirus system, or amino acids 22-558 (or a similar fragment, such as 22-555) fused to Fc expressed from CHO cells; 125 nM to 0.49 nM) may be injected in HBS-P buffer (0.01M HEPES pH7.4, 0.15M NaCl, 0.005% surfactant P20) at 25°C with a flow rate of 30  $\mu$ l/min. Association rates ( $k_{on}$ ) and dissociation rates ( $k_{off}$ ) may be calculated using a 1:1 Langmuir binding model (BIAcore™ Evaluation Software version 3.2). The equilibrium dissociation constant ( $K_d$ ) may be calculated as the ratio  $k_{off}/k_{on}$ . If the on-rate exceeds  $10^6 \text{ M}^{-1} \text{ s}^{-1}$  by the surface plasmon resonance assay above, then the on-rate may 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 spectrophotometer (Aviv Instruments) or a 8000-series SLM-Aminco® spectrophotometer (ThermoSpectronic) with a stirred cuvette.

[0149] In some embodiments, the anti-LGR5 antibody binds LGR5 with an affinity of about any of  $\leq 5 \text{ nM}$ , or  $\leq 4 \text{ nM}$ , or  $\leq 3 \text{ nM}$ , or  $\leq 2 \text{ nM}$ , or  $\leq 1 \text{ nM}$ . In some embodiments, the anti-LGR5 antibody binds LGR5 with an affinity of about  $\leq 5$ . In some embodiments, the anti-LGR5 antibody binds LGR5 with an affinity of about  $\leq 4 \text{ nM}$ . In some embodiments, the anti-LGR5 antibody binds LGR5 with an affinity of about  $\leq 3 \text{ nM}$ . In some embodiments, the anti-LGR5 antibody binds LGR5 with an affinity of about  $\leq 2 \text{ nM}$ . In some embodiments, LGR5 is human LGR5. In some embodiments, LGR5 is human LGR5 or cynomolgus monkey LGR5.

[0150] As is understood by one skilled in the art, reference to “about” a value or parameter includes (and describes) embodiments that are direct to that value or parameter per se. For example, description referring to “about X” includes description of “X”.

*(c) does not significantly disrupt the binding of R-spondin (RSPO) to LGR5*

[0151] Methods of determining the ability of an anti-LGR5 antibody to disrupt the binding of an RSPO to LGR5 are known in the art. In some embodiments, the ability of an anti-LGR5 antibody to significantly disrupt the binding of an R-spondon (RSPO) to LGR5 may be determined by flow cytometry. In some embodiments, for example, 293 cells expressing LGR5 may be contacted with fluorescently-labeled RSPO, such as RSPO1, RSPO2, RSPO3, and/or RSPO4, in the presence and absence of an anti-LGR5 antibody. Binding of RSPO to the 293 cells may be detected using

fluorescence-activated cell sorting (FACS). In some embodiments, a decrease in RSPO binding in the presence of an anti-LGR5 antibody of less than about 25% relative to RSPO binding in the presence of a control antibody, indicates that the anti-LGR5 antibody does not significantly disrupt binding of RSPO to LGR5.

[0152] In some embodiments, the ability of an anti-LGR5 antibody to significantly disrupt the binding of an R-spondon (RSPO) to LGR5 may be determined by BIAcore assay. In some embodiments, for example, LGR5 extracellular domain may be immobilized on CM5 chips, *e.g.*, as described herein, and binding of RSPO, such as RSPO1, RSPO2, RSPO3, and/or RSPO4, to the immobilized LGR5 may be determined in the presence and absence of an anti-LGR5 antibody. In some embodiments, a decrease in RSPO binding in the presence of an anti-LGR5 antibody of less than about 25% relative to RSPO binding in the presence of a control antibody, indicates that the anti-LGR5 antibody does not significantly disrupt binding of RSPO to LGR5.

[0153] In some embodiments, the RSPO is selected from RSPO1, RSPO2, RSPO3, and RSPO4. In some embodiments, the antibody disrupts binding by less than about 25%, less than about 20%, less than about 15%, or less than about 10%. In some embodiments, the antibody does not detectably disrupt binding of an RSPO to LGR5. In some embodiments, LGR5 is human LGR5. In some embodiments, LGR5 is human LGR5 or cynomolgus monkey LGR5.

*(d) does not significantly disrupt wnt/beta-catenin signaling*

[0154] Methods of determining ability of an anti-LGR5 antibody to disrupt wnt/beta-catenin signaling are known in the art. In some embodiments, the ability of an anti-LGR5 antibody to significantly disrupt wnt/beta-catenin signaling may be determined using a reporter gene assay. In some embodiments, for example, a reporter construct comprising a reporter gene (such as, for example, a luciferase gene) under the control of a wnt/beta-catenin responsive promoter (such as, for example, a promoter comprising multimerized TCF/LEF DNA-binding sites) may be transfected into cells that express LGR5. The cells are then contacted with a Wnt ligand, such as Wnt3a, and an RSPO, such as RSPO1, RSPO2, RSPO3, and/or RSPO4, in the presence and absence of an anti-LGR5 antibody, and luciferase expression is measured. In some embodiments, a decrease in luciferase expression in the presence of antibody of less than about 25% relative to luciferase expression in the presence of a control antibody, indicates that the anti-LGR5 antibody does not significantly disrupt beta-catenin signaling.

[0155] In some embodiments, the antibody disrupts beta-catenin signaling by less than about 25%, less than about 20%, less than about 15%, or less than about 10%. In some embodiments, the antibody does not detectably disrupt beta-catenin signaling. In some embodiments, LGR5 is human LGR5. In some embodiments, LGR5 is human LGR5 or cynomolgus monkey LGR5.

*(e) does not significantly disrupt RSPO activation of LGR5 signaling*

[0156] Methods of determining ability of an anti-LGR5 antibody to disrupt RSPO activation of LGR5 are known in the art. In some embodiments, the ability of an anti-LGR5 antibody to

significantly disrupt RSPO activation of LGR5 signaling may be determined using a reporter gene assay. In some embodiments, for example, a reporter construct comprising a reporter gene (such as, for example, a luciferase gene) under the control of a beta-catenin responsive promoter (such as, for example, a promoter comprising multimerized TCF/LEF DNA-binding sites) may be transfected into cells that express LGR5. The cells may be then contacted with a Wnt ligand, such as Wnt3a, in the presence and absence of an RSPO, such as RSPO1, RSPO2, RSPO3, and/or RSPO4, and the activation of LGR5 signaling may be measured as the increase in luciferase expression in the presence of the RSPO. The activation of LGR5 signaling may also be measured in the presence and absence of an anti-LGR5 antibody. In some embodiments, a decrease in the activation of LGR5 signaling in the presence of RSPO1, RSPO2, RSPO3, and/or RSPO4 of less than about 25% when the cells are contacted with an anti-LGR5 antibody versus a control antibody, indicates that the anti-LGR5 antibody does not significantly disrupt RSPO activation of LGR5 signaling.

[0157] In some embodiments, the RSPO is selected from RSPO1, RSPO2, RSPO3, and RSPO4. In some embodiments, the antibody disrupts RSPO activation of LGR5 signaling by less than about 25%, less than about 20%, less than about 15%, or less than about 10%. In some embodiments, the antibody does not detectably disrupt RSPO activation of LGR5 signaling. In some embodiments, LGR5 is human LGR5. In some embodiments, LGR5 is human LGR5 or cynomolgus monkey LGR5.

*(f) activates caspase 3 cleavage*

[0158] Methods of determining ability of an anti-LGR5 antibody to activate caspase 3 cleavage are known in the art. In some embodiments, the ability of an anti-LGR5 antibody to activate caspase 3 cleavage may be determined in a rodent xenograft model. In some embodiments, the presence of cleaved caspase 3 may be measured as a function of tumor area, for example, in formalin fixed paraffin embedded (FFPE) tissue collected from tumorigenesis model mice that were administered an anti-LGR5 antibody. The presence of cleaved caspase 3 may be determined, in some embodiments, using immunohistochemistry. Further, in some embodiments, caspase 3 cleavage may be determined as a percent positive tumor area.

[0159] In some embodiments, an anti-LGR5 antibody increases the percentage of caspase 3 positive tumor area by about any of at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100% (i.e., the percentage of positive tumor area doubles).

*(g) recognizes both human and rodent LGR5*

[0160] Methods of determining the ability of an anti-LGR5 antibody to bind human and rodent LGR5 are known in the art. In some embodiments, human and rodent LGR5 polypeptides are expressed in 293 cells and binding of the antibody to the LGR5-expressing 293 cells is tested by FACS. In some embodiments, rodent LGR5 is mouse or rat LGR5. In some embodiments, rodent LGR5 is mouse LGR5.

*(h) recognizes human LGR5 but not rodent LGR5*

[0161] Methods of determining the ability of an anti-LGR5 antibody to bind human but not rodent LGR5 are known in the art. In some embodiments, human and rodent LGR5 polypeptides are expressed in 293 cells and binding of the antibody to the LGR5-expressing 293 cells is tested by FACS. In some embodiments, rodent LGR5 is mouse or rat LGR5. In some embodiments, rodent LGR5 is mouse LGR5.

*(i) does not significantly inhibit tumor growth in its unconjugated form*

[0162] Methods of determining the ability of an anti-LGR5 antibody to inhibit tumor growth in its unconjugated form are known in the art. Inhibition of tumor growth in a xenograft model or murine 1 tumorigenesis model is determined relative to a vehicle control or control antibody.

[0163] In some embodiments, an anti-LGR5 antibody inhibits tumor growth in its unconjugated form by less than about 25%, less than about 20%, less than about 15%, or less than about 10%. In some embodiments, an anti-LGR5 antibody does not detectably inhibit tumor growth in its unconjugated form.

*(j) does not induce stem cell differentiation*

[0164] Methods of determining the ability of an anti-LGR5 antibody to induce stem cell differentiation are known in the art. In some embodiments, stem cell differentiation may be assayed by determining ability to differentiation of crypt base columnar cells (CBCs), which are fast-cycling stem cells in the small intestine that express LGR5, into, for example, enterocytes, goblet cells, and/or enteroendocrine cells, in the presence and absence of an anti-LGR5 antibody. In some embodiments, an anti-LGR5 antibody is considered to not induce stem cell differentiation if about any of less than 25%, less than 20%, less than 15%, or less than 10% of a population of CBCs differentiates in the presence of the anti-LGR5 antibody under conditions in which a control antibody also induces stem cell differentiation in less than about 25% of a population of CBCs.

[0165] In some embodiments, an anti-LGR5 antibody immunoconjugate inhibits tumor growth through a primary mechanism that is not inducing stem cell differentiation. In some such embodiments, the anti-LGR5 antibody immunoconjugate inhibits tumor growth through cytotoxic activity mediated through a cytotoxic agent conjugated to the antibody in the immunoconjugate.

*Exemplary anti-LGR5 Antibodies*

[0166] In some embodiments, provided herein are exemplary, but nonlimiting, anti-LGR5 antibodies for use in the methods described herein. In some embodiments of any of the methods, the anti-LGR5 antibody comprises at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 30; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 31; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 27; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 28; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 29.

[0167] In some embodiments of any of the methods, the anti-LGR5 antibody comprises (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 30, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 31, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 32; and/or (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 27, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 28, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 29.

[0168] In some embodiments, an anti-LGR5 antibody comprises HVRs as in any of the above embodiments, and further comprises a heavy chain framework FR3 sequence selected from SEQ ID NOs: 40 to 43. In some embodiments, an anti-LGR5 antibody comprises HVRs as in any of the above embodiments, and further comprises a heavy chain framework FR3 sequence of SEQ ID NO: 41. In some such embodiments, the heavy chain variable domain framework is a modified human VH<sub>1</sub> framework having an FR3 sequence selected from SEQ ID NOs: 40 to 43. In some such embodiments, the heavy chain variable domain framework is a modified human VH<sub>1</sub> framework having an FR3 sequence of SEQ ID NO: 41.

[0169] In some embodiments, an anti-LGR5 antibody comprises HVRs as in any of the above embodiments, and further comprises a light chain framework FR3 sequence of SEQ ID NO: 36. In some such embodiments, the heavy chain variable domain framework is a modified VL kappa IV consensus (VL<sub>KIV</sub>) framework having an FR3 sequence of SEQ ID NO: 36.

[0170] In another aspect, In some embodiments of any of the methods, the anti-LGR5 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: 6 and SEQ ID NO: 5, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO: 8 and SEQ ID NO: 7, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO: 10 and SEQ ID NO: 9, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO: 12 and SEQ ID NO: 11, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO: 14 and SEQ ID NO: 13, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO: 16 and SEQ ID NO: 15, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO: 18 and SEQ ID NO: 17, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO: 20 and SEQ ID NO: 19, respectively, including post-translational modifications of those sequences.



[0171] In one aspect, in some embodiments of any of the methods, the anti-LGR5 antibody comprises at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 60; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 61; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 62; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 57; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 58; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 59.

[0172] In another aspect, in some embodiments of any of the methods, the anti-LGR5 antibody comprises (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 60, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 61, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 62; and/or (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 57, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 58, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 59.

[0173] In another aspect, in some embodiments of any of the methods, the anti-LGR5 antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 60; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 61; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 62; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 57; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 58; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO: 59.

[0174] In another aspect, an anti-LGR5 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: 26 and SEQ ID NO: 25, respectively, including post-translational modifications of those sequences.

[0175] In some embodiments, in some embodiments of any of the methods, the anti-LGR5 antibody comprises at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 48; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 49; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 50; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 45; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 46; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 47.

[0176] In another aspect, in some embodiments of any of the methods, the anti-LGR5 antibody comprises (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 48, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 49, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 50; and/or (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 45, (ii) HVR-L2

comprising the amino acid sequence of SEQ ID NO: 46, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 47.

[0177] In another aspect, in some embodiments of any of the methods, the anti-LGR5 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: 22 and SEQ ID NO: 21, respectively, including post-translational modifications of those sequences.

[0178] In some embodiments, in some embodiments of any of the methods, the anti-LGR5 antibody comprises at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 54; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 55; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 56; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 51; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 52; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 53.

[0179] In another aspect, in some embodiments of any of the methods, the anti-LGR5 antibody comprises (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 54, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 55, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 56; and/or (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 51, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 52, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 53.

[0180] In another aspect, an anti-LGR5 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: 24 and SEQ ID NO: 23, respectively, including post-translational modifications of those sequences.

[0181] In a further aspect, in some embodiments of any of the methods, the anti-LGR5 antibody binds to the same epitope as an anti-LGR5 antibody provided herein. For example, in certain embodiments, an antibody is provided that binds to the same epitope as an anti-LGR5 antibody comprising a VH sequence of SEQ ID NO: 24 and a VL sequence of SEQ ID NO: 23. In certain embodiments, an antibody is provided that binds to the same epitope as an anti-LGR5 antibody comprising a VH sequence of SEQ ID NO: 22 and a VL sequence of SEQ ID NO: 21. In certain embodiments, an antibody is provided that binds to an epitope of SEQ ID NO: 67 from, within, or overlapping amino acids 324-423. In some embodiments, an antibody is provided that binds to an epitope from, within, or overlapping amino acids 303-402. In certain embodiments, an antibody is provided that binds to an epitope of SEQ ID NO: 67 from, within, or overlapping amino acids 324-555. In some embodiments, an antibody is provided that binds to an epitope from, within, or overlapping amino acids 303-534. For example, in certain embodiments, an antibody is provided that

binds to the same epitope as an anti-LGR5 antibody comprising a VH sequence of SEQ ID NO: 26 and a VL sequence of SEQ ID NO: 25. In certain embodiments, an antibody is provided that binds to an epitope of SEQ ID NO: 67 from, within, or overlapping amino acids 22-123. In certain embodiments, an antibody is provided that binds to an epitope from, within, or overlapping amino acids 1-102. For example, in certain embodiments, an antibody is provided that binds to the same epitope as an anti-LGR5 antibody comprising a VH sequence of SEQ ID NO: 8 and a VL sequence of SEQ ID NO: 7. In certain embodiments, an antibody is provided that binds to an epitope of SEQ ID NO: 67 from, within, or overlapping amino acids 22-323. In some embodiments, an antibody is provided that binds to an epitope from, within, or overlapping amino acids 1-312.

[0182] In any of the above embodiments, an anti-LGR5 antibody is humanized. In one embodiment, an anti-LGR5 antibody comprises HVRs as in any of the above embodiments, and further comprises a human acceptor framework, *e.g.* a human immunoglobulin framework or a human consensus framework. In certain embodiments, the human acceptor framework is the human VL kappa IV consensus (VL<sub>KIV</sub>) framework and/or the VH framework VH<sub>I</sub>. In certain embodiments, the human acceptor framework is the human VL kappa IV consensus (VL<sub>KIV</sub>) framework and/or the VH framework VH<sub>I</sub> comprising an R71S mutation and an A78V mutation in heavy chain framework region FR3.

[0183] In a further aspect, an anti-LGR5 antibody according to any of the above embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody. In one embodiment, an anti-LGR5 antibody is an antibody fragment, *e.g.*, a Fv, Fab, Fab', scFv, diabody, or F(ab')<sub>2</sub> fragment. In another embodiment, the antibody is a substantially full length antibody, *e.g.*, an IgG1 antibody or other antibody class or isotype as defined herein.

[0184] In a further aspect, an anti-LGR5 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***

[0185] In certain embodiments, an antibody provided herein has a dissociation constant (K<sub>d</sub>) of  $\leq 1\mu\text{M}$ ,  $\leq 100\text{ nM}$ ,  $\leq 10\text{ nM}$ ,  $\leq 1\text{ nM}$ ,  $\leq 0.1\text{ nM}$ ,  $\leq 0.01\text{ nM}$ , or  $\leq 0.001\text{ nM}$ , and optionally is  $\geq 10^{-13}\text{ M}$ . (*e.g.*  $10^{-8}\text{ M}$  or less, *e.g.* from  $10^{-8}\text{ M}$  to  $10^{-13}\text{ M}$ , *e.g.*, from  $10^{-9}\text{ M}$  to  $10^{-13}\text{ M}$ ).

[0186] In one embodiment, K<sub>d</sub> 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 (<sup>125</sup>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<sup>®</sup> multi-well plates (Thermo Scientific) are coated overnight with 5  $\mu\text{g/ml}$  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 [ $^{125}\text{I}$ ]-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<sup>®</sup>) in PBS. When the plates have dried, 150  $\mu\text{l}$ /well of scintillant (MICROSCINT-20<sup>™</sup>; Packard) is added, and the plates are counted on a TOPCOUNT<sup>™</sup> 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.

[0187] According to another embodiment,  $K_d$  is measured using surface plasmon resonance assays using a BIACORE<sup>®</sup>-2000 or a BIACORE<sup>®</sup>-3000 (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at  $\sim 10$  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  $\mu\text{g}/\text{ml}$  ( $\sim 0.2 \mu\text{M}$ ) before injection at a flow rate of 5  $\mu\text{l}/\text{minute}$  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<sup>™</sup>) surfactant (PBST) at 25°C at a flow rate of approximately 25  $\mu\text{l}/\text{min}$ . Association rates ( $k_{\text{on}}$ ) and dissociation rates ( $k_{\text{off}}$ ) are calculated using a simple one-to-one Langmuir binding model (BIACORE<sup>®</sup> Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant ( $K_d$ ) is calculated as the ratio  $k_{\text{off}}/k_{\text{on}}$ . *See, e.g.*, Chen et al., *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds  $10^6 \text{ M}^{-1} \text{ s}^{-1}$  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 spectrophotometer (Aviv Instruments) or a 8000-series SLM-AMINCO<sup>™</sup> spectrophotometer (ThermoSpectronic) with a stirred cuvette.

## 2. Antibody Fragments

[0188] 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')<sub>2</sub>, 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, Rosenberg 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')<sub>2</sub> fragments comprising salvage receptor binding epitope residues and having increased *in vivo* half-life, *see* U.S. Patent No. 5,869,046.

[0189] 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).

[0190] 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).

[0191] 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

[0192] 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.

[0193] 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.

[0194] 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).

[0195] 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

[0196] 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).

[0197] 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.

[0198] 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).

[0199] 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

[0200] Antibodies for use in the methods described herein 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).

[0201] 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.

[0202] Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

### 6. *Multispecific Antibodies*

[0203] 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 LGR5 and the other is for any other antigen. In certain embodiments, one of the binding specificities is for LGR5 and the other is for CD3. *See, e.g.*, U.S. Patent No. 5,821,337. In certain embodiments, bispecific antibodies may bind to two different epitopes of LGR5. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express LGR5. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

[0204] 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).

[0205] Engineered antibodies with three or more functional antigen binding sites, including “Octopus antibodies,” are also included herein (*see, e.g.* US 2006/0025576A1).

[0206] The antibody or fragment herein also includes a “Dual Acting FAb” or “DAF” comprising an antigen binding site that binds to LGR5 as well as another, different antigen (*see*, US 2008/0069820, for example).

### 7. *Antibody Variants*

[0207] 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**

[0208] 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 "preferred 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**

| <b>Original Residue</b> | <b>Exemplary Substitutions</b>      | <b>Preferred Substitutions</b> |
|-------------------------|-------------------------------------|--------------------------------|
| Ala (A)                 | Val; Leu; Ile                       | Val                            |
| 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                            |
| 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.

[0209] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0210] 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).

[0211] 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.

[0212] 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.

[0213] 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 is used 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.

[0214] 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**

[0215] 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.

[0216] 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 may be made in order to create antibody variants with certain improved properties.

[0217] 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  $\pm$

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).

[0218] 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**

[0219] 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.

[0220] In certain embodiments, 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 is contemplated as useful in the methods described herein. *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(R)III 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, ACTITM 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)).

[0221] 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).

[0222] 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).)

[0223] 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).

[0224] 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).

[0225] 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). [0226] *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) Cysteine engineered antibody variants**

[0227] 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.

[0228] Further exemplary V205C cysteine engineered thiomabs comprise a light chain comprising a variable region selected from SEQ ID NOs: 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 23 and a constant region of SEQ ID NO: 80; and a heavy chain comprising a variable region selected from SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 and a human heavy chain constant region, such as an IgG1. Further exemplary A118C cysteine engineered thiomabs comprise a light chain comprising a variable region selected from SEQ ID NOs: 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 23 and a human light chain constant region, such as a kappa light chain constant region; and a heavy chain comprising a variable region selected from SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 and a constant region. Further exemplary S400C cysteine engineered thiomabs comprise a light chain comprising a variable region selected from SEQ ID NOs: 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 23 and a human light chain constant region, such as a kappa light chain constant region; and a heavy chain comprising a variable region selected from SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 and a constant region.

#### **e) Antibody Derivatives**

[0229] 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, propylene glycol homopolymers, polypropylene 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.

[0230] 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.

### **C. Recombinant Methods and Compositions**

[0231] 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-LGR5 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-LGR5 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).

[0232] For recombinant production of an anti-LGR5 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).

[0233] 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.

[0234] 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).

[0235] 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.

[0236] 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).

[0237] 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<sup>-</sup> 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).



#### **D. Assays**

[0238] Anti-LGR5 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.

[0239] In one aspect, an antibody is tested for its antigen binding activity, *e.g.*, by known methods such as ELISA, BIAcore®, FACS, or Western blot.

[0240] In another aspect, competition assays may be used to identify an antibody that competes with any of the antibodies described herein for binding to LGR5. In certain embodiments, such a competing antibody binds to the same epitope (*e.g.*, a linear or a conformational epitope) that is bound by an antibody described herein. 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).

[0241] In an exemplary competition assay, immobilized LGR5 is incubated in a solution comprising a first labeled antibody that binds to LGR5 (*e.g.*, any of the antibodies described herein) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to LGR5. The second antibody may be present in a hybridoma supernatant. As a control, immobilized LGR5 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 LGR5, excess unbound antibody is removed, and the amount of label associated with immobilized LGR5 is measured. If the amount of label associated with immobilized LGR5 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 LGR5. *See* Harlow and Lane (1988) *Antibodies: A Laboratory Manual* ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

#### **E. Immunoconjugates**

[0242] Provided herein are also anti-LGR5 antibodies for use herein comprising an anti-LGR5 antibody herein conjugated to one or more cytotoxic agents (also referred to as "immunoconjugates"), such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (*e.g.*, protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes (*i.e.*, a radioconjugate).

[0243] Immunoconjugates allow for the targeted delivery of a drug moiety to a tumor, and, in some embodiments intracellular accumulation therein, where systemic administration of unconjugated drugs may result in unacceptable levels of toxicity to normal cells (Polakis P. (2005) *Current Opinion in Pharmacology* 5:382-387).

[0244] Antibody-drug conjugates (ADC) are targeted chemotherapeutic molecules which combine properties of both antibodies and cytotoxic drugs by targeting potent cytotoxic drugs to antigen-expressing tumor cells (Teicher, B.A. (2009) *Current Cancer Drug Targets* 9:982-1004), thereby enhancing the therapeutic index by maximizing efficacy and minimizing off-target toxicity (Carter,

P.J. and Senter P.D. (2008) *The Cancer Jour.* 14(3):154-169; Chari, R.V. (2008) *Acc. Chem. Res.* 41:98-107 .

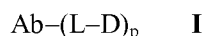
[0245] The ADC compounds for use herein include those with anticancer activity. In some embodiments, the ADC compounds include an antibody conjugated, i.e. covalently attached, to the drug moiety. In some embodiments, the antibody is covalently attached to the drug moiety through a linker. The antibody-drug conjugates (ADC) selectively deliver an effective dose of a drug to tumor tissue whereby greater selectivity, i.e. a lower efficacious dose, may be achieved while increasing the therapeutic index (“therapeutic window”).

[0246] The drug moiety (D) of the antibody-drug conjugates (ADC) may include any compound, moiety or group that has a cytotoxic or cytostatic effect. Drug moieties may impart their cytotoxic and cytostatic effects by mechanisms including but not limited to tubulin binding, DNA binding or intercalation, and inhibition of RNA polymerase, protein synthesis, and/or topoisomerase. Exemplary drug moieties include, but are not limited to, a maytansinoid, dolastatin, auristatin, calicheamicin, pyrrolobenzodiazepine (PBD), nemorubicin and its derivatives, PNU-159682, anthracycline, duocarmycin, vinca alkaloid, taxane, trichothecene, CC1065, camptothecin, elinafide, and stereoisomers, isosteres, analogs, and derivatives thereof that have cytotoxic activity. Nonlimiting examples of such immunoconjugates are discussed in further detail below.

### ***1. Exemplary Antibody-drug Conjugates***

[0247] An exemplary embodiment of an antibody-drug conjugate (ADC) compound comprises an antibody (Ab) which targets a tumor cell, a drug moiety (D), and a linker moiety (L) that attaches Ab to D. In some embodiments, the antibody is attached to the linker moiety (L) through one or more amino acid residues, such as lysine and/or cysteine.

[0248] An exemplary ADC has Formula I:



where p is 1 to about 20. In some embodiments, the number of drug moieties that can be conjugated to an antibody is limited by the number of free cysteine residues. In some embodiments, free cysteine residues are introduced into the antibody amino acid sequence by the methods described herein.

Exemplary ADC of Formula I include, but are not limited to, antibodies that have 1, 2, 3, or 4 engineered cysteine amino acids (Lyon, R. et al (2012) *Methods in Enzym.* 502:123-138). In some embodiments, one or more free cysteine residues are already present in an antibody, without the use of engineering, in which case the existing free cysteine residues may be used to conjugate the antibody to a drug. In some embodiments, an antibody is exposed to reducing conditions prior to conjugation of the antibody in order to generate one or more free cysteine residues.

#### ***a) Exemplary Linkers***

[0249] A “Linker” (L) is a bifunctional or multifunctional moiety that can be used to link one or more drug moieties (D) to an antibody (Ab) to form an antibody-drug conjugate (ADC) of Formula I. In some embodiments, antibody-drug conjugates (ADC) can be prepared using a Linker having

reactive functionalities for covalently attaching to the drug and to the antibody. For example, in some embodiments, a cysteine thiol of an antibody (Ab) can form a bond with a reactive functional group of a linker or a drug-linker intermediate to make an ADC.

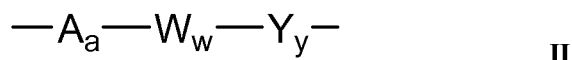
[0250] In one aspect, a linker has a functionality that is capable of reacting with a free cysteine present on an antibody to form a covalent bond. Nonlimiting exemplary such reactive functionalities include maleimide, haloacetamides,  $\alpha$ -haloacetyl, activated esters such as succinimide esters, 4-nitrophenyl esters, pentafluorophenyl esters, tetrafluorophenyl esters, anhydrides, acid chlorides, sulfonyl chlorides, isocyanates, and isothiocyanates. *See, e.g.*, the conjugation method at page 766 of Klussman, et al (2004), *Bioconjugate Chemistry* 15(4):765-773, and the Examples herein.

[0251] In some embodiments, a linker has a functionality that is capable of reacting with an electrophilic group present on an antibody. Exemplary such electrophilic groups include, but are not limited to, aldehyde and ketone carbonyl groups. In some embodiments, a heteroatom of the reactive functionality of the linker can react with an electrophilic group on an antibody and form a covalent bond to an antibody unit. Nonlimiting exemplary such reactive functionalities include, but are not limited to, hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide.

[0252] A linker may comprise one or more linker components. Exemplary linker components include 6-maleimidocaproyl ("MC"), maleimidopropanoyl ("MP"), valine-citrulline ("val-cit" or "vc"), alanine-phenylalanine ("ala-phe"), p-aminobenzoyloxycarbonyl (a "PAB"), N-Succinimidyl 4-(2-pyridylthio) pentanoate ("SPP"), and 4-(N-maleimidomethyl) cyclohexane-1 carboxylate ("MCC"). Various linker components are known in the art, some of which are described below.

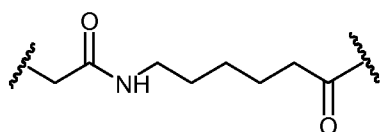
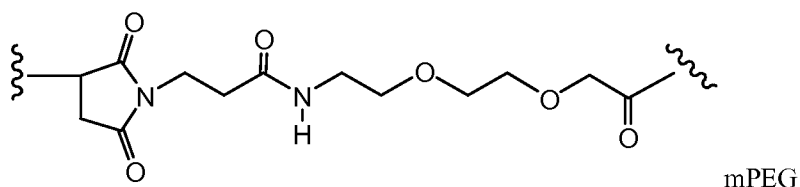
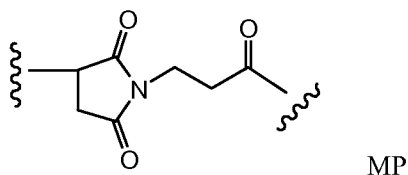
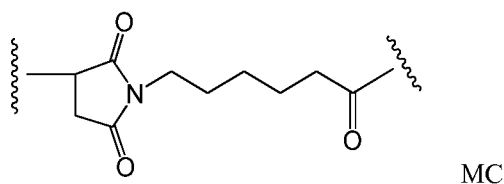
[0253] A linker may be a "cleavable linker," facilitating release of a drug. Nonlimiting exemplary cleavable linkers include acid-labile linkers (*e.g.*, comprising hydrazone), protease-sensitive (*e.g.*, peptidase-sensitive) linkers, photolabile linkers, or disulfide-containing linkers (Chari et al., *Cancer Research* 52:127-131 (1992); US 5208020).

[0254] In certain embodiments, a linker has the following Formula II:



wherein A is a "stretcher unit", and a is an integer from 0 to 1; W is an "amino acid unit", and w is an integer from 0 to 12; Y is a "spacer unit", and y is 0, 1, or 2. An ADC comprising the linker of Formula II has the Formula I(A): Ab-(A<sub>a</sub>-W<sub>w</sub>-Y<sub>y</sub>-D)<sub>p</sub>, wherein Ab, D, and p are defined as above for Formula I. Exemplary embodiments of such linkers are described in U.S. Patent No. 7,498,298, which is expressly incorporated herein by reference.

[0255] In some embodiments, a linker component comprises a "stretcher unit" (A) that links an antibody to another linker component or to a drug moiety. Nonlimiting exemplary stretcher units are shown below (wherein the wavy line indicates sites of covalent attachment to an antibody, drug, or additional linker components):



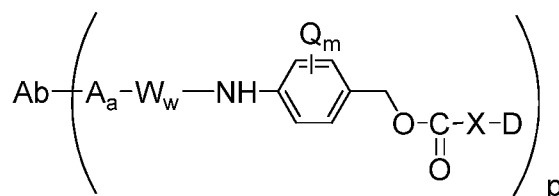
[0256] In some embodiments, a linker component comprises an “amino acid unit” (W). In some such embodiments, the amino acid unit allows for cleavage of the linker by a protease, thereby facilitating release of the drug from the immunoconjugate upon exposure to intracellular proteases, such as lysosomal enzymes (Doronina et al. (2003) *Nat. Biotechnol.* 21:778-784). Exemplary amino acid units include, but are not limited to, dipeptides, tripeptides, tetrapeptides, and pentapeptides. Exemplary dipeptides include, but are not limited to, valine-citrulline (vc or val-cit), alanine-phenylalanine (af or ala-phe); phenylalanine-lysine (fk or phe-lys); phenylalanine-homolysine (phe-homolys); and N-methyl-valine-citrulline (Me-val-cit). Exemplary tripeptides include, but are not limited to, glycine-valine-citrulline (gly-val-cit) and glycine-glycine-glycine (gly-gly-gly). An amino acid unit may comprise amino acid residues that occur naturally and/or minor amino acids and/or non-naturally occurring amino acid analogs, such as citrulline. Amino acid units can be designed and optimized for enzymatic cleavage by a particular enzyme, for example, a tumor-associated protease, cathepsin B, C and D, or a plasmin protease.

[0257] Typically, peptide-type linkers can be prepared by forming a peptide bond between two or more amino acids and/or peptide fragments. Such peptide bonds can be prepared, for example,

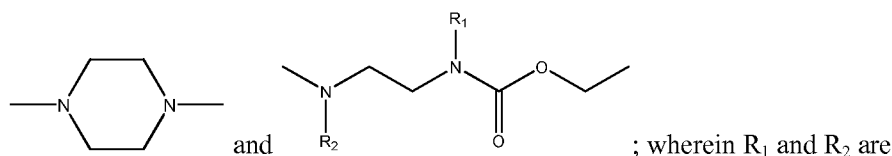
according to a liquid phase synthesis method (e.g., E. Schröder and K. Lübke (1965) "The Peptides", volume 1, pp 76-136, Academic Press).

[0258] In some embodiments, a linker component comprises a "spacer unit" (Y) that links the antibody to a drug moiety, either directly or through a stretcher unit and/or an amino acid unit. A spacer unit may be "self-immolative" or a "non-self-immolative." A "non-self-immolative" spacer unit is one in which part or all of the spacer unit remains bound to the drug moiety upon cleavage of the ADC. Examples of non-self-immolative spacer units include, but are not limited to, a glycine spacer unit and a glycine-glycine spacer unit. In some embodiments, enzymatic cleavage of an ADC containing a glycine-glycine spacer unit by a tumor-cell associated protease results in release of a glycine-glycine-drug moiety from the remainder of the ADC. In some such embodiments, the glycine-glycine-drug moiety is subjected to a hydrolysis step in the tumor cell, thus cleaving the glycine-glycine spacer unit from the drug moiety.

[0259] A "self-immolative" spacer unit allows for release of the drug moiety. In certain embodiments, a spacer unit of a linker comprises a p-aminobenzyl unit. In some such embodiments, a p-aminobenzyl alcohol is attached to an amino acid unit via an amide bond, and a carbamate, methylcarbamate, or carbonate is made between the benzyl alcohol and the drug (Hamann et al. (2005) *Expert Opin. Ther. Patents* (2005) 15:1087-1103). In some embodiments, the spacer unit comprises p-aminobenzylloxycarbonyl (PAB). In some embodiments, an ADC comprising a self-immolative linker has the structure:



wherein Q is -C<sub>1</sub>-C<sub>8</sub> alkyl, -O-(C<sub>1</sub>-C<sub>8</sub> alkyl), -halogen, -nitro, or -cyano; m is an integer ranging from 0 to 4; X may be one or more additional spacer units or may be absent; and p ranges from 1 to about 20. In some embodiments, p ranges from 1 to 10, 1 to 7, 1 to 5, or 1 to 4. Nonlimiting exemplary X spacer units include:



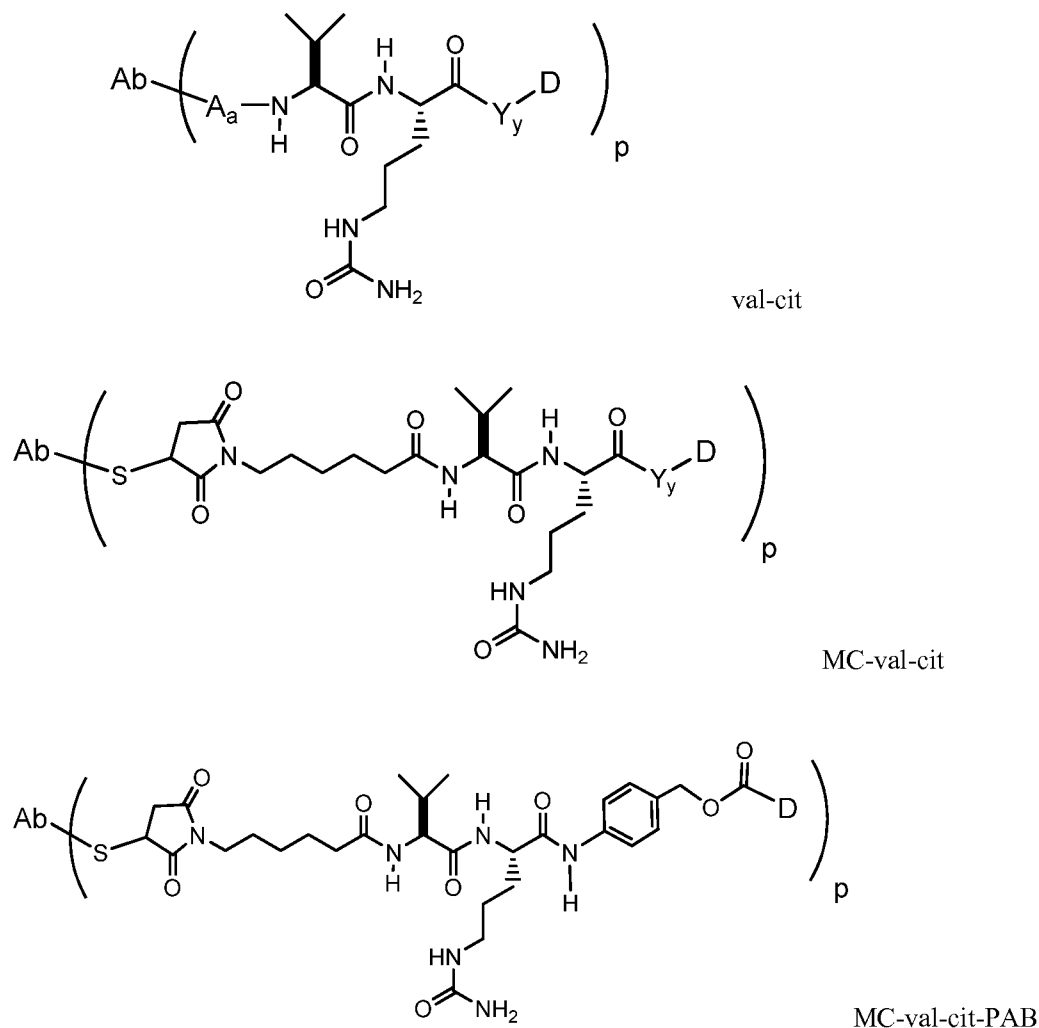
independently selected from H and C<sub>1</sub>-C<sub>6</sub> alkyl. In some embodiments, R<sub>1</sub> and R<sub>2</sub> are each -CH<sub>3</sub>.

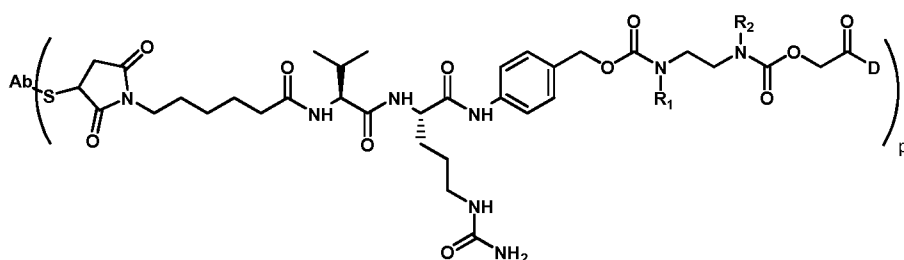
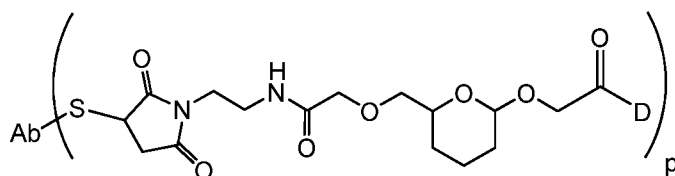
[0260] Other examples of self-immolative spacers include, but are not limited to, aromatic compounds that are electronically similar to the PAB group, such as 2-aminoimidazol-5-methanol derivatives (U.S. Patent No. 7,375,078; Hay et al. (1999) *Bioorg. Med. Chem. Lett.* 9:2237) and ortho- or para-aminobenzylacetals. In some embodiments, spacers can be used that undergo cyclization upon amide bond hydrolysis, such as substituted and unsubstituted 4-aminobutyric acid amides (Rodrigues

et al (1995) *Chemistry Biology* 2:223), appropriately substituted bicyclo[2.2.1] and bicyclo[2.2.2] ring systems (Storm et al (1972) *J. Amer. Chem. Soc.* 94:5815) and 2-aminophenylpropionic acid amides (Amsberry, et al (1990) *J. Org. Chem.* 55:5867). Linkage of a drug to the  $\alpha$ -carbon of a glycine residue is another example of a self-immolative spacer that may be useful in ADC (Kingsbury et al (1984) *J. Med. Chem.* 27:1447).

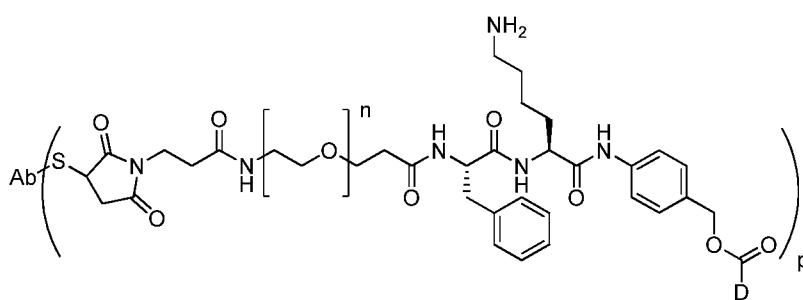
[0261] In some embodiments, linker L may be a dendritic type linker for covalent attachment of more than one drug moiety to an antibody through a branching, multifunctional linker moiety (Sun et al (2002) *Bioorganic & Medicinal Chemistry Letters* 12:2213-2215; Sun et al (2003) *Bioorganic & Medicinal Chemistry* 11:1761-1768). Dendritic linkers can increase the molar ratio of drug to antibody, i.e. loading, which is related to the potency of the ADC. Thus, where an antibody bears only one reactive cysteine thiol group, a multitude of drug moieties may be attached through a dendritic linker.

[0262] Nonlimiting exemplary linkers are shown below in the context of an ADC of Formula I:





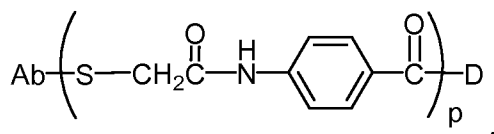
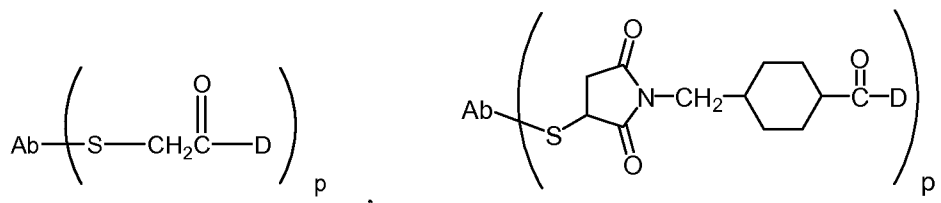
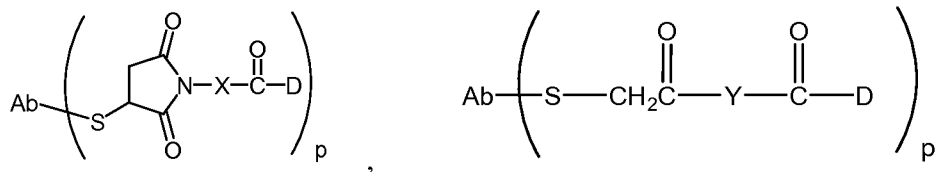
; wherein  $\text{R}_1$  and  $\text{R}_2$  are independently selected from H and  $\text{C}_1\text{-C}_6$  alkyl. In some embodiments,  $\text{R}_1$  and  $\text{R}_2$  are each  $\text{-CH}_3$ .



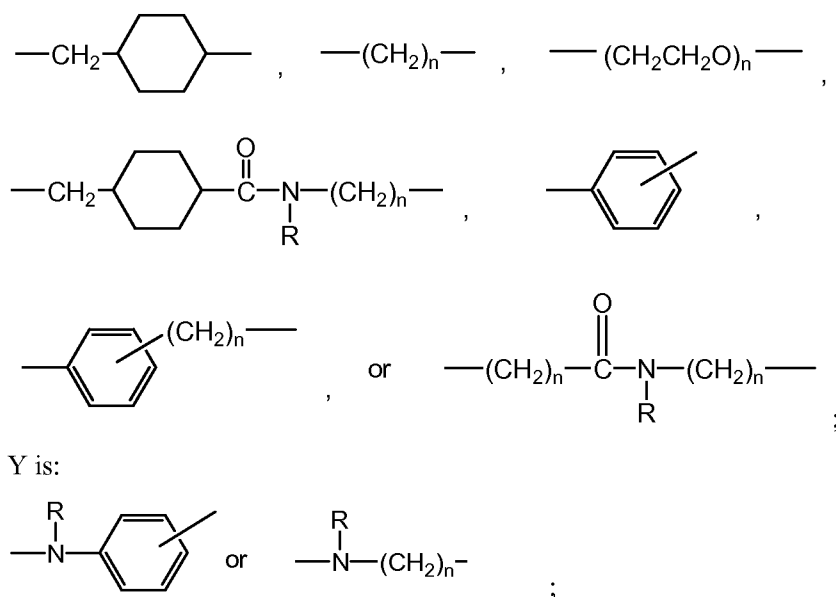
Phe-Lys-PAB-Ab;

wherein  $n$  is 0 to 12. In some embodiments,  $n$  is 2 to 10. In some embodiments,  $n$  is 4 to 8.

[0263] Further nonlimiting exemplary ADCs include the structures:



where X is:



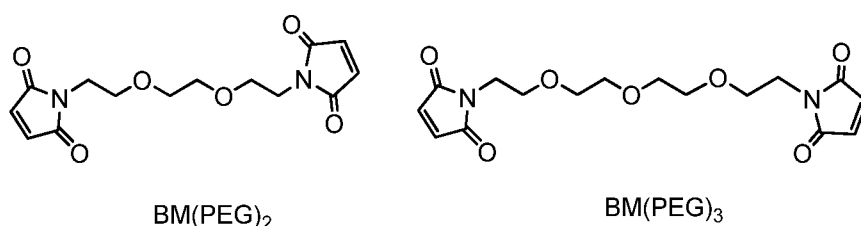
each R is independently H or C<sub>1</sub>–C<sub>6</sub> alkyl; and n is 1 to 12.

[0264] In some embodiments, a linker is substituted with groups that modulate solubility and/or reactivity. As a nonlimiting example, a charged substituent such as sulfonate (–SO<sub>3</sub><sup>–</sup>) or ammonium may increase water solubility of the linker reagent and facilitate the coupling reaction of the linker reagent with the antibody and/or the drug moiety, or facilitate the coupling reaction of Ab-L (antibody-linker intermediate) with D, or D-L (drug-linker intermediate) with Ab, depending on the synthetic route employed to prepare the ADC. In some embodiments, a portion of the linker is coupled to the antibody and a portion of the linker is coupled to the drug, and then the Ab-(linker portion)<sup>a</sup> is coupled to drug-(linker portion)<sup>b</sup> to form the ADC of Formula I.

[0265] The compounds expressly contemplate, but are not limited to, ADC prepared with the following linker reagents: bis-maleimido-trioxyethylene glycol (BMPEO), N-(β-maleimidopropoxy)-N-hydroxy succinimide ester (BMPS), N-(ε-maleimidocaproyloxy) succinimide ester (EMCS), N-[γ-maleimidobutyroxy]succinimide ester (GMBS), 1,6-hexane-bis-vinylsulfone (HBVS), succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxy-(6-amidocaproate) (LC-SMCC), m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), 4-(4-N-Maleimidophenyl)butyric acid hydrazide (MPBH), succinimidyl 3-(bromoacetamido)propionate (SBAP), succinimidyl iodoacetate (SIA), succinimidyl (4-iodoacetyl)aminobenzoate (SIAB), N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), N-succinimidyl-4-(2-pyridylthio)pentanoate (SPP), succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), succinimidyl 4-(p-maleimidophenyl)butyrate (SMPB), succinimidyl 6-[(β-maleimidopropionamido)hexanoate] (SMPH), iminothiolane (IT), sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and succinimidyl-(4-vinylsulfone)benzoate (SVSB), and including bis-maleimide reagents: dithiobismaleimidoethane (DTME), 1,4-Bismaleimidobutane (BMB), 1,4-Bismaleimidyl-2,3-dihydroxybutane (BMDB), bismaleimidoethane (BMH), bismaleimidoethane



(BMOE), BM(PEG)<sub>2</sub> (shown below), and BM(PEG)<sub>3</sub> (shown below); bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). In some embodiments, bis-maleimide reagents allow the attachment of the thiol group of a cysteine in the antibody to a thiol-containing drug moiety, linker, or linker-drug intermediate. Other functional groups that are reactive with thiol groups include, but are not limited to, iodoacetamide, bromoacetamide, vinyl pyridine, disulfide, pyridyl disulfide, isocyanate, and isothiocyanate.



[0266] Certain useful linker reagents can be obtained from various commercial sources, such as Pierce Biotechnology, Inc. (Rockford, IL), Molecular Biosciences Inc. (Boulder, CO), or synthesized in accordance with procedures described in the art; for example, in Toki et al (2002) *J. Org. Chem.* 67:1866-1872; Dubowchik, et al. (1997) *Tetrahedron Letters*, 38:5257-60; Walker, M.A. (1995) *J. Org. Chem.* 60:5352-5355; Frisch et al (1996) *Bioconjugate Chem.* 7:180-186; US 6214345; WO 02/088172; US 2003130189; US2003096743; WO 03/026577; WO 03/043583; and WO 04/032828.

[0267] Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. *See, e.g.*, WO94/11026.

## b) Exemplary Drug Moieties

### (1) *Maytansine and maytansinoids*

[0268] In some embodiments, an immunoconjugate comprises an antibody conjugated to one or more maytansinoid molecules. Maytansinoids are derivatives of maytansine, and are mitototic inhibitors which act by inhibiting tubulin polymerization. Maytansine was first isolated from the east African shrub *Maytenus serrata* (U.S. Patent No. 3896111). Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (U.S. Patent No. 4,151,042). Synthetic maytansinoids are disclosed, for example, in U.S. Patent Nos. 4,137,230; 4,248,870; 4,256,746; 4,260,608; 4,265,814; 4,294,757; 4,307,016; 4,308,268; 4,308,269; 4,309,428; 4,313,946; 4,315,929; 4,317,821; 4,322,348; 4,331,598; 4,361,650; 4,364,866; 4,424,219; 4,450,254; 4,362,663; and 4,371,533.

[0269] Maytansinoid drug moieties are attractive drug moieties in antibody-drug conjugates because they are: (i) relatively accessible to prepare by fermentation or chemical modification or derivatization

of fermentation products, (ii) amenable to derivatization with functional groups suitable for conjugation through non-disulfide linkers to antibodies, (iii) stable in plasma, and (iv) effective against a variety of tumor cell lines.

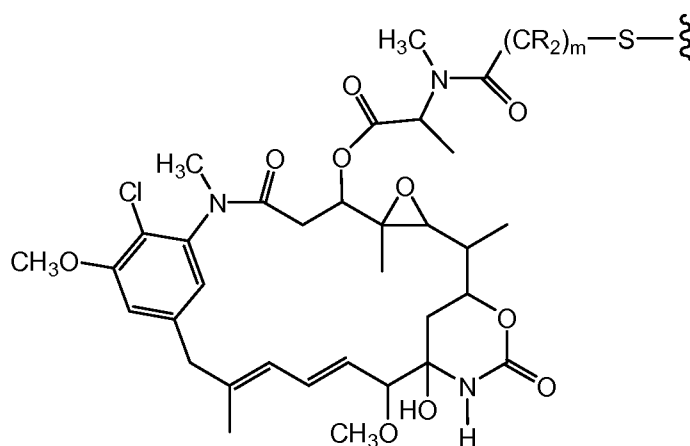
[0270] Certain maytansinoids suitable for use as maytansinoid drug moieties are known in the art and can be isolated from natural sources according to known methods or produced using genetic engineering techniques (*see, e.g.*, Yu et al (2002) PNAS 99:7968-7973). Maytansinoids may also be prepared synthetically according to known methods.

[0271] Exemplary maytansinoid drug moieties include, but are not limited to, those having a modified aromatic ring, such as: C-19-dechloro (US Pat. No. 4256746) (prepared, for example, by lithium aluminum hydride reduction of ansamytocin P2); C-20-hydroxy (or C-20-demethyl) +/-C-19-dechloro (US Pat. Nos. 4361650 and 4307016) (prepared, for example, by demethylation using *Streptomyces* or *Actinomyces* or dechlorination using LAH); and C-20-demethoxy, C-20-acyloxy (-OCOR), +/-dechloro (U.S. Pat. No. 4,294,757) (prepared, for example, by acylation using acyl chlorides), and those having modifications at other positions of the aromatic ring.

[0272] Exemplary maytansinoid drug moieties also include those having modifications such as: C-9-SH (US Pat. No. 4424219) (prepared, for example, by the reaction of maytansinol with H<sub>2</sub>S or P<sub>2</sub>S<sub>5</sub>); C-14-alkoxymethyl(demethoxy/CH<sub>2</sub> OR)(US 4331598); C-14-hydroxymethyl or acyloxymethyl (CH<sub>2</sub>OH or CH<sub>2</sub>OAc) (US Pat. No. 4450254) (prepared, for example, from *Nocardia*); C-15-hydroxy/acyloxy (US 4364866) (prepared, for example, by the conversion of maytansinol by *Streptomyces*); C-15-methoxy (US Pat. Nos. 4313946 and 4315929) (for example, isolated from *Trewia nudiflora*); C-18-N-demethyl (US Pat. Nos. 4362663 and 4322348) (prepared, for example, by the demethylation of maytansinol by *Streptomyces*); and 4,5-deoxy (US 4371533) (prepared, for example, by the titanium trichloride/LAH reduction of maytansinol).

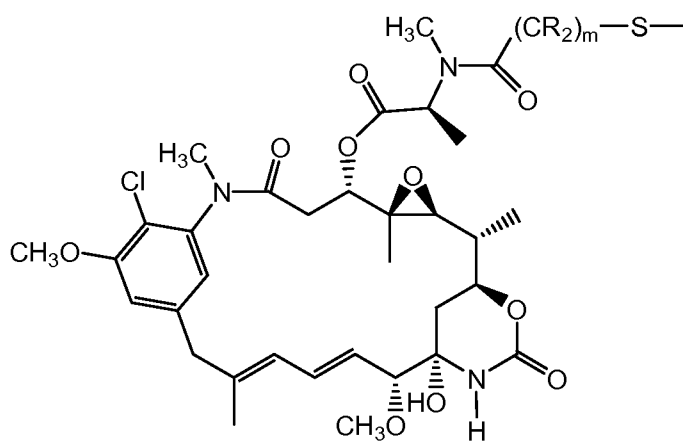
[0273] Many positions on maytansinoid compounds are useful as the linkage position. For example, an ester linkage may be formed by reaction with a hydroxyl group using conventional coupling techniques. In some embodiments, the reaction may occur at the C-3 position having a hydroxyl group, the C-14 position modified with hydroxymethyl, the C-15 position modified with a hydroxyl group, and the C-20 position having a hydroxyl group. In some embodiments, the linkage is formed at the C-3 position of maytansinol or a maytansinol analogue.

[0274] Maytansinoid drug moieties include those having the structure:

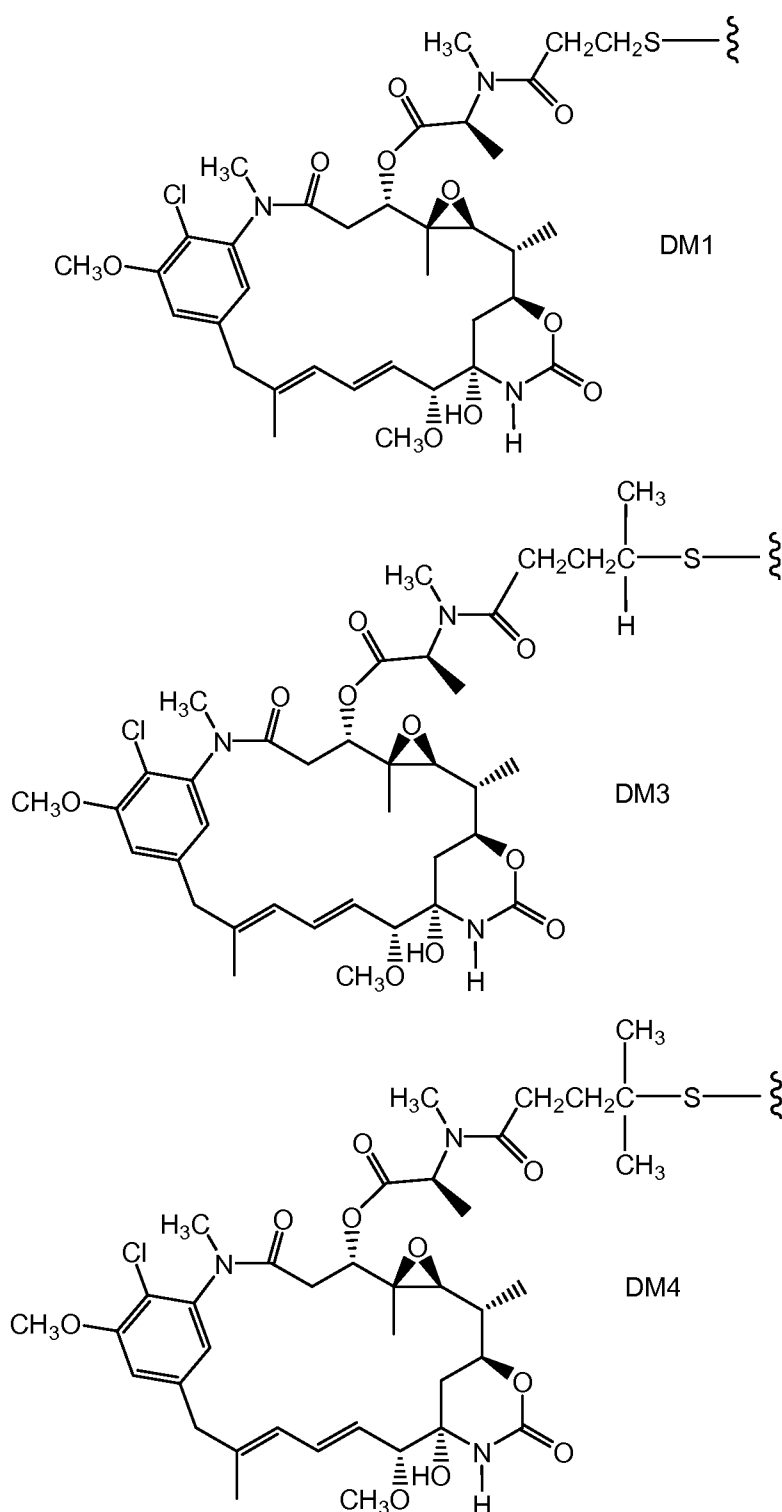


where the wavy line indicates the covalent attachment of the sulfur atom of the maytansinoid drug moiety to a linker of an ADC. Each R may independently be H or a C<sub>1</sub>-C<sub>6</sub> alkyl. The alkylene chain attaching the amide group to the sulfur atom may be methanyl, ethanyl, or propyl, i.e., m is 1, 2, or 3 (US 633410; US 5208020; Chari et al (1992) *Cancer Res.* 52:127-131; Liu et al (1996) *Proc. Natl. Acad. Sci USA* 93:8618-8623).

[0275] All stereoisomers of the maytansinoid drug moiety are contemplated for the ADC, *i.e.* any combination of *R* and *S* configurations at the chiral carbons (US 7276497; US 6913748; US 6441163; US 633410 (RE39151); US 5208020; Widdison et al (2006) *J. Med. Chem.* 49:4392-4408, which are incorporated by reference in their entirety). In some embodiments, the maytansinoid drug moiety has the following stereochemistry:

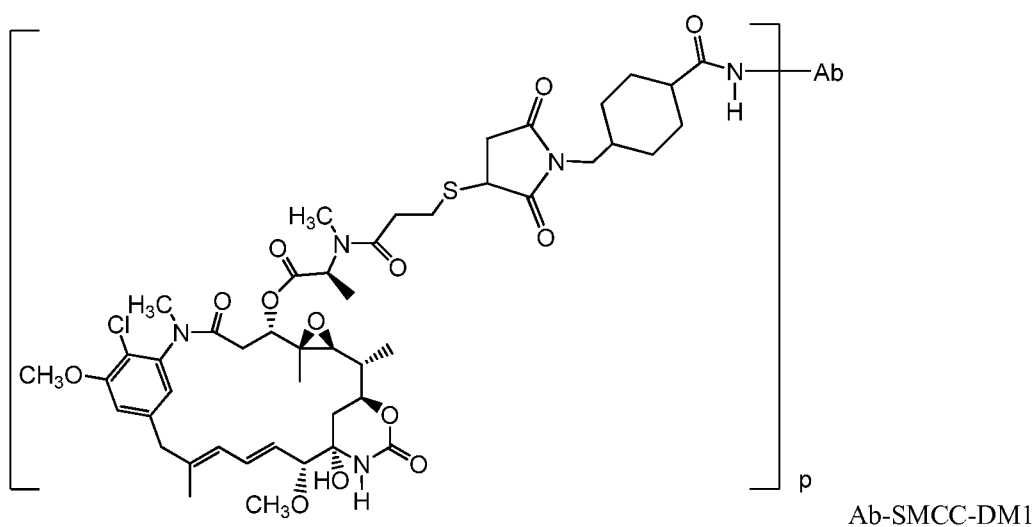
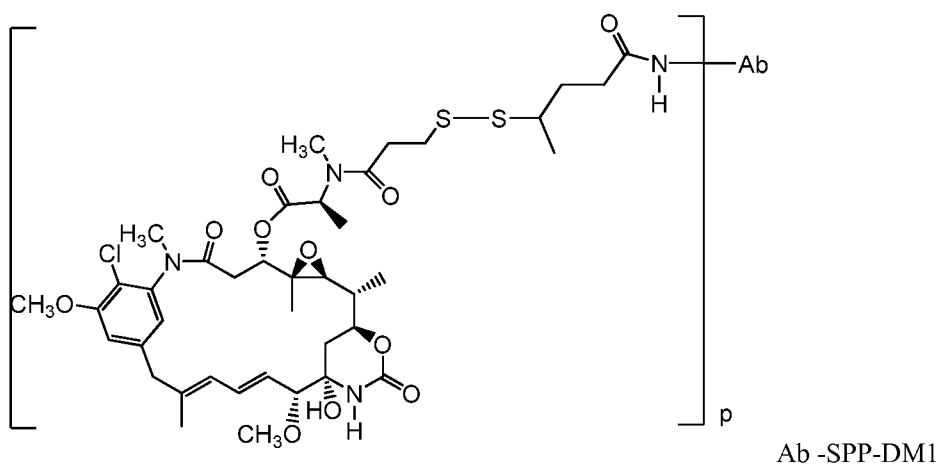


[0276] Exemplary embodiments of maytansinoid drug moieties include, but are not limited to, DM1; DM3; and DM4, having the structures:

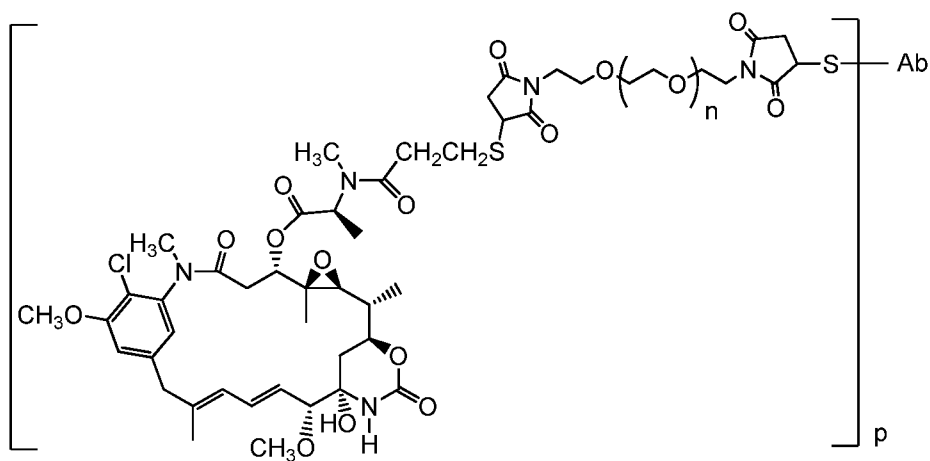


wherein the wavy line indicates the covalent attachment of the sulfur atom of the drug to a linker (L) of an antibody-drug conjugate.

[0277] Other exemplary maytansinoid antibody-drug conjugates have the following structures and abbreviations (wherein Ab is antibody and p is 1 to about 20. In some embodiments, p is 1 to 10, p is 1 to 7, p is 1 to 5, or p is 1 to 4):



[0278] Exemplary antibody-drug conjugates where DM1 is linked through a BMPEO linker to a thiol group of the antibody have the structure and abbreviation:



where Ab is antibody; n is 0, 1, or 2; and p is 1 to about 20. In some embodiments, p is 1 to 10, p is 1 to 7, p is 1 to 5, or p is 1 to 4.

[0279] Immunoconjugates containing maytansinoids, methods of making the same, and their therapeutic use are disclosed, for example, in U.S. Patent Nos. 5,208,020 and 5,416,064; US 2005/0276812 A1; and European Patent EP 0 425 235 B1, the disclosures of which are hereby expressly incorporated by reference. *See also* Liu et al. *Proc. Natl. Acad. Sci. USA* 93:8618-8623 (1996); and Chari et al. *Cancer Research* 52:127-131 (1992).

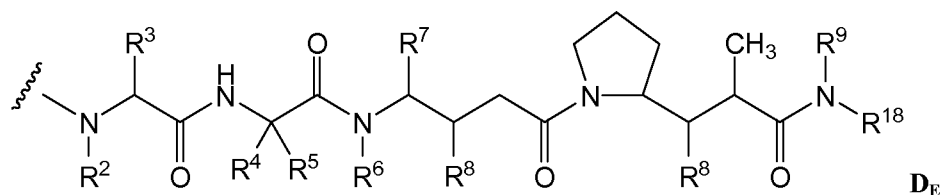
[0280] In some embodiments, antibody-maytansinoid conjugates may be prepared by chemically linking an antibody to a maytansinoid molecule without significantly diminishing the biological activity of either the antibody or the maytansinoid molecule. *See, e.g.*, U.S. Patent No. 5,208,020 (the disclosure of which is hereby expressly incorporated by reference). In some embodiments, ADC with an average of 3-4 maytansinoid molecules conjugated per antibody molecule has shown efficacy in enhancing cytotoxicity of target cells without negatively affecting the function or solubility of the antibody. In some instances, even one molecule of toxin/antibody is expected to enhance cytotoxicity over the use of naked antibody.

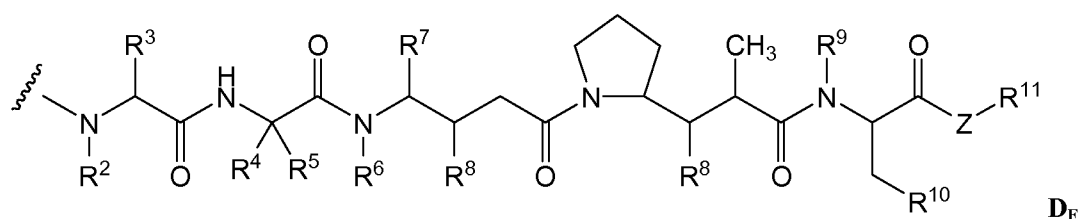
[0281] Exemplary linking groups for making antibody-maytansinoid conjugates include, for example, those described herein and those disclosed in U.S. Patent No. 5,208,020; EP Patent 0 425 235 B1; Chari et al. *Cancer Research* 52:127-131 (1992); US 2005/0276812 A1; and US 2005/016993 A1, the disclosures of which are hereby expressly incorporated by reference.

## (2) Auristatins and dolastatins

[0282] Drug moieties include dolastatins, auristatins, and analogs and derivatives thereof (US 5635483; US 5780588; US 5767237; US 6124431). Auristatins are derivatives of the marine mollusk compound dolastatin-10. While not intending to be bound by any particular theory, dolastatins and auristatins have been shown to interfere with microtubule dynamics, GTP hydrolysis, and nuclear and cellular division (Woyke et al (2001) *Antimicrob. Agents and Chemother.* 45(12):3580-3584) and have anticancer (US 5663149) and antifungal activity (Pettit et al (1998) *Antimicrob. Agents Chemother.* 42:2961-2965). The dolastatin/auristatin drug moiety may be attached to the antibody through the N (amino) terminus or the C (carboxyl) terminus of the peptidic drug moiety (WO 02/088172; Doronina et al (2003) *Nature Biotechnology* 21(7):778-784; Francisco et al (2003) *Blood* 102(4):1458-1465).

[0283] Exemplary auristatin embodiments include the N-terminus linked monomethylauristatin drug moieties D<sub>E</sub> and D<sub>F</sub>, disclosed in US 7498298 and US 7659241, the disclosures of which are expressly incorporated by reference in their entirety:





wherein the wavy line of D<sub>E</sub> and D<sub>F</sub> indicates the covalent attachment site to an antibody or antibody-linker component, and independently at each location:

R<sup>2</sup> is selected from H and C<sub>1</sub>-C<sub>8</sub> alkyl;

R<sup>3</sup> is selected from H, C<sub>1</sub>-C<sub>8</sub> alkyl, C<sub>3</sub>-C<sub>8</sub> carbocycle, aryl, C<sub>1</sub>-C<sub>8</sub> alkyl-aryl, C<sub>1</sub>-C<sub>8</sub> alkyl-(C<sub>3</sub>-C<sub>8</sub> carbocycle), C<sub>3</sub>-C<sub>8</sub> heterocycle and C<sub>1</sub>-C<sub>8</sub> alkyl-(C<sub>3</sub>-C<sub>8</sub> heterocycle);

R<sup>4</sup> is selected from H, C<sub>1</sub>-C<sub>8</sub> alkyl, C<sub>3</sub>-C<sub>8</sub> carbocycle, aryl, C<sub>1</sub>-C<sub>8</sub> alkyl-aryl, C<sub>1</sub>-C<sub>8</sub> alkyl-(C<sub>3</sub>-C<sub>8</sub> carbocycle), C<sub>3</sub>-C<sub>8</sub> heterocycle and C<sub>1</sub>-C<sub>8</sub> alkyl-(C<sub>3</sub>-C<sub>8</sub> heterocycle);

R<sup>5</sup> is selected from H and methyl;

or R<sup>4</sup> and R<sup>5</sup> jointly form a carbocyclic ring and have the formula -(CR<sup>a</sup>R<sup>b</sup>)<sub>n</sub>- wherein R<sup>a</sup> and R<sup>b</sup> are independently selected from H, C<sub>1</sub>-C<sub>8</sub> alkyl and C<sub>3</sub>-C<sub>8</sub> carbocycle and n is selected from 2, 3, 4, 5 and 6;

R<sup>6</sup> is selected from H and C<sub>1</sub>-C<sub>8</sub> alkyl;

R<sup>7</sup> is selected from H, C<sub>1</sub>-C<sub>8</sub> alkyl, C<sub>3</sub>-C<sub>8</sub> carbocycle, aryl, C<sub>1</sub>-C<sub>8</sub> alkyl-aryl, C<sub>1</sub>-C<sub>8</sub> alkyl-(C<sub>3</sub>-C<sub>8</sub> carbocycle), C<sub>3</sub>-C<sub>8</sub> heterocycle and C<sub>1</sub>-C<sub>8</sub> alkyl-(C<sub>3</sub>-C<sub>8</sub> heterocycle);

each R<sup>8</sup> is independently selected from H, OH, C<sub>1</sub>-C<sub>8</sub> alkyl, C<sub>3</sub>-C<sub>8</sub> carbocycle and O-(C<sub>1</sub>-C<sub>8</sub> alkyl);

R<sup>9</sup> is selected from H and C<sub>1</sub>-C<sub>8</sub> alkyl;

R<sup>10</sup> is selected from aryl or C<sub>3</sub>-C<sub>8</sub> heterocycle;

Z is O, S, NH, or NR<sup>12</sup>, wherein R<sup>12</sup> is C<sub>1</sub>-C<sub>8</sub> alkyl;

R<sup>11</sup> is selected from H, C<sub>1</sub>-C<sub>20</sub> alkyl, aryl, C<sub>3</sub>-C<sub>8</sub> heterocycle, -(R<sup>13</sup>O)<sub>m</sub>-R<sup>14</sup>, or -(R<sup>13</sup>O)<sub>m</sub>-CH(R<sup>15</sup>)<sub>2</sub>;

m is an integer ranging from 1-1000;

R<sup>13</sup> is C<sub>2</sub>-C<sub>8</sub> alkyl;

R<sup>14</sup> is H or C<sub>1</sub>-C<sub>8</sub> alkyl;

each occurrence of R<sup>15</sup> is independently H, COOH, -(CH<sub>2</sub>)<sub>n</sub>-N(R<sup>16</sup>)<sub>2</sub>, -(CH<sub>2</sub>)<sub>n</sub>-SO<sub>3</sub>H, or -(CH<sub>2</sub>)<sub>n</sub>-SO<sub>3</sub>-C<sub>1</sub>-C<sub>8</sub> alkyl;

each occurrence of R<sup>16</sup> is independently H, C<sub>1</sub>-C<sub>8</sub> alkyl, or -(CH<sub>2</sub>)<sub>n</sub>-COOH;

R<sup>18</sup> is selected from -C(R<sup>8</sup>)<sub>2</sub>-C(R<sup>8</sup>)<sub>2</sub>-aryl, -C(R<sup>8</sup>)<sub>2</sub>-C(R<sup>8</sup>)<sub>2</sub>-(C<sub>3</sub>-C<sub>8</sub> heterocycle), and -C(R<sup>8</sup>)<sub>2</sub>-C(R<sup>8</sup>)<sub>2</sub>-(C<sub>3</sub>-C<sub>8</sub> carbocycle); and

n is an integer ranging from 0 to 6.

[0284] In one embodiment,  $R^3$ ,  $R^4$  and  $R^7$  are independently isopropyl or sec-butyl and  $R^5$  is -H or methyl. In an exemplary embodiment,  $R^3$  and  $R^4$  are each isopropyl,  $R^5$  is -H, and  $R^7$  is sec-butyl.

[0285] In yet another embodiment,  $R^2$  and  $R^6$  are each methyl, and  $R^9$  is -H.

[0286] In still another embodiment, each occurrence of  $R^8$  is -OCH<sub>3</sub>.

[0287] In an exemplary embodiment,  $R^3$  and  $R^4$  are each isopropyl,  $R^2$  and  $R^6$  are each methyl,  $R^5$  is -H,  $R^7$  is sec-butyl, each occurrence of  $R^8$  is -OCH<sub>3</sub>, and  $R^9$  is -H.

[0288] In one embodiment, Z is -O- or -NH-.

[0289] In one embodiment,  $R^{10}$  is aryl.

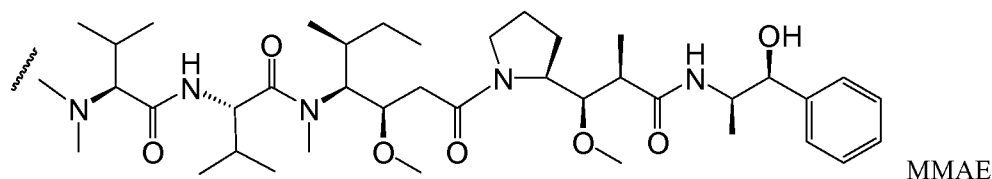
[0290] In an exemplary embodiment,  $R^{10}$  is -phenyl.

[0291] In an exemplary embodiment, when Z is -O-,  $R^{11}$  is -H, methyl or t-butyl.

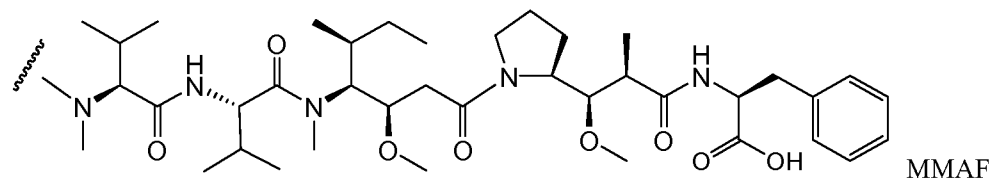
[0292] In one embodiment, when Z is -NH,  $R^{11}$  is -CH( $R^{15}$ )<sub>2</sub>, wherein  $R^{15}$  is -(CH<sub>2</sub>)<sub>n</sub>-N( $R^{16}$ )<sub>2</sub>, and  $R^{16}$  is -C<sub>1</sub>-C<sub>8</sub> alkyl or -(CH<sub>2</sub>)<sub>n</sub>-COOH.

[0293] In another embodiment, when Z is -NH,  $R^{11}$  is -CH( $R^{15}$ )<sub>2</sub>, wherein  $R^{15}$  is -(CH<sub>2</sub>)<sub>n</sub>-SO<sub>3</sub>H.

[0294] An exemplary auristatin embodiment of formula D<sub>E</sub> is MMAE, wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody-drug conjugate:

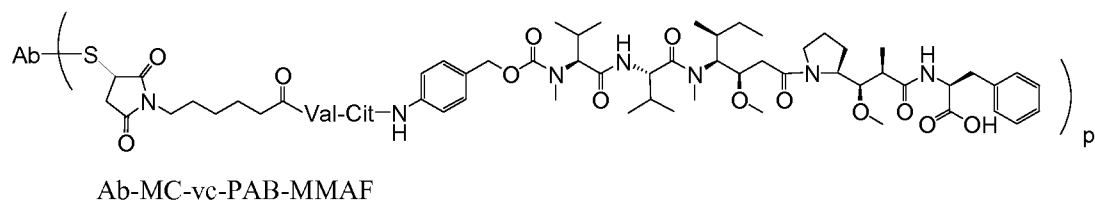


[0295] An exemplary auristatin embodiment of formula D<sub>F</sub> is MMAF, wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody-drug conjugate:

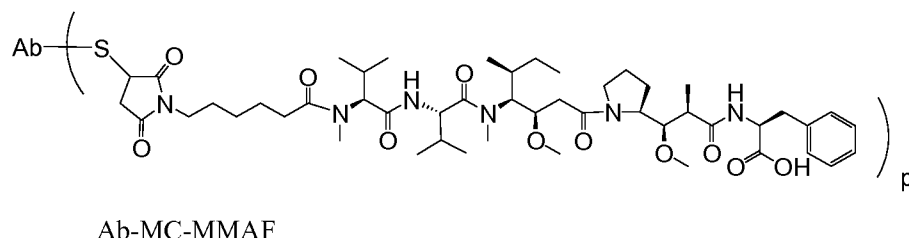
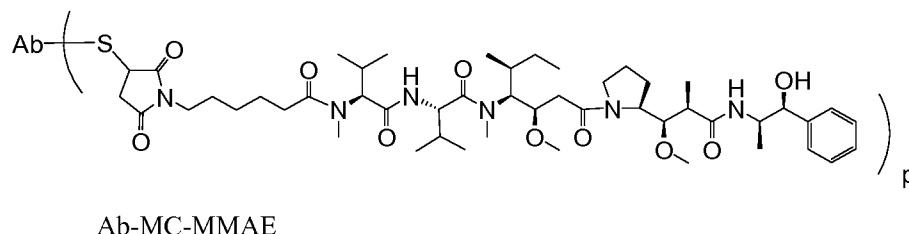
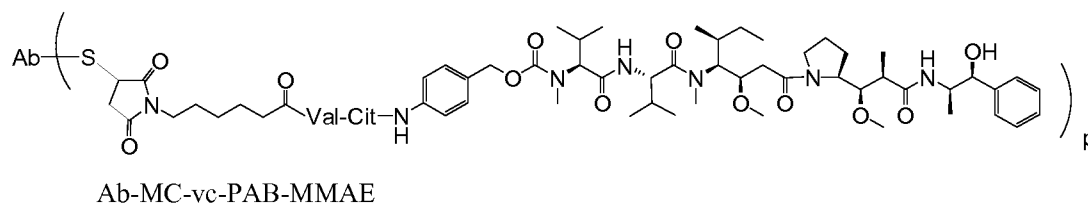


[0296] Other exemplary embodiments include monomethylvaline compounds having phenylalanine carboxy modifications at the C-terminus of the pentapeptide auristatin drug moiety (WO 2007/008848) and monomethylvaline compounds having phenylalanine sidechain modifications at the C-terminus of the pentapeptide auristatin drug moiety (WO 2007/008603).

[0297] Nonlimiting exemplary embodiments of ADC of Formula I comprising MMAE or MMAF and various linker components have the following structures and abbreviations (wherein “Ab” is an antibody; p is 1 to about 8, “Val-Cit” is a valine-citrulline dipeptide; and “S” is a sulfur atom:







[0298] Nonlimiting exemplary embodiments of ADCs of Formula I comprising MMAF and various linker components further include Ab-MC-PAB-MMAF and Ab-PAB-MMAF. Immunoconjugates comprising MMAF attached to an antibody by a linker that is not proteolytically cleavable have been shown to possess activity comparable to immunoconjugates comprising MMAF attached to an antibody by a proteolytically cleavable linker (Doronina et al. (2006) *Bioconjugate Chem.* 17:114-124). In some such embodiments, drug release is believed to be effected by antibody degradation in the cell.

[0299] Typically, peptide-based drug moieties can be prepared by forming a peptide bond between two or more amino acids and/or peptide fragments. Such peptide bonds can be prepared, for example, according to a liquid phase synthesis method (*see, e.g.,* E. Schröder and K. Lübke, "The Peptides", volume 1, pp 76-136, 1965, Academic Press). Auristatin/dolastatin drug moieties may, in some embodiments, be prepared according to the methods of: US 7498298; US 5635483; US 5780588; Pettit et al (1989) *J. Am. Chem. Soc.* 111:5463-5465; Pettit et al (1998) *Anti-Cancer Drug Design* 13:243-277; Pettit, G.R., et al. *Synthesis*, 1996, 719-725; Pettit et al (1996) *J. Chem. Soc. Perkin Trans. 1* 5:859-863; and Doronina (2003) *Nat. Biotechnol.* 21(7):778-784.

[0300] In some embodiments, auristatin/dolastatin drug moieties of formulas D<sub>E</sub> such as MMAE, and D<sub>F</sub>, such as MMAF, and drug-linker intermediates and derivatives thereof, such as MC-MMAF, MC-MMAE, MC-vc-PAB-MMAF, and MC-vc-PAB-MMAE, may be prepared using methods described

in US 7498298; Doronina et al. (2006) *Bioconjugate Chem.* 17:114-124; and Doronina et al. (2003) *Nat. Biotech.* 21:778-784 and then conjugated to an antibody of interest.

(3) *Calicheamicin*

[0301] In some embodiments, the immunoconjugate comprises an antibody conjugated to one or more calicheamicin molecules. The calicheamicin family of antibiotics, and analogues thereof, are capable of producing double-stranded DNA breaks at sub-picomolar concentrations (Hinman et al., (1993) *Cancer Research* 53:3336-3342; Lode et al., (1998) *Cancer Research* 58:2925-2928).

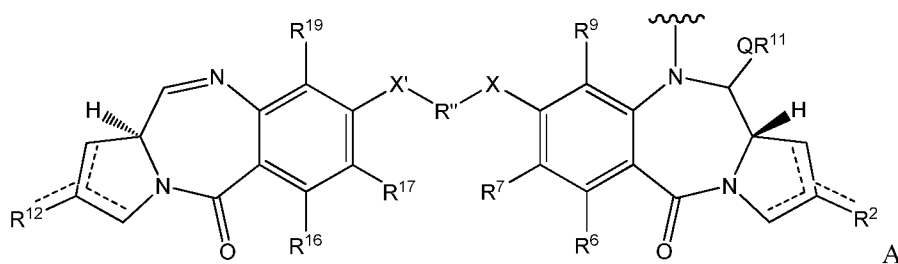
Calicheamicin has intracellular sites of action but, in certain instances, does not readily cross the plasma membrane. Therefore, cellular uptake of these agents through antibody-mediated internalization may, in some embodiments, greatly enhance their cytotoxic effects. Nonlimiting exemplary methods of preparing antibody-drug conjugates with a calicheamicin drug moiety are described, for example, in US 5712374; US 5714586; US 5739116; and US 5767285.

(4) *Pyrrolobenzodiazepines*

[0302] In some embodiments, an ADC comprises a pyrrolobenzodiazepine (PBD). In some embodiments, PBD dimers recognize and bind to specific DNA sequences. The natural product anthramycin, a PBD, was first reported in 1965 (Leimgruber, et al., (1965) *J. Am. Chem. Soc.*, 87:5793-5795; Leimgruber, et al., (1965) *J. Am. Chem. Soc.*, 87:5791-5793). Since then, a number of PBDs, both naturally-occurring and analogues, have been reported (Thurston, et al., (1994) *Chem. Rev.* 1994, 433-465 including dimers of the tricyclic PBD scaffold (US 6884799; US 7049311; US 7067511; US 7265105; US 7511032; US 7528126; US 7557099). Without intending to be bound by any particular theory, it is believed that the dimer structure imparts the appropriate three-dimensional shape for isohelicity with the minor groove of B-form DNA, leading to a snug fit at the binding site (Kohn, In *Antibiotics III*. Springer-Verlag, New York, pp. 3-11 (1975); Hurley and Needham-VanDevanter, (1986) *Acc. Chem. Res.*, 19:230-237). Dimeric PBD compounds bearing C2 aryl substituents have been shown to be useful as cytotoxic agents (Hartley et al (2010) *Cancer Res.* 70(17):6849-6858; Antonow (2010) *J. Med. Chem.* 53(7):2927-2941; Howard et al (2009) *Bioorganic and Med. Chem. Letters* 19(22):6463-6466).

[0303] PBD dimers have been conjugated to antibodies and the resulting ADC shown to have anti-cancer properties. Nonlimiting exemplary linkage sites on the PBD dimer include the five-membered pyrrolo ring, the tether between the PBD units, and the N10-C11 imine group (WO 2009/016516; US 2009/304710; US 2010/047257; US 2009/036431; US 2011/0256157; WO 2011/130598).

[0304] Nonlimiting exemplary PBD dimer components of ADCs are of Formula A:



and salts and solvates thereof, wherein:

the wavy line indicates the covalent attachment site to the linker;

the dotted lines indicate the optional presence of a double bond between C1 and C2 or C2 and C3;

$R^2$  is independently selected from H, OH, =O, =CH<sub>2</sub>, CN, R, OR, =CH-R<sup>D</sup>, =C(R<sup>D</sup>)<sub>2</sub>, O-SO<sub>2</sub>-R, CO<sub>2</sub>R and COR, and optionally further selected from halo or dihalo, wherein R<sup>D</sup> is independently selected from R, CO<sub>2</sub>R, COR, CHO, CO<sub>2</sub>H, and halo;

$R^6$  and  $R^9$  are independently selected from H, R, OH, OR, SH, SR, NH<sub>2</sub>, NHR, NRR', NO<sub>2</sub>, Me<sub>3</sub>Sn and halo;

$R^7$  is independently selected from H, R, OH, OR, SH, SR, NH<sub>2</sub>, NHR, NRR', NO<sub>2</sub>, Me<sub>3</sub>Sn and halo;

Q is independently selected from O, S and NH;

$R^{11}$  is either H, or R or, where Q is O, SO<sub>3</sub>M, where M is a metal cation;

R and R' are each independently selected from optionally substituted C<sub>1-8</sub> alkyl, C<sub>1-12</sub> alkyl, C<sub>3-8</sub> heterocyclyl, C<sub>3-20</sub> heterocycle, and C<sub>5-20</sub> aryl groups, and optionally in relation to the group NRR', R and R' together with the nitrogen atom to which they are attached form an optionally substituted 4-, 5-, 6- or 7-membered heterocyclic ring;

$R^{12}$ ,  $R^{16}$ ,  $R^{19}$  and  $R^{17}$  are as defined for  $R^2$ ,  $R^6$ ,  $R^9$  and  $R^7$  respectively;

R'' is a C<sub>3-12</sub> alkylene group, which chain may be interrupted by one or more heteroatoms, *e.g.* O, S, N(H), NMe and/or aromatic rings, *e.g.* benzene or pyridine, which rings are optionally substituted; and

X and X' are independently selected from O, S and N(H).

[0305] In some embodiments, R and R' are each independently selected from optionally substituted C<sub>1-12</sub> alkyl, C<sub>3-20</sub> heterocycle, and C<sub>5-20</sub> aryl groups, and optionally in relation to the group NRR', R and R' together with the nitrogen atom to which they are attached form an optionally substituted 4-, 5-, 6- or 7-membered heterocyclic ring.

[0306] In some embodiments,  $R^9$  and  $R^{19}$  are H.

[0307] In some embodiments,  $R^6$  and  $R^{16}$  are H.

[0308] In some embodiments,  $R^7$  and  $R^{17}$  are both  $OR^{7A}$ , where  $R^{7A}$  is optionally substituted  $C_{1-4}$  alkyl. In some embodiments,  $R^{7A}$  is Me. In some embodiments,  $R^{7A}$  is  $CH_2Ph$ , where Ph is a phenyl group.

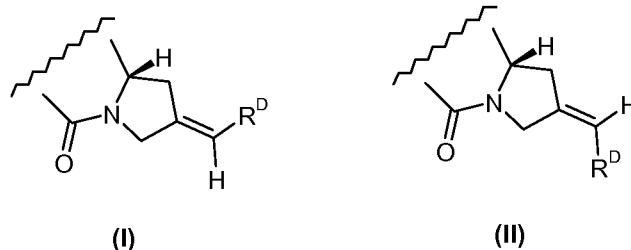
[0309] In some embodiments, X is O.

[0310] In some embodiments,  $R^{11}$  is H.

[0311] In some embodiments, there is a double bond between C2 and C3 in each monomer unit.

[0312] In some embodiments,  $R^2$  and  $R^{12}$  are independently selected from H and R. In some embodiments,  $R^2$  and  $R^{12}$  are independently R. In some embodiments,  $R^2$  and  $R^{12}$  are independently optionally substituted  $C_{5-20}$  aryl or  $C_{5-7}$  aryl or  $C_{8-10}$  aryl. In some embodiments,  $R^2$  and  $R^{12}$  are independently optionally substituted phenyl, thienyl, naphthyl, pyridyl, quinoliny, or isoquinoliny. In some embodiments,  $R^2$  and  $R^{12}$  are independently selected from  $=O$ ,  $=CH_2$ ,  $=CH-R^D$ , and  $=C(R^D)_2$ . In some embodiments,  $R^2$  and  $R^{12}$  each  $=CH_2$ . In some embodiments,  $R^2$  and  $R^{12}$  are each H. In some embodiments,  $R^2$  and  $R^{12}$  are each  $=O$ . In some embodiments,  $R^2$  and  $R^{12}$  are each  $=CF_2$ . In some embodiments,  $R^2$  and/or  $R^{12}$  are independently  $=C(R^D)_2$ . In some embodiments,  $R^2$  and/or  $R^{12}$  are independently  $=CH-R^D$ .

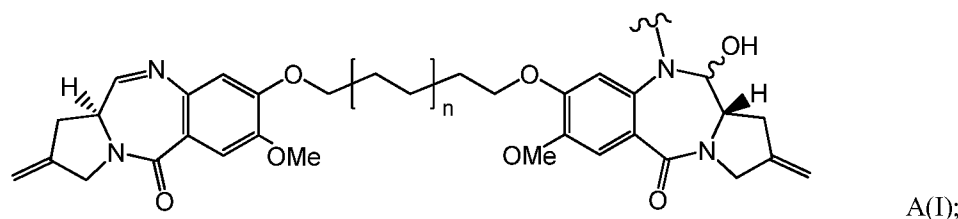
[0313] In some embodiments, when  $R^2$  and/or  $R^{12}$  is  $=CH-R^D$ , each group may independently have either configuration shown below:



In some embodiments, a  $=CH-R^D$  is in configuration (I).

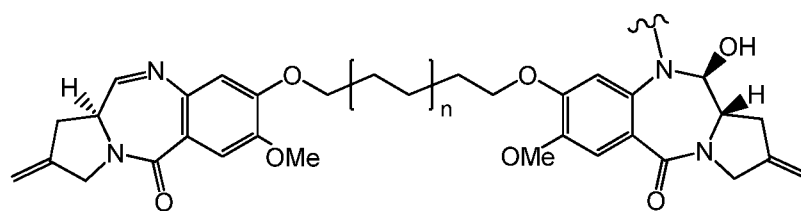
[0314] In some embodiments,  $R''$  is a  $C_3$  alkylene group or a  $C_5$  alkylene group.

[0315] In some embodiments, an exemplary PBD dimer component of an ADC has the structure of Formula A(I):



wherein n is 0 or 1.

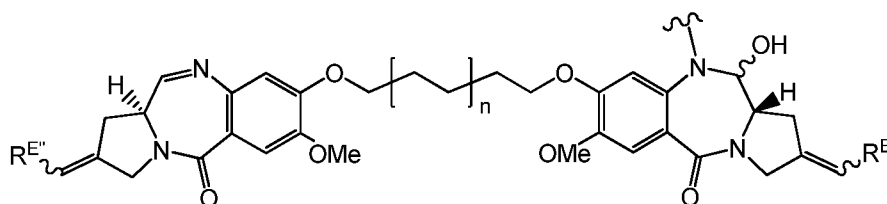
[0316] In some embodiments, an exemplary PBD dimer component of an ADC has the structure of Formula A(II):



A(II);

wherein  $n$  is 0 or 1.

[0317] In some embodiments, an exemplary PBD dimer component of an ADC has the structure of Formula A(III):



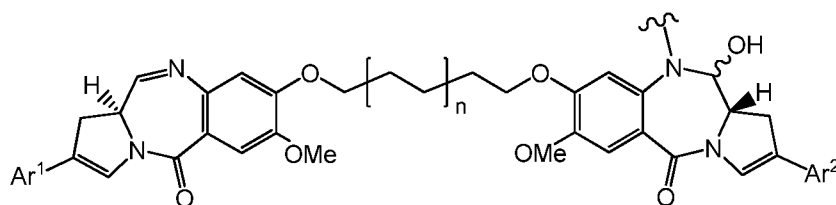
A(III);

wherein  $R^E$  and  $R^{E''}$  are each independently selected from H or  $R^D$ , wherein  $R^D$  is defined as above; and

wherein  $n$  is 0 or 1.

[0318] In some embodiments,  $n$  is 0. In some embodiments,  $n$  is 1. In some embodiments,  $R^E$  and/or  $R^{E''}$  is H. In some embodiments,  $R^E$  and  $R^{E''}$  are H. In some embodiments,  $R^E$  and/or  $R^{E''}$  is  $R^D$ , wherein  $R^D$  is optionally substituted  $C_{1-12}$  alkyl. In some embodiments,  $R^E$  and/or  $R^{E''}$  is  $R^D$ , wherein  $R^D$  is methyl.

[0319] In some embodiments, an exemplary PBD dimer component of an ADC has the structure of Formula A(IV):

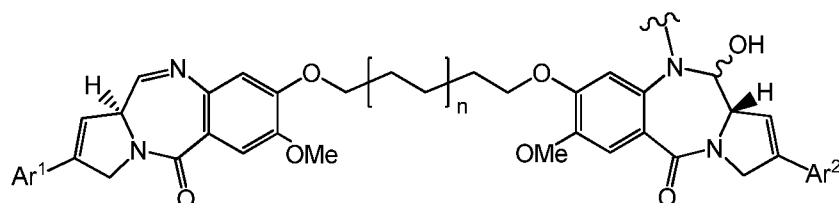


A(IV);

wherein  $Ar^1$  and  $Ar^2$  are each independently optionally substituted  $C_{5-20}$  aryl; wherein  $Ar^1$  and  $Ar^2$  may be the same or different; and

wherein  $n$  is 0 or 1.

[0320] In some embodiments, an exemplary PBD dimer component of an ADC has the structure of Formula A(V):



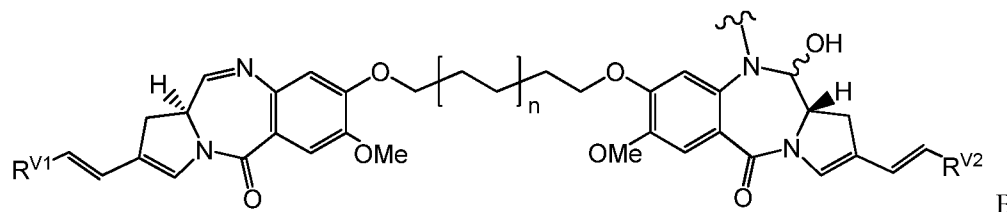
A(V);

wherein Ar<sup>1</sup> and Ar<sup>2</sup> are each independently optionally substituted C<sub>5-20</sub> aryl; wherein Ar<sup>1</sup> and Ar<sup>2</sup> may be the same or different; and

wherein n is 0 or 1.

[0321] In some embodiments, Ar<sup>1</sup> and Ar<sup>2</sup> are each independently selected from optionally substituted phenyl, furanyl, thiophenyl and pyridyl. In some embodiments, Ar<sup>1</sup> and Ar<sup>2</sup> are each independently optionally substituted phenyl. In some embodiments, Ar<sup>1</sup> and Ar<sup>2</sup> are each independently optionally substituted thien-2-yl or thien-3-yl. In some embodiments, Ar<sup>1</sup> and Ar<sup>2</sup> are each independently optionally substituted quinolinyl or isoquinolinyl. The quinolinyl or isoquinolinyl group may be bound to the PBD core through any available ring position. For example, the quinolinyl may be quinolin-2-yl, quinolin-3-yl, quinolin-4yl, quinolin-5-yl, quinolin-6-yl, quinolin-7-yl and quinolin-8-yl. In some embodiments, the quinolinyl is selected from quinolin-3-yl and quinolin-6-yl. The isoquinolinyl may be isoquinolin-1-yl, isoquinolin-3-yl, isoquinolin-4yl, isoquinolin-5-yl, isoquinolin-6-yl, isoquinolin-7-yl and isoquinolin-8-yl. In some embodiments, the isoquinolinyl is selected from isoquinolin-3-yl and isoquinolin-6-yl.

[0322] Further nonlimiting exemplary PBD dimer components of ADCs are of Formula B:



and salts and solvates thereof, wherein:

the wavy line indicates the covalent attachment site to the linker;

the wavy line connected to the OH indicates the S or R configuration;

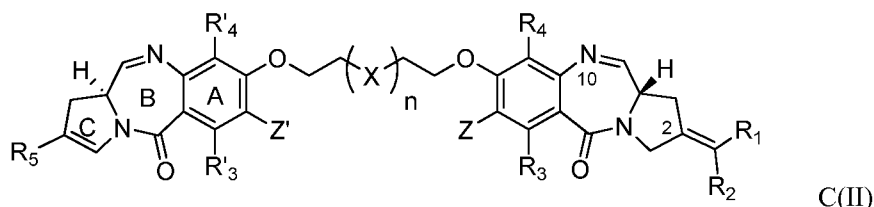
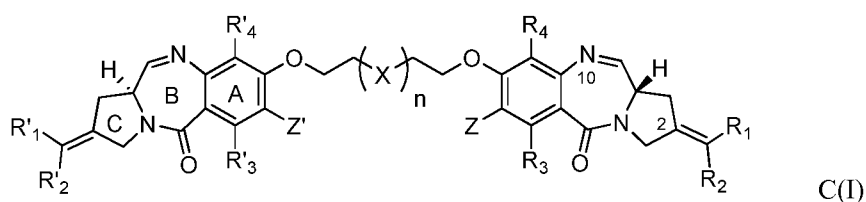
R<sup>V1</sup> and R<sup>V2</sup> are independently selected from H, methyl, ethyl and phenyl (which phenyl may be optionally substituted with fluoro, particularly in the 4 position) and C<sub>5-6</sub> heterocyclyl; wherein R<sup>V1</sup> and R<sup>V2</sup> may be the same or different; and

n is 0 or 1.

[0323] In some embodiments, R<sup>V1</sup> and R<sup>V2</sup> are independently selected from H, phenyl, and 4-fluorophenyl.

[0324] In some embodiments, a linker may be attached at one of various sites of the PBD dimer drug moiety, including the N10 imine of the B ring, the C-2 endo/exo position of the C ring, or the tether unit linking the A rings (*see* structures C(I) and C(II) below).

[0325] Nonlimiting exemplary PBD dimer components of ADCs include Formulas C(I) and C(II):



[0326] Formulas C(I) and C(II) are shown in their N10-C11 imine form. Exemplary PBD drug moieties also include the carbinolamine and protected carbinolamine forms as well, as shown in the table below:

|       |               |                         |
|-------|---------------|-------------------------|
|       |               |                         |
| Imine | Carbinolamine | Protected Carbinolamine |

wherein:

X is CH<sub>2</sub> (n = 1 to 5), N, or O;

Z and Z' are independently selected from OR and NR<sub>2</sub>, where R is a primary, secondary or tertiary alkyl chain containing 1 to 5 carbon atoms;

R<sub>1</sub>, R'<sub>1</sub>, R<sub>2</sub> and R'<sub>2</sub> are each independently selected from H, C<sub>1</sub>-C<sub>8</sub> alkyl, C<sub>2</sub>-C<sub>8</sub> alkenyl, C<sub>2</sub>-C<sub>8</sub> alkynyl, C<sub>5-20</sub> aryl (including substituted aryls), C<sub>5-20</sub> heteroaryl groups, -NH<sub>2</sub>, -NHMe, -OH, and -SH, where, in some embodiments, alkyl, alkenyl and alkynyl chains comprise up to 5 carbon atoms;

R<sub>3</sub> and R'<sub>3</sub> are independently selected from H, OR, NHR, and NR<sub>2</sub>, where R is a primary, secondary or tertiary alkyl chain containing 1 to 5 carbon atoms;

R<sub>4</sub> and R'<sub>4</sub> are independently selected from H, Me, and OMe;

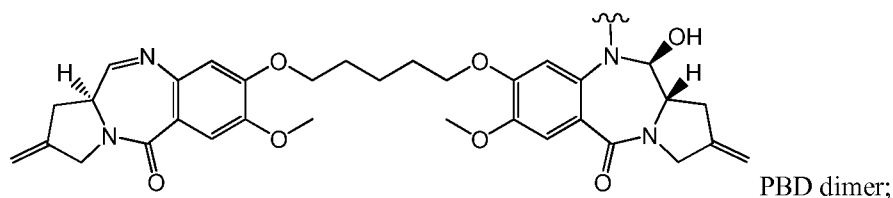
R<sub>5</sub> is selected from C<sub>1</sub>-C<sub>8</sub> alkyl, C<sub>2</sub>-C<sub>8</sub> alkenyl, C<sub>2</sub>-C<sub>8</sub> alkynyl, C<sub>5-20</sub> aryl (including aryls substituted by halo, nitro, cyano, alkoxy, alkyl, heterocyclyl) and C<sub>5-20</sub> heteroaryl groups, where, in some embodiments, alkyl, alkenyl and alkynyl chains comprise up to 5 carbon atoms;

R<sub>11</sub> is H, C<sub>1</sub>-C<sub>8</sub> alkyl, or a protecting group (such as acetyl, trifluoroacetyl, t-butoxycarbonyl (BOC), benzyloxycarbonyl (CBZ), 9-fluorenylmethylenoxycarbonyl (Fmoc), or a moiety comprising a self-immolating unit such as valine-citrulline-PAB);

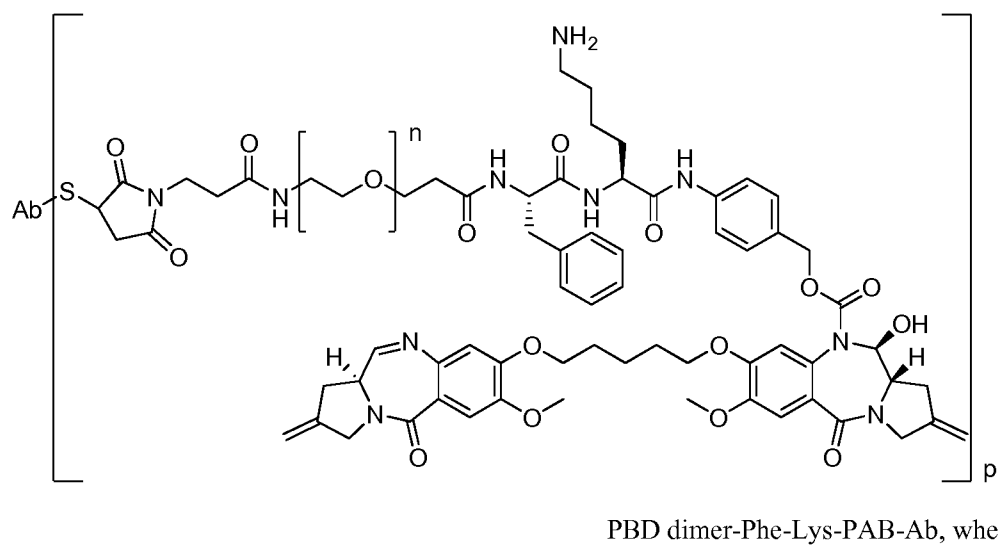
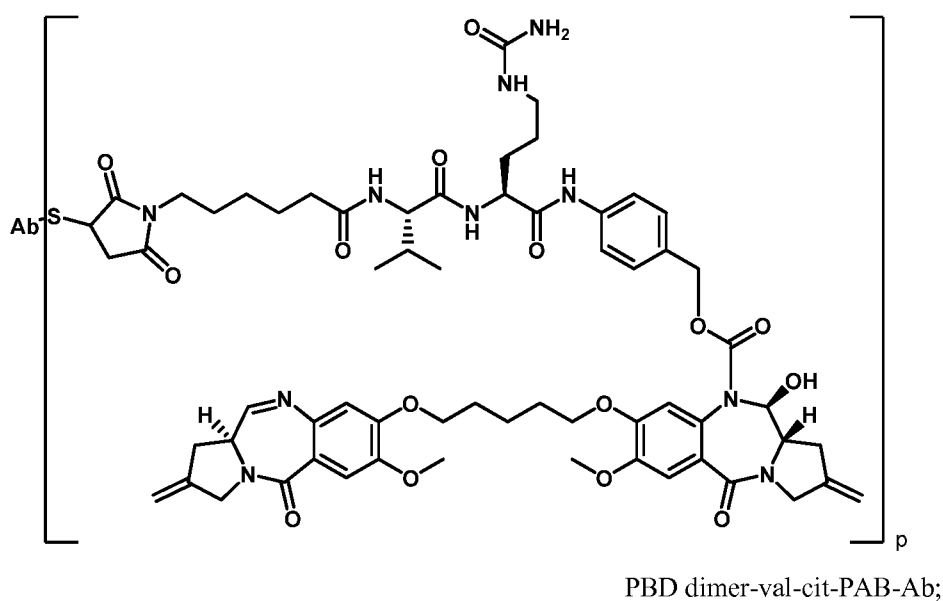
R<sub>12</sub> is H, C<sub>1</sub>-C<sub>8</sub> alkyl, or a protecting group;

wherein a hydrogen of one of R<sub>1</sub>, R'<sub>1</sub>, R<sub>2</sub>, R'<sub>2</sub>, R<sub>5</sub>, or R<sub>12</sub> or a hydrogen of the -OCH<sub>2</sub>CH<sub>2</sub>(X)<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>O- spacer between the A rings is replaced with a bond connected to the linker of the ADC.

[0327] Exemplary PBD dimer portions of ADC include, but are not limited to (the wavy line indicates the site of covalent attachment to the linker):



[0328] Nonlimiting exemplary embodiments of ADCs comprising PBD dimers have the following structures:



n is 0 to 12. In some embodiments, n is 2 to 10. In some embodiments, n is 4 to 8. In some embodiments, n is selected from 4, 5, 6, 7, and 8.



[0329] The linkers of PBD dimer-val-cit-PAB-Ab and the PBD dimer-Phe-Lys-PAB-Ab are protease cleavable, while the linker of PBD dimer-maleimide-acetal is acid-labile.

[0330] PBD dimers and ADC comprising PBD dimers may be prepared according to methods known in the art. *See, e.g.*, WO 2009/016516; US 2009/304710; US 2010/047257; US 2009/036431; US 2011/0256157; WO 2011/130598.

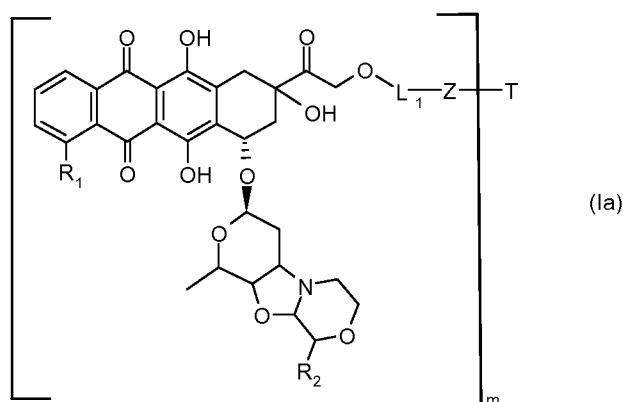
#### (5) Anthracyclines

[0331] In some embodiments, an ADC comprising anthracycline. Anthracyclines are antibiotic compounds that exhibit cytotoxic activity. While not intending to be bound by any particular theory, studies have indicated that anthracyclines may operate to kill cells by a number of different mechanisms, including: 1) intercalation of the drug molecules into the DNA of the cell thereby inhibiting DNA-dependent nucleic acid synthesis; 2) production by the drug of free radicals which then react with cellular macromolecules to cause damage to the cells, and/or 3) interactions of the drug molecules with the cell membrane (*see, e.g.*, C. Peterson et al., "Transport And Storage Of Anthracycline In Experimental Systems And Human Leukemia" in Anthracycline Antibiotics In Cancer Therapy; N.R. Bachur, "Free Radical Damage" *id.* at pp.97-102). Because of their cytotoxic potential anthracyclines have been used in the treatment of numerous cancers such as leukemia, breast carcinoma, lung carcinoma, ovarian adenocarcinoma and sarcomas (*see e.g.*, P.H- Wiernik, in Anthracycline: Current Status And New Developments p 11).

[0332] Nonlimiting exemplary anthracyclines include doxorubicin, epirubicin, idarubicin, daunomycin, nemorubicin, and derivatives thereof. Immunoconjugates and prodrugs of daunorubicin and doxorubicin have been prepared and studied (Kratz et al (2006) *Current Med. Chem.* 13:477-523; Jeffrey et al (2006) *Bioorganic & Med. Chem. Letters* 16:358-362; Torgov et al (2005) *Bioconj. Chem.* 16:717-721; Nagy et al (2000) *Proc. Natl. Acad. Sci. USA* 97:829-834; Dubowchik et al (2002) *Bioorg. & Med. Chem. Letters* 12:1529-1532; King et al (2002) *J. Med. Chem.* 45:4336-4343; EP 0328147; US 6630579). The antibody-drug conjugate BR96-doxorubicin reacts specifically with the tumor-associated antigen Lewis-Y and has been evaluated in phase I and II studies (Saleh et al (2000) *J. Clin. Oncology* 18:2282-2292; Ajani et al (2000) *Cancer Jour.* 6:78-81; Tolcher et al (1999) *J. Clin. Oncology* 17:478-484).

[0333] PNU-159682 is a potent metabolite (or derivative) of nemorubicin (Quintieri, et al. (2005) *Clinical Cancer Research* 11(4):1608-1617). Nemorubicin is a semisynthetic analog of doxorubicin with a 2-methoxymorpholino group on the glycoside amino of doxorubicin and has been under clinical evaluation (Grandi et al (1990) *Cancer Treat. Rev.* 17:133; Ripamonti et al (1992) *Brit. J. Cancer* 65:703; ), including phase II/III trials for hepatocellular carcinoma (Sun et al (2003) *Proceedings of the American Society for Clinical Oncology* 22, Abs1448; Quintieri (2003) *Proceedings of the American Association of Cancer Research*, 44:1st Ed, Abs 4649; Pacciarini et al (2006) *Jour. Clin. Oncology* 24:14116).

[0334] A nonlimiting exemplary ADC comprising nemorubicin or nemorubicin derivatives is shown in Formula Ia:



wherein  $R_1$  is hydrogen atom, hydroxy or methoxy group and  $R_2$  is a  $C_1$ - $C_5$  alkoxy group, or a pharmaceutically acceptable salt thereof;

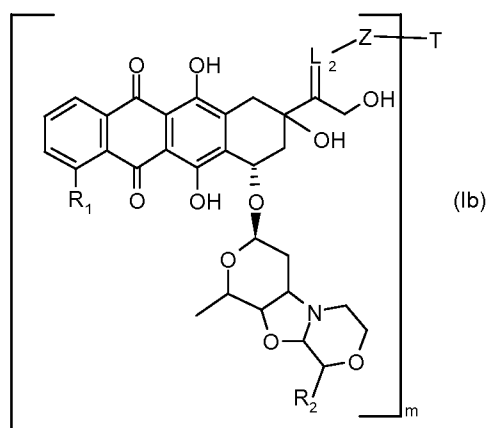
$L_1$  and  $Z$  together are a linker ( $L$ ) as described herein;

$T$  is an antibody ( $Ab$ ) as described herein; and

$m$  is 1 to about 20. In some embodiments,  $m$  is 1 to 10, 1 to 7, 1 to 5, or 1 to 4.

[0335] In some embodiments,  $R_1$  and  $R_2$  are both methoxy ( $-OMe$ ).

[0336] A further nonlimiting exemplary ADC comprising nemorubicin or nemorubicin derivatives is shown in Formula Ib:



wherein  $R_1$  is hydrogen atom, hydroxy or methoxy group and  $R_2$  is a  $C_1$ - $C_5$  alkoxy group, or a pharmaceutically acceptable salt thereof;

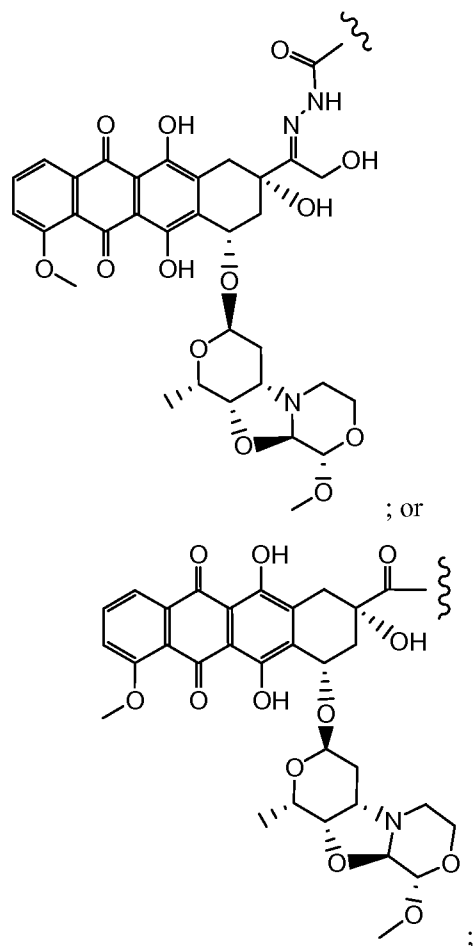
$L_2$  and  $Z$  together are a linker ( $L$ ) as described herein;

$T$  is an antibody ( $Ab$ ) as described herein; and

$m$  is 1 to about 20. In some embodiments,  $m$  is 1 to 10, 1 to 7, 1 to 5, or 1 to 4.

[0337] In some embodiments,  $R_1$  and  $R_2$  are both methoxy ( $-OMe$ ).

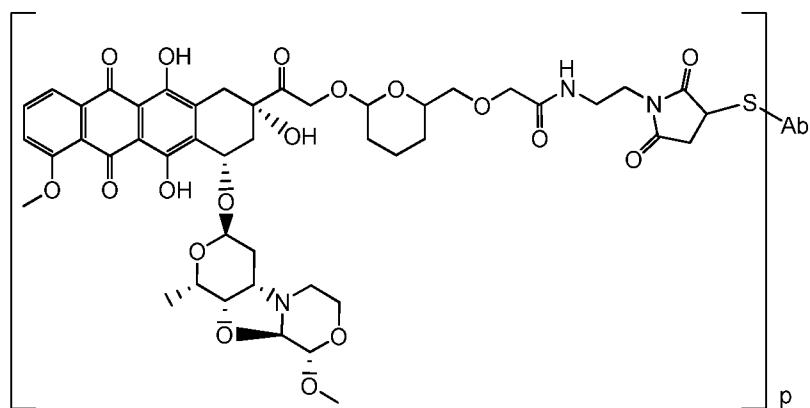
[0338] In some embodiments, the nemorubicin component of a nemorubicin-containing ADC is PNU-159682. In some such embodiments, the drug portion of the ADC may have one of the following structures:



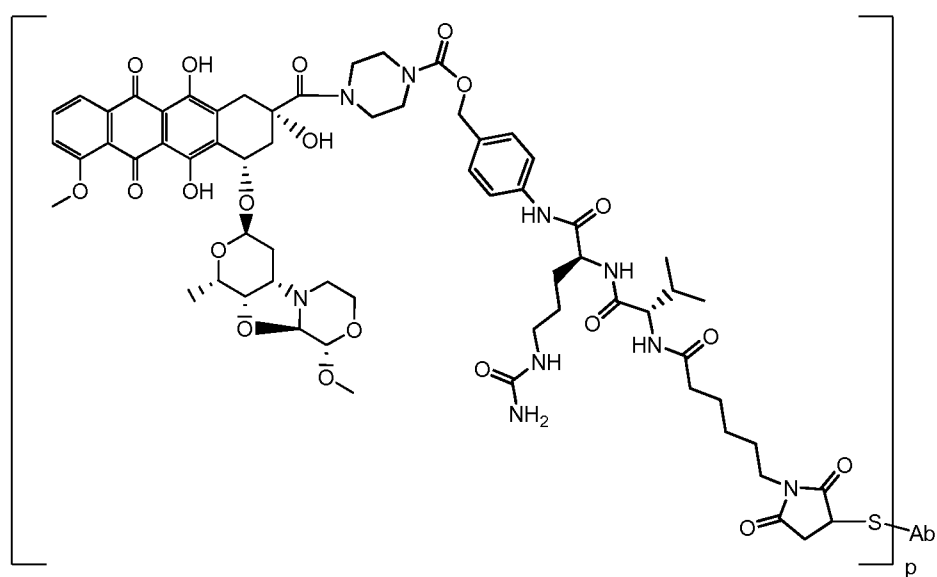
wherein the wavy line indicates the attachment to the linker (L).

[0339] Anthracyclines, including PNU-159682, may be conjugated to antibodies through several linkage sites and a variety of linkers (US 2011/0076287; WO2009/099741; US 2010/0034837; WO 2010/009124), including the linkers described herein.

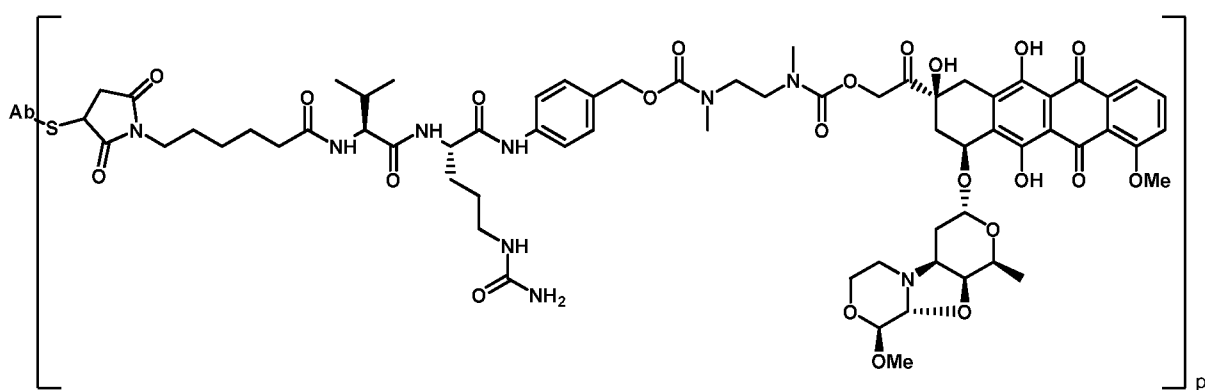
[0340] Exemplary ADCs comprising a nemorubicin and linker include, but are not limited to:



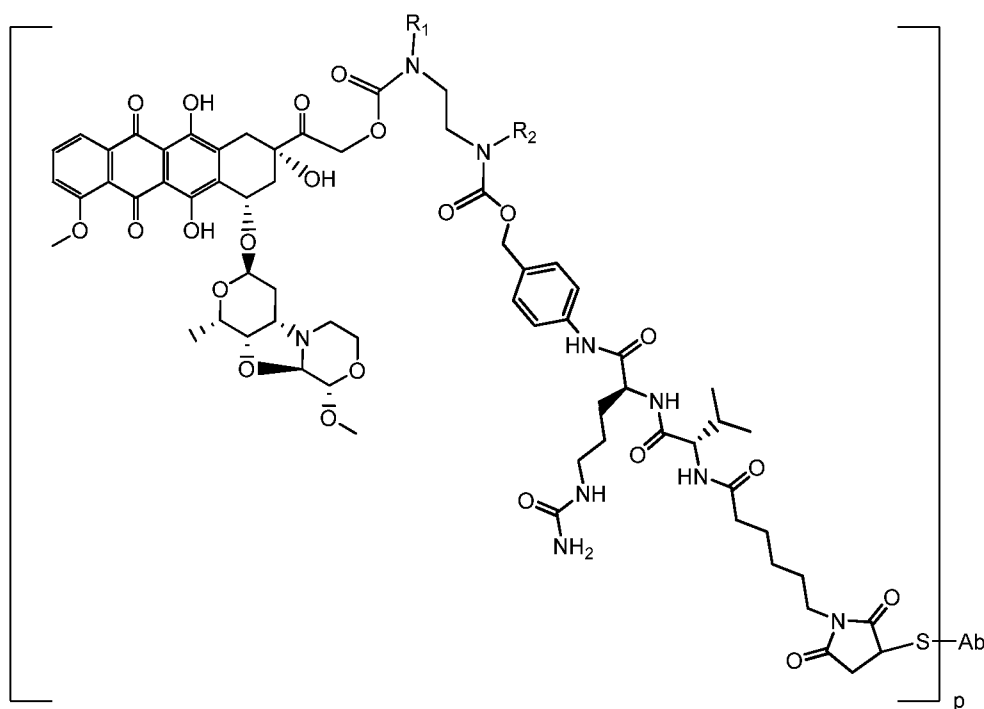
PNU-159682 maleimide acetal-Ab;



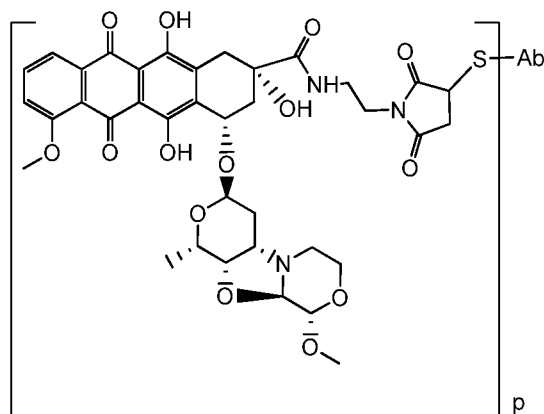
PNU-159682-val-cit-PAB-Ab;



PNU-159682-val-cit-PAB-spacer-Ab;

PNU-159682-val-cit-PAB-spacer(R<sup>1</sup>R<sup>2</sup>)-Ab, wherein:

R<sub>1</sub> and R<sub>2</sub> are independently selected from H and C<sub>1</sub>-C<sub>6</sub> alkyl; and



PNU-159682-maleimide-Ab.

[0341] The linker of PNU-159682 maleimide acetal-Ab is acid-labile, while the linkers of PNU-159682-val-cit-PAB-Ab, PNU-159682-val-cit-PAB-spacer-Ab, and PNU-159682-val-cit-PAB-spacer(R<sup>1</sup>R<sup>2</sup>)-Ab are protease cleavable.

#### (6) Other Drug Moieties

[0342] Drug moieties also include geldanamycin (Mandler et al (2000) *J. Nat. Cancer Inst.* 92(19):1573-1581; Mandler et al (2000) *Bioorganic & Med. Chem. Letters* 10:1025-1028; Mandler et al (2002) *Bioconjugate Chem.* 13:786-791); and enzymatically active toxins and fragments thereof, including, but not limited to, diphtheria A chain, nonbinding active fragments of diphtheria toxin,

exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. *See, e.g.*, WO 93/21232.

[0343] Drug moieties also include compounds with nucleolytic activity (*e.g.*, a ribonuclease or a DNA endonuclease).

[0344] In certain embodiments, an immunoconjugate may comprise a highly radioactive atom. A variety of radioactive isotopes are available for the production of radioconjugated antibodies.

Examples include At<sup>211</sup>, I<sup>131</sup>, I<sup>125</sup>, Y<sup>90</sup>, Re<sup>186</sup>, Re<sup>188</sup>, Sm<sup>153</sup>, Bi<sup>212</sup>, P<sup>32</sup>, Pb<sup>212</sup> and radioactive isotopes of Lu. In some embodiments, when an immunoconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example Tc<sup>99</sup> or I<sup>123</sup>, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, MRI), such as zirconium-89, iodine-123, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron. Zirconium-89 may be complexed to various metal chelating agents and conjugated to antibodies, *e.g.*, for PET imaging (WO 2011/056983).

[0345] The radio- or other labels may be incorporated in the immunoconjugate in known ways. For example, a peptide may be biosynthesized or chemically synthesized using suitable amino acid precursors comprising, for example, one or more fluorine-19 atoms in place of one or more hydrogens. In some embodiments, labels such as Tc<sup>99</sup>, I<sup>123</sup>, Re<sup>186</sup>, Re<sup>188</sup> and In<sup>111</sup> can be attached via a cysteine residue in the antibody. In some embodiments, yttrium-90 can be attached via a lysine residue of the antibody. In some embodiments, the IODOGEN method (Fraker et al (1978) *Biochem. Biophys. Res. Commun.* 80: 49-57 can be used to incorporate iodine-123. "Monoclonal Antibodies in Immunoscintigraphy" (Chatal, CRC Press 1989) describes certain other methods.

[0346] In certain embodiments, an immunoconjugate may comprise an antibody conjugated to a prodrug-activating enzyme. In some such embodiments, a prodrug-activating enzyme converts a prodrug (*e.g.*, a peptidyl chemotherapeutic agent, *see* WO 81/01145) to an active drug, such as an anti-cancer drug. Such immunoconjugates are useful, in some embodiments, in antibody-dependent enzyme-mediated prodrug therapy ("ADEPT"). Enzymes that may be conjugated to an antibody include, but are not limited to, alkaline phosphatases, which are useful for converting phosphate-containing prodrugs into free drugs; arylsulfatases, which are useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase, which is useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), which are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, which are useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as  $\beta$ -galactosidase and neuraminidase, which are useful for converting glycosylated prodrugs into free drugs;  $\beta$ -lactamase, which is useful for converting drugs derivatized with  $\beta$ -lactams

into free drugs; and penicillin amidases, such as penicillin V amidase and penicillin G amidase, which are useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. In some embodiments, enzymes may be covalently bound to antibodies by recombinant DNA techniques well known in the art. *See, e.g.,* Neuberger et al., *Nature* 312:604-608 (1984).

**c) Drug Loading**

[0347] Drug loading is represented by  $p$ , the average number of drug moieties per antibody in a molecule of Formula I. Drug loading may range from 1 to 20 drug moieties ( $D$ ) per antibody. ADCs of Formula I include collections of antibodies conjugated with a range of drug moieties, from 1 to 20. The average number of drug moieties per antibody in preparations of ADC from conjugation reactions may be characterized by conventional means such as mass spectroscopy, ELISA assay, and HPLC. The quantitative distribution of ADC in terms of  $p$  may also be determined. In some instances, separation, purification, and characterization of homogeneous ADC where  $p$  is a certain value from ADC with other drug loadings may be achieved by means such as reverse phase HPLC or electrophoresis.

[0348] For some antibody-drug conjugates,  $p$  may be limited by the number of attachment sites on the antibody. For example, where the attachment is a cysteine thiol, as in certain exemplary embodiments above, an antibody may have only one or several cysteine thiol groups, or may have only one or several sufficiently reactive thiol groups through which a linker may be attached. In certain embodiments, higher drug loading, *e.g.*  $p > 5$ , may cause aggregation, insolubility, toxicity, or loss of cellular permeability of certain antibody-drug conjugates. In certain embodiments, the average drug loading for an ADC ranges from 1 to about 8; from about 2 to about 6; or from about 3 to about 5. Indeed, it has been shown that for certain ADCs, the optimal ratio of drug moieties per antibody may be less than 8, and may be about 2 to about 5 (US 7498298).

[0349] In certain embodiments, fewer than the theoretical maximum of drug moieties are conjugated to an antibody during a conjugation reaction. An antibody may contain, for example, lysine residues that do not react with the drug-linker intermediate or linker reagent, as discussed below. Generally, antibodies do not contain many free and reactive cysteine thiol groups which may be linked to a drug moiety; indeed most cysteine thiol residues in antibodies exist as disulfide bridges. In certain embodiments, an antibody may be reduced with a reducing agent such as dithiothreitol (DTT) or tricarboylethylphosphine (TCEP), under partial or total reducing conditions, to generate reactive cysteine thiol groups. In certain embodiments, an antibody is subjected to denaturing conditions to reveal reactive nucleophilic groups such as lysine or cysteine.

[0350] The loading (drug/antibody ratio) of an ADC may be controlled in different ways, and for example, by: (i) limiting the molar excess of drug-linker intermediate or linker reagent relative to antibody, (ii) limiting the conjugation reaction time or temperature, and (iii) partial or limiting reductive conditions for cysteine thiol modification.

[0351] It is to be understood that where more than one nucleophilic group reacts with a drug-linker intermediate or linker reagent, then the resulting product is a mixture of ADC compounds with a distribution of one or more drug moieties attached to an antibody. The average number of drugs per antibody may be calculated from the mixture by a dual ELISA antibody assay, which is specific for antibody and specific for the drug. Individual ADC molecules may be identified in the mixture by mass spectroscopy and separated by HPLC, *e.g.* hydrophobic interaction chromatography (*see, e.g.*, McDonagh et al (2006) *Prot. Engr. Design & Selection* 19(7):299-307; Hamblett et al (2004) *Clin. Cancer Res.* 10:7063-7070; Hamblett, K.J., et al. "Effect of drug loading on the pharmacology, pharmacokinetics, and toxicity of an anti-CD30 antibody-drug conjugate," Abstract No. 624, American Association for Cancer Research, 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004; Alley, S.C., et al. "Controlling the location of drug attachment in antibody-drug conjugates," Abstract No. 627, American Association for Cancer Research, 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004). In certain embodiments, a homogeneous ADC with a single loading value may be isolated from the conjugation mixture by electrophoresis or chromatography.

**d) Certain Methods of Preparing Immunoconjugates**

[0352] An ADC of Formula I may be prepared by several routes employing organic chemistry reactions, conditions, and reagents known to those skilled in the art, including: (1) reaction of a nucleophilic group of an antibody with a bivalent linker reagent to form Ab-L via a covalent bond, followed by reaction with a drug moiety D; and (2) reaction of a nucleophilic group of a drug moiety with a bivalent linker reagent, to form D-L, via a covalent bond, followed by reaction with a nucleophilic group of an antibody. Exemplary methods for preparing an ADC of Formula I via the latter route are described in US 7498298, which is expressly incorporated herein by reference.

[0353] Nucleophilic groups on antibodies include, but are not limited to: (i) N-terminal amine groups, (ii) side chain amine groups, *e.g.* lysine, (iii) side chain thiol groups, *e.g.* cysteine, and (iv) sugar hydroxyl or amino groups where the antibody is glycosylated. Amine, thiol, and hydroxyl groups are nucleophilic and capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; and (iii) aldehydes, ketones, carboxyl, and maleimide groups. Certain antibodies have reducible interchain disulfides, *i.e.* cysteine bridges. Antibodies may be made reactive for conjugation with linker reagents by treatment with a reducing agent such as DTT (dithiothreitol) or tricarboylethylphosphine (TCEP), such that the antibody is fully or partially reduced. Each cysteine bridge will thus form, theoretically, two reactive thiol nucleophiles. Additional nucleophilic groups can be introduced into antibodies through modification of lysine residues, *e.g.*, by reacting lysine residues with 2-iminothiolane (Traut's reagent), resulting in conversion of an amine into a thiol. Reactive thiol groups may also be



introduced into an antibody by introducing one, two, three, four, or more cysteine residues (*e.g.*, by preparing variant antibodies comprising one or more non-native cysteine amino acid residues).

[0354] Antibody-drug conjugates may also be produced by reaction between an electrophilic group on an antibody, such as an aldehyde or ketone carbonyl group, with a nucleophilic group on a linker reagent or drug. Useful nucleophilic groups on a linker reagent include, but are not limited to, hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide. In one embodiment, an antibody is modified to introduce electrophilic moieties that are capable of reacting with nucleophilic substituents on the linker reagent or drug. In another embodiment, the sugars of glycosylated antibodies may be oxidized, *e.g.* with periodate oxidizing reagents, to form aldehyde or ketone groups which may react with the amine group of linker reagents or drug moieties. The resulting imine Schiff base groups may form a stable linkage, or may be reduced, *e.g.* by borohydride reagents to form stable amine linkages. In one embodiment, reaction of the carbohydrate portion of a glycosylated antibody with either galactose oxidase or sodium meta-periodate may yield carbonyl (aldehyde and ketone) groups in the antibody that can react with appropriate groups on the drug (Hermanson, *Bioconjugate Techniques*). In another embodiment, antibodies containing N-terminal serine or threonine residues can react with sodium meta-periodate, resulting in production of an aldehyde in place of the first amino acid (Geoghegan & Stroh, (1992) *Bioconjugate Chem.* 3:138-146; US 5362852). Such an aldehyde can be reacted with a drug moiety or linker nucleophile.

[0355] Exemplary nucleophilic groups on a drug moiety include, but are not limited to: amine, thiol, hydroxyl, hydrazide, oxime, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide groups capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; (iii) aldehydes, ketones, carboxyl, and maleimide groups.

[0356] Nonlimiting exemplary cross-linker reagents that may be used to prepare ADC are described herein in the section titled "Exemplary Linkers." Methods of using such cross-linker reagents to link two moieties, including a proteinaceous moiety and a chemical moiety, are known in the art. In some embodiments, a fusion protein comprising an antibody and a cytotoxic agent may be made, *e.g.*, by recombinant techniques or peptide synthesis. A recombinant DNA molecule may comprise regions encoding the antibody and cytotoxic portions of the conjugate either adjacent to one another or separated by a region encoding a linker peptide which does not destroy the desired properties of the conjugate.

[0357] In yet another embodiment, an antibody may be conjugated to a "receptor" (such as streptavidin) for utilization in tumor pre-targeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (*e.g.*, avidin) which is conjugated to a cytotoxic agent (*e.g.*, a drug or radionucleotide).

## F. Methods and Compositions for Diagnostics and Detection

[0358] In certain embodiments, any of the anti-LGR5 antibodies provided herein is useful in any of the methods described herein for detecting the presence of LGR5 in a biological sample. The term “detecting” as used herein encompasses quantitative or qualitative detection. A “biological sample” comprises, *e.g.*, a cell or tissue (*e.g.*, biopsy material, including cancerous or potentially cancerous basal cell carcinoma).

[0359] In one embodiment, an anti-LGR5 antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of LGR5 in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with an anti-LGR5 antibody as described herein under conditions permissive for binding of the anti-LGR5 antibody to LGR5, and detecting whether a complex is formed between the anti-LGR5 antibody and LGR5 in the biological sample. Such method may be an *in vitro* or *in vivo* method. In one embodiment, an anti-LGR5 antibody is used to select subjects eligible for therapy with an anti-LGR5 antibody, *e.g.* where LGR5 is a biomarker for selection of patients. In a further embodiment, the biological sample is a cell or tissue (*e.g.*, biopsy material, including cancerous or potentially cancerous basal cell carcinoma tissue).

[0360] In a further embodiment, an anti-LGR5 antibody is used *in vivo* to detect, *e.g.*, by *in vivo* imaging, an LGR5-positive cancer in a subject, *e.g.*, for the purposes of diagnosing, prognosing, or staging cancer, determining the appropriate course of therapy, or monitoring response of a cancer to therapy. One method known in the art for *in vivo* detection is immuno-positron emission tomography (immuno-PET), as described, *e.g.*, in van Dongen et al., *The Oncologist* 12:1379-1389 (2007) and Verel et al., *J. Nucl. Med.* 44:1271-1281 (2003). In such embodiments, a method is provided for detecting an LGR5-positive cancer in a subject, the method comprising administering a labeled anti-LGR5 antibody to a subject having or suspected of having an LGR5-positive cancer, and detecting the labeled anti-LGR5 antibody in the subject, wherein detection of the labeled anti-LGR5 antibody indicates an LGR5-positive cancer in the subject. In certain of such embodiments, the labeled anti-LGR5 antibody comprises an anti-LGR5 antibody conjugated to a positron emitter, such as  $^{68}\text{Ga}$ ,  $^{18}\text{F}$ ,  $^{64}\text{Cu}$ ,  $^{86}\text{Y}$ ,  $^{76}\text{Br}$ ,  $^{89}\text{Zr}$ , and  $^{124}\text{I}$ . In a particular embodiment, the positron emitter is  $^{89}\text{Zr}$ .

[0361] In further embodiments, a method of diagnosis or detection comprises contacting a first anti-LGR5 antibody immobilized to a substrate with a biological sample to be tested for the presence of LGR5, exposing the substrate to a second anti-LGR5 antibody, and detecting whether the second anti-LGR5 is bound to a complex between the first anti-LGR5 antibody and LGR5 in the biological sample. A substrate may be any supportive medium, *e.g.*, glass, metal, ceramic, polymeric beads, slides, chips, and other substrates. In certain embodiments, a biological sample comprises a cell or tissue (*e.g.*, biopsy material, including cancerous or potentially cancerous basal cell carcinoma tissue). In certain embodiments, the first or second anti-LGR5 antibody is any of the antibodies described herein. In some such embodiments, the second anti-LGR5 antibody may be 8E11 or antibodies

derived from 8E11, *e.g.*, as described herein. In some such embodiments, the second anti-LGR5 antibody may be YW353 or antibodies derived from YW353, *e.g.*, as described herein. In some embodiments, the first or second anti-LGR5 antibody is selected from 3G12 and 2H6 and antibodies derived from 3G12 and/or 2H6, *e.g.*, as described herein.

[0362] Exemplary disorders that may be diagnosed or detected according to any of the above embodiments include LGR5-positive cancers. In some embodiments, an LGR5-positive cancer is a cancer that receives an anti-LGR5 immunohistochemistry (IHC) or in situ hybridization (ISH) score greater than “0,” which corresponds to very weak or no staining in >90% of tumor cells, under the conditions described herein in Example B. In another embodiment, an LGR5-positive cancer expresses LGR5 at a 1+, 2+ or 3+ level, as defined under the conditions described herein in Example B. In some embodiments, an LGR5-positive cancer is a cancer that expresses LGR5 according to a reverse-transcriptase PCR (RT-PCR) assay that detects LGR5 mRNA. In some embodiments, the RT-PCR is quantitative RT-PCR.

[0363] In certain embodiments, labeled anti-LGR5 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  $^{32}\text{P}$ ,  $^{14}\text{C}$ ,  $^{125}\text{I}$ ,  $^3\text{H}$ , and  $^{131}\text{I}$ , fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luciferases, *e.g.*, firefly luciferase and bacterial luciferase (U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase,  $\beta$ -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. In another embodiment, a label is a positron emitter. Positron emitters include but are not limited to  $^{68}\text{Ga}$ ,  $^{18}\text{F}$ ,  $^{64}\text{Cu}$ ,  $^{86}\text{Y}$ ,  $^{76}\text{Br}$ ,  $^{89}\text{Zr}$ , and  $^{124}\text{I}$ . In a particular embodiment, a positron emitter is  $^{89}\text{Zr}$ .

#### **G. Pharmaceutical Formulations**

[0364] Pharmaceutical formulations of an anti-LGR5 antibody including immunoconjugates as described herein are prepared by mixing such antibody including immunoconjugate 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 dextrans; 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 interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX<sup>®</sup>, 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.

[0365] Exemplary lyophilized antibody including immunoconjugate formulations are described in US Patent No. 6,267,958. Aqueous antibody including immunoconjugate formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

[0366] The formulation herein may also contain more than one active ingredient as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other.

[0367] 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-(methylmethacrylate) 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).

[0368] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody including immunoconjugate, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules.

[0369] 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.

## **H. Articles of Manufacture**

[0370] In another aspect, 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 disorder 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 including immunoconjugate. 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 including immunoconjugate; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. In some embodiments, the second container with a composition contained therein, wherein the composition comprises an inhibitor of the hedgehog pathway. In some embodiments, the inhibitor of the hedgehog pathway is an antagonist of smoothened. In some embodiments, the inhibitor of the hedgehog pathway is a cyclopamine-competitive antagonist of smoothened. In some embodiments, the antagonist of smoothened is 2-chloro-N-[4-chloro-3-(pyridin-2-yl)phenyl]-4-(methylsulfonyl)benzamide or a salt thereof. In some embodiments, the antagonist of smoothened is 2-chloro-N-[4-chloro-3-(pyridin-2-yl)phenyl]-4-(methylsulfonyl)benzamide. In some embodiments, the antagonist of smoothened is vismodegib.

[0371] The article of manufacture in this embodiment 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 or dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

### III. EXAMPLES

[0372] 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*

##### *LGR5<sup>DTR<sup>EGFP</sup></sup> Construction*

[0373] For *LGR5*, a 7,213 bp fragment (assembly NCBI37/mm9, chr10:115,020,315-115,027,527) from a C57BL/6 BAC (RP23 library) was cloned into plasmid pBlight-TK25. To generate the DTR-EGFP KI vector for *LGR5*, a DTR-EGFP-pA-loxP-Neo-loxP cassette was synthesized (Blue Heron/Origene, DTR-EGFP sequence was based on that described previously<sup>27</sup>, and inserted right after the ATG of *LGR5* (chr10:115,024,547, reverse strand), deleting the remainder of exon 1 and splice donor of intron 1 (a 212 bp deletion). To generate the CreERT2 KI vector, a dsRed2-IRES-

CreERT2-pA-Frt-neo-Frt cassette was synthesized (Blue Heron/Origene) and inserted at the same position as the DTR-EGFP cassette. The final vectors were confirmed by DNA sequencing.

[0374] The *LGR5* KI vectors were linearized with NotI and C57BL/6 C2 embryonic stem cells were targeted using standard methods (G418-positive and gancyclovir-negative selection). Positive clones were identified using PCR and taqman analysis, and confirmed by sequencing of the modified locus. Correctly targeted embryonic stem cells were transfected with a Cre or Flpe plasmid, respectively, to remove the Neo cassette. The modified embryonic stem cells were then injected into blastocysts using standard techniques, and germline transmission was obtained after crossing the resulting chimaeras with C57BL/6N females.

#### *DT cell ablation in Superficial Basal Cell Carcinoma (BCC)*

[0375]  $K14^{CreER/+};p53^{loxP/loxP};Ptch^{loxP/loxP};LGR5^{DTReGFP/+}$  mice were generated by breeding  $p53^{loxP/loxP};Ptch^{loxP/loxP};LGR5^{DTReGFP/+}$  mice with  $p53^{loxP/loxP};Ptch^{loxP/+};K14^{CreER/+}$ . Animals showing telltale signs of Superficial BCC disease such as loss of fur, scruffy coat, thickening of the ears or tail skin were put on study. Animals were typically placed on study at 7 weeks of age.

$K14^{CreER/+};p53^{loxP/loxP};Ptch^{loxP/loxP};LGR5^{DTReGFP/+}$  mice were treated with the regimes as follows. To test the effects of DT treatments on residual superficial BCC, mice were treated Vismodegib (GDC-0449) po bid at 75 mg/kg for 38 days with concomitant with Diphtheria Toxin (DT) at 50 ug/kg every other day for the final 8 days starting at day 29. Control mice were treated with Vismodegib (GDC-0449) po bid at 75 mg/kg for 38 days concomitant with 200 uL IP saline for the final 8 days of treatment. In addition, animals showing signs of disease (listed above) were treated with saline or DT alone at 50ug/kg every other day for 5 days to test the effect of single DT treatments.

#### *Histology and Immunofluorescent Staining*

[0376] Harvested skin from treated animals was processed for Hematoxylin and Eosin (H&E) staining using established protocols. Alternatively, backskin from treated animals was fixed overnight in 4% paraformaldehyde/PBS solution, washed, incubated in 30% sucrose/PBS solution overnight, and embedded in frozen OCT. Sections were then cut at 8 um from the frozen blocks and stained for the apoptosis marker cleaved caspase 3 (1:1000), GFP (1:1000), or Keratin 5 (1:1000). Sections were counterstained with DAPI.

#### *Results*

[0377] Strong GFP expression was observed in superficial BCC tumors in animals treated with saline alone. This result is consistent with observations from in situ hybridization experiments revealing that *LGR5* is expressed in superficial BCC. In these control mice, apoptotic cells marked by CC3 staining were scarce and isolated to hair follicles. In mice treated with DT for a total of 5 days, a significant CC3 staining in tumors was observed as well as in hair follicles. No significant apoptosis was observed in basal cells of the epidermis suggesting that DT treatments specifically targeted tumors and hair follicles.

[0378] To determine whether DT treatments could target residual BCC, mice were treated for 28 days with Vismodegib and then co-treated with Vismodegib and DT for an additional 8 days. In at least one animal, residual disease was scarce as revealed by Keratin 5 staining. Minimal CC3 staining suggested that residual disease had been largely eliminated in this animal. In other mice, we observed targeted apoptosis occurring in residual tumors not originally cleared from the skin by the Smoothed antagonist Vismodegib.

[0379] H&E staining was performed on a variety of tissues from treated and control mice. A marked decrease was observed in the thickening of ear tissue treated with Vismodegib plus DT compared to treatment with Vismodegib alone suggesting that additional ablation of *LGR5* positive cells with DT treatments was beneficial in removing residual disease.

[0380] 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.

**Table of Sequences**

| SEQ ID NO | Description                                    | Sequence  |
|-----------|--|---|
| 5         | hu8E11.v1<br>light chain<br>variable<br>region | DIVMTQSPDS LAVSLGERAT INCRASESVD NYGNSFMHWY<br>QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS<br>SLQAEDVAVY YCQQNYEDPF TFGQGTKVEI KR       |
| 6         | hu8E11.v1<br>heavy chain<br>variable<br>region | EVQLVQSGAE VKKPGASVKV SCKASGYTFS AYWIEWVRQA<br>PGQGLEWIGE ILPGSDSTDY NEKFKVRVTI TSDTSTSTVY<br>LELSSLRSED TAVYYCARGG HYGSLDYWGQ GTLVTVSS |
| 7         | hu8E11.v2<br>light chain<br>variable<br>region | DIVMTQSPDS LAVSLGERAT INCRASESVD NYGNSFMHWY<br>QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS<br>SLQAEDVAVY YCQQNYEDPF TFGQGTKVEI KR       |
| 8         | hu8E11.v2<br>heavy chain<br>variable<br>region | EVQLVQSGAE VKKPGASVKV SCKASGYTFS AYWIEWVRQA<br>PGQGLEWIGE ILPGSDSTDY NEKFKVRATF TSDTSTSTVY<br>LELSSLRSED TAVYYCARGG HYGSLDYWGQ GTLVTVSS |
| 9         | hu8E11.v3<br>light chain<br>variable<br>region | DIVMTQSPDS LAVSLGERAT INCRASESVD NYGNSFMHWY<br>QQKPGQPPKL LIYLASNLES GVPDRFSGSG SRTDFTLTIS<br>SLQAEDVAVY YCQQNYEDPF TFGQGTKVEI KR       |
| 10        | hu8E11.v3<br>heavy chain<br>variable<br>region | EVQLVQSGAE VKKPGASVKV SCKASGYTFS AYWIEWVRQA<br>PGQGLEWIGE ILPGSDSTDY NEKFKVRVTI TSDTSTSTVY<br>LELSSLRSED TAVYYCARGG HYGSLDYWGQ GTLVTVSS |
| 11        | hu8E11.v4<br>light chain<br>variable<br>region | DIVMTQSPDS LAVSLGERAT INCRASESVD NYGNSFMHWY<br>QQKPGQPPKL LIYLASNLES GVPDRFSGSG SRTDFTLTIS<br>SLQAEDVAVY YCQQNYEDPF TFGQGTKVEI KR       |
| 12        | hu8E11.v4<br>heavy chain<br>variable<br>region | EVQLVQSGAE VKKPGASVKV SCKASGYTFS AYWIEWVRQA<br>PGQGLEWIGE ILPGSDSTDY NEKFKVRATF TSDTSTSTVY<br>LELSSLRSED TAVYYCARGG HYGSLDYWGQ GTLVTVSS |
| 13        | hu8E11.v5<br>light chain<br>variable<br>region | DIVMTQSPDS LAVSLGERAT INCRASESVD NYGNSFMHWY<br>QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS<br>SLQAEDVAVY YCQQNYEDPF TFGQGTKVEI KR       |
| 14        | hu8E11.v5<br>heavy chain<br>variable<br>region | EVQLVQSGAE VKKPGASVKV SCKASGYTFS AYWIEWVRQA<br>PGQGLEWIGE ILPGSDSTDY NEKFKVRVTI TRDTSTSTAY<br>LELSSLRSED TAVYYCARGG HYGSLDYWGQ GTLVTVSS |
| 15        | hu8E11.v6<br>light chain<br>variable<br>region | DIVMTQSPDS LAVSLGERAT INCRASESVD NYGNSFMHWY<br>QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS<br>SLQAEDVAVY YCQQNYEDPF TFGQGTKVEI KR       |
| 16        | hu8E11.v6<br>heavy chain<br>variable<br>region | EVQLVQSGAE VKKPGASVKV SCKASGYTFS AYWIEWVRQA<br>PGQGLEWIGE ILPGSDSTDY NEKFKVRVTI TADTSTSTAY<br>LELSSLRSED TAVYYCARGG HYGSLDYWGQ GTLVTVSS |
| 17        | hu8E11.v7<br>light chain<br>variable<br>region | DIVMTQSPDS LAVSLGERAT INCRASESVD NYGNSFMHWY<br>QQKPGQPPKL LIYLASNLES GVPDRFSGSG SRTDFTLTIS<br>SLQAEDVAVY YCQQNYEDPF TFGQGTKVEI KR       |
| 18        | hu8E11.v7<br>heavy chain<br>variable<br>region | EVQLVQSGAE VKKPGASVKV SCKASGYTFS AYWIEWVRQA<br>PGQGLEWIGE ILPGSDSTDY NEKFKVRVTI TRDTSTSTAY<br>LELSSLRSED TAVYYCARGG HYGSLDYWGQ GTLVTVSS |



|    |  |   |
|----|--|---|
| 19 | hu8E11.v8<br>light chain<br>variable<br>region   | DIVMTQSPDS LAVSLGERAT INCREASESVD NYGNSFMHWY<br>QQKPGQPPKL LIYLASNLES GVPDRFSGSG SRTDFTLTIS<br>SLQAEDVAVY YCQQNYEDPF TFGQGTKVEI KR      |
| 20 | hu8E11.v8<br>heavy chain<br>variable<br>region   | EVQLVQSGAE VKKPGASVKV SCKASGYTFS AYWIEWVRQA<br>PGQGLEWIGE ILPGSDSTDY NEKFKVRVTI TADTSTSTAY<br>LELSSLRSED TAVYYCARGG HYGSLDYWGQ GTLVTVSS |
| 25 | YW353 light<br>chain variable<br>region  | DIQMTQSPSS LSASVGDRVT ITCRASQDVS TAVAWYQQKP<br>GKAPKLLIYS ASFLYSGVPS RFSGSGSGTD FTLTISSLQP<br>EDFATYYCQQ SYTTPPTFGQ GTKVEIKR            |
| 26 | YW353 heavy<br>chain variable<br>region  | EVQLVESGGG LVQPGGSLRL SCAASGFTFT SYSISWVRQA<br>PGKGLEWVAE IYPPGGYTDY ADSVKGRFTI SADTSKNTAY<br>LQMNSLRAED TAVYYCAKAR LFFDYWGQGT LVTVSS   |
| 27 | mu8E11 HVR L1  | RASESVDNYG NSFMH  |
| 28 | mu8E11 HVR L2  | LASNLES   |
| 29 | mu8E11 HVR L3  | QQNYEDPFT   |
| 30 | mu8E11 HVR H1  | GYTFSAYWIE  |
| 31 | mu8E11 HVR H2  | EILPGSDSTD YNEKFKV  |
| 32 | mu8E11 HVR H3  | GGHYGSLDY   |
| 33 | Hu8E11 light<br>chain (LC)<br>framework 1<br>(FR1)                                       | DIVMTQSPDS LAVSLGERAT INC   |
| 34 | Hu8E11 LC FR2  | WYQQKPGQPP KLLIY  |
| 35 | Hu8E11.v1 LC<br>FR3<br>Hu8E11.v2 LC<br>FR3<br>Hu8E11.v5 LC<br>FR3<br>Hu8E11.v6 LC<br>FR3 | GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YC   |
| 36 | Hu8E11.v3 LC<br>FR3<br>Hu8E11.v4 LC<br>FR3<br>Hu8E11.v7 LC<br>FR3<br>Hu8E11.v8 LC<br>FR3 | GVPDRFSGSG SRTDFTLTIS SLQAEDVAVY YC   |
| 37 | Hu8E11 LC FR4  | FGQGTKVEIK R  |
| 38 | Hu8E11 heavy<br>chain (HC)<br>framework1<br>(FR1)  | EVQLVQSGAE VKKPGASVKV SCKAS   |
| 39 | Hu8E11 HC FR2  | WVRQAPGQGL EWIG   |
| 40 | Hu8E11.v1 HC<br>FR3<br>Hu8E11.v3 HC<br>FR3   | RVTITSDTST STVYLELSSL RSED TAVYYC AR  |
| 41 | Hu8E11.v2 HC<br>FR3<br>Hu8E11.v4 HC<br>FR3   | RATFTSDTST STVYLELSSL RSED TAVYYC AR  |
| 42 | Hu8E11.v5 HC<br>FR3<br>Hu8E11.v7 HC<br>FR3   | RVTITRDTST STAYLELSSL RSED TAVYYC AR  |
| 43 | Hu8E11.v6 HC   | RVTITADTST STAYLELSSL RSED TAVYYC AR  |

|    |  |  |
|----|--|--|
|    | FR3<br>Hu8E11.v8 HC<br>FR3                           |  |
| 44 | Hu8E11 HC FR4  | WGQGLTVTVS S   |
| 45 | mu3G12 HVR L1  | RSSQSLVHSN GNTYLQ  |
| 46 | mu3G12 HVR L2  | KVSNRFS  |
| 47 | mu3G12 HVR L3  | SQSTHFPYT  |
| 48 | mu3G12 HVR H1  | VDTFNSYWMH   |
| 49 | mu3G12 HVR H2  | EINPSNGRTN YIEKFKN   |
| 50 | mu3G12 HVR H3  | GWYFDV   |
| 51 | mu2H6 HVR L1   | KSSQSLNLSG NQKNYLT   |
| 52 | mu2H6 HVR L2   | WASTRES  |
| 53 | mu2H6 HVR L3   | QNDYSFPFT  |
| 54 | mu2H6 HVR H1   | GYSFTGYTMN   |
| 55 | mu2H6 HVR H2   | LINCYNNGTN YNQKFKG   |
| 56 | mu2H6 HVR H3   | GGSTMITPRF AY  |
| 57 | YW353 HVR L1   | RASQDVSTAV A   |
| 58 | YW353 HVR L2   | SASFLYS  |
| 59 | YW353 HVR L3   | QOSYTTPT   |
| 60 | YW353 HVR H1   | GFTFTSYSIS   |
| 61 | YW353 HVR H2   | EIYPPGGYTD YADSVKG   |
| 62 | YW353 HVR H3   | ARLFFDY  |
| 63 | hu8E11.v2<br>light chain                             | DIVMTQSPDS LAVSLGERAT INCRASESVD NYGNSFMHWY<br>QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS<br>SLQAEDVAVY YCQQNYEDPF TFGQGTKVEI KRTVAAPSVF<br>IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS<br>GNSQESVTEQ DSKDSTYSLS STLTLKADY EKHKVYACEV<br>THQGLSPVT KSFNRGEC   |
| 64 | hu8E11.v2<br>heavy chain                             | EVQLVQSGAE VKKPGASVKV SCKASGYTFS AYWIEWVRQA<br>PGQGLEWIGE ILPGSDSTDY NEKFKVRATF TSDTSTSTVY<br>LELSSLRSED TAVYYCARGG HYGSLDYWGQ GTLTVTVSSAS<br>TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN<br>SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTQTYI<br>CNVNHKPSNT KVDKKVEPKS CDKHTCPCPC PAPELLGGPS<br>VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV<br>DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY<br>KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSREEMT<br>KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTPPVLD<br>SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSPGK |
| 65 | YW353 light<br>chain                                 | DIQMTQSPSS LSASVGRVT ITCRASQDVS TAVAWYQQKP<br>GKAPKLLIYS ASFLYSGVPS RFGSGSGSGTD FTLTISSLQP<br>EDFATYYCQQ SYTTPPTFGQ GTKVEIKRTV AAPSVFIFPP<br>SDEQLKSGTA SVVCLLNIFY PREAKVQWKV DNALQSGNSQ<br>ESVTEQDSKD STYSLSTLT LSKADYEKKH VYACEVTHQG<br>LSPVTKSFN RGEK   |
| 66 | YW353 heavy<br>chain                                 | EVQLVESGGG LVPQGGSLRL SCAASGFTFT SYSISWVRQA<br>PGKGLEWVAE IYPPGGYTDY ADSVKGRFTI SADTSKNATAY<br>LQMNSLRAED TAVYYCAKAR LFFDYWGQGT LVTVSSASTK<br>GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG<br>ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS SLGTQTYICN<br>VNHKPSNTKV DKKVEPKSCD KHTCPCPCPA PELLGGPSVF<br>LFPPKPKDNL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG<br>VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC<br>KVSNAKALP IEKTISKAKG QPREPQVYTL PPSREEMTKN<br>QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPVLDSD<br>GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK |
| 67 | Human LGR5<br>precursor;<br>LGR5_human<br>NP_003658; | MDTSRLGVLL SLPVLLQLAT GGSSPRSGVL LRGCPHCHC<br>EPDGRMLLRV DCSDLGLSEL PSNLSVFTSY LDLSMNNISQ<br>LLPNPLPSLR FLEELRLAGN ALTYIPKGAF TGLYSLKVLM<br>LQNNQLRHVP TEALQNLRLSL QSLRLDANHI SYVPPSCFSG   |

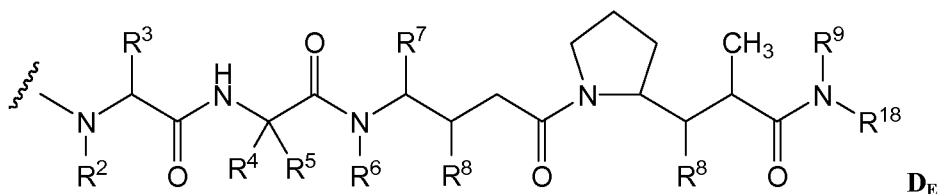
|  |             |            |            |            |
|--|-------------|------------|------------|------------|
| signal<br>sequence =<br>amino acids 1-<br>21 | LHSLRHLWLD  | DNALTEIPVQ | AFRSLSALQA | MTLALNKIHH |
|  | IPDYAFGNLS  | SLVVLHLHNN | RIHSLGKKCF | DGLHSLETLD |
|  | LNYNLDEFEP  | TAIRTLNLK  | ELGFHSNNIR | SIPEKAFVGN |
|  | PSLITIHFYD  | NPIQFVGRSA | FQHLPELRTL | TLNGASQITE |
|  | FPDLTG TANL | ESLTLTGAQI | SSLPQTVCNQ | LPNLQVLDLS |
|  | YNLLEDLPSF  | SVCQKLQKID | LRHNEIYEIK | VDTFQQLLSL |
|  | RSLNLAWNKI  | AIHPNAFST  | LPSLIKLDLS | SNLLSSFPIT |
|  | GLHGLTHLKL  | TGNHALQSLI | SSENFPELV  | IEMPYAYQCC |
|  | AFGVCENAYK  | ISNQWNKGDN | SSMDDLHKKD | AGMFQAQDER |
|  | DLEDFLLD FE | EDLKALHSVQ | CSPSPGPFKP | CEHLLDGWLI |
|  | RIGVWTIAVL  | ALTCNALVTS | TVFRSPLYIS | PIKLLIGVIA |
|  | AVNMLTG VSS | AVLAGVDAFT | FGSFARHGAW | WENGVGCHVI |
|  | GFLSIFASES  | SVFLTLAAL  | ERGFSVKYSA | KFETKAPFSS |
|  | LKVIILLCAL  | LALTMAAVPL | LGGSKYGASP | LCLPLPFGEF |
|  | STMGYMVALI  | LLNSLCFLMM | TIAYTKLYCN | LDKGDLENIW |
|  | DCSMVKHIAL  | LLFTNCILNC | PVAFLSFSSL | INLTFISPEV |
|  | IKFILLVVVP  | LPACLNPLLY | ILFNPHFKED | LVSLRKQTYV |
|  | WTRSKHPSLM  | SINSDDVEKQ | SCDSTQALVT | FTSSSITYDL |
|  | PPSSVPSPAY  | PVTESCHLSS | VAFVPCCL   |            |

## WHAT IS CLAIMED IS:

- 1) A method of treating a hedgehog-related disease in an individual comprising administering to the individual an effective amount of an anti-LGR5 antibody.
- 2) A method of treating hedgehog-related disease in an individual comprising administering to the individual an effective amount of an anti-LGR5 antibody and an effective amount of an inhibitor of the hedgehog pathway.
- 3) The method of claim 2, wherein the respective amounts the anti-LGR5 antibody and the inhibitor of the hedgehog pathway are effective to increase the period of response to therapy and/or delay the recurrence and/or development of resistance compared to treatment with the inhibitor of the hedgehog pathway alone.
- 4) A method of increasing efficacy of a treatment of a hedgehog-related disease comprising an inhibitor of the hedgehog pathway in an individual, wherein the method comprises administering to the individual an effective amount of an anti-LGR5 antibody and an effective amount of the inhibitor of the hedgehog pathway.
- 5) A method of treating a hedgehog-related disease in an individual wherein the treatment comprises administering to the individual an effective amount of an anti-LGR5 antibody and an effective amount of an inhibitor of the hedgehog pathway, and wherein the treatment has increased efficacy compared to a standard treatment comprising administering an effective amount of the inhibitor of the hedgehog pathway without (in the absence of) the anti-LGR5 antibody.
- 6) A method of delaying and/or preventing development of the recurrence and/or resistance of a hedgehog-related disease to an inhibitor of the hedgehog pathway in an individual, comprising administering to the individual an effective amount of an anti-LGR5 antibody and an effective amount of the inhibitor of the hedgehog pathway.
- 7) A method of increasing sensitivity to an inhibitor of the hedgehog pathway in an individual with a hedgehog-related disease comprising administering to the individual an effective amount of an anti-LGR5 antibody and an effective amount of the inhibitor of the hedgehog pathway.
- 8) A method of extending the period of sensitivity an inhibitor of the hedgehog pathway in an individual with a hedgehog-related disease comprising administering to the individual an effective amount of an anti-LGR5 antibody and an effective amount of the inhibitor of the hedgehog pathway.
- 9) A method of extending the duration of response to an inhibitor of the hedgehog pathway in an individual with a hedgehog-related disease comprising administering to the individual an effective amount of an anti-LGR5 antibody and an effective amount of the inhibitor of the hedgehog pathway.
- 10) The method of any one of claims 1-9, wherein the anti-LGR5 antibody is a monoclonal antibody.
- 11) The method of any one of claims 1-10, wherein the anti-LGR5 antibody binds an epitope within amino acids 22-323 of SEQ ID NO: 67 or within amino acids 22-123 of SEQ ID NO: 67 and binds to LGR5 with an affinity of  $\leq 5$  nM.

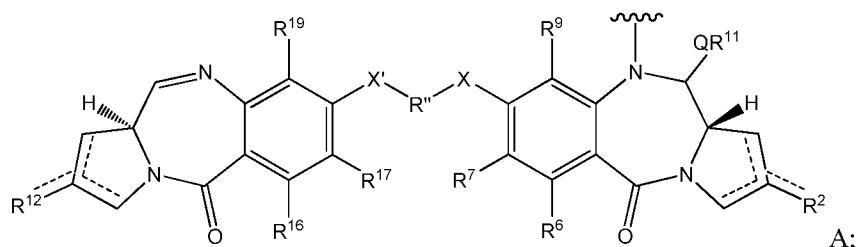
- 12) The method of any one of claims 1-11, wherein the anti-LGR5 antibody is a human, humanized, or chimeric antibody.
- 13) The method of any one of claims 1-12, wherein the anti-LGR5 antibody is an antibody fragment that binds human LGR5 of SEQ ID NO: 67.
- 14) The method of any one of claims 1-13, wherein the anti-LGR5 antibody comprises:
- a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 30, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 31, and HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32; and
  - b) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 27, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 28, and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 29.
- 15) The method of any one of claims 1-13, wherein the anti-LGR5 antibody comprises:
- a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 60, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 61, and HVR-H3 comprising the amino acid sequence of SEQ ID NO: 62; and
  - b) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 57, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 58, and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 59.
- 16) The method of any one of claims 1-13, wherein the anti-LGR5 antibody comprises:
- a) a VH sequence of SEQ ID NO: 6 and a VL sequence of SEQ ID NO: 5;
  - b) a VH sequence of SEQ ID NO: 8 and a VL sequence of SEQ ID NO: 7;
  - c) a VH sequence of SEQ ID NO: 10 and a VL sequence of SEQ ID NO: 9;
  - d) a VH sequence of SEQ ID NO: 12 and a VL sequence of SEQ ID NO: 11;
  - e) a VH sequence of SEQ ID NO: 14 and a VL sequence of SEQ ID NO: 13;
  - f) a VH sequence of SEQ ID NO: 16 and a VL sequence of SEQ ID NO: 15;
  - g) a VH sequence of SEQ ID NO: 18 and a VL sequence of SEQ ID NO: 17;
  - h) a VH sequence of SEQ ID NO: 20 and a VL sequence of SEQ ID NO: 19; or
  - i) a VH sequence of SEQ ID NO: 26 and a VL sequence of SEQ ID NO: 25.
- 17) The method of any one of claims 1-16, wherein the anti-LGR5 antibody is conjugated to a cytotoxic agent.
- 18) The method of any one of claims 1-17, wherein the anti-LGR5 antibody has the formula Ab-(L-D)<sub>p</sub>, wherein:
- (a) Ab is the anti-LGR5 antibody;
  - (b) L is a linker;
  - (c) D is a drug selected from a maytansinoid, an auristatin, a calicheamicin, a pyrrolobenzodiazepine, and a nemorubicin derivative; and
  - (d) p ranges from 1-8.

- 19) The method of claim 18, wherein D is an auristatin.
- 20) The method of claim 18, wherein D has formula D<sub>E</sub>



and wherein R<sup>2</sup> and R<sup>6</sup> are each methyl, R<sup>3</sup> and R<sup>4</sup> are each isopropyl, R<sup>5</sup> is H, R<sup>7</sup> is sec-butyl, each R<sup>8</sup> is independently selected from CH<sub>3</sub>, O-CH<sub>3</sub>, OH, and H; R<sup>9</sup> is H; and R<sup>18</sup> is -C(R<sup>8</sup>)<sub>2</sub>-C(R<sup>8</sup>)<sub>2</sub>-aryl.

- 21) The method of claim 18, wherein the drug is MMAE.
- 22) The method of claim 18, wherein D is a pyrrolobenzodiazepine of Formula A:



wherein the dotted lines indicate the optional presence of a double bond between C1 and C2 or C2 and C3;

R<sup>2</sup> is independently selected from H, OH, =O, =CH<sub>2</sub>, CN, R, OR, =CH-R<sup>D</sup>, =C(R<sup>D</sup>)<sub>2</sub>,

O-SO<sub>2</sub>-R, CO<sub>2</sub>R and COR, and optionally further selected from halo or dihalo, wherein R<sup>D</sup> is independently selected from R, CO<sub>2</sub>R, COR, CHO, CO<sub>2</sub>H, and halo;

R<sup>6</sup> and R<sup>9</sup> are independently selected from H, R, OH, OR, SH, SR, NH<sub>2</sub>, NHR, NRR', NO<sub>2</sub>, Me<sub>3</sub>Sn and halo;

R<sup>7</sup> is independently selected from H, R, OH, OR, SH, SR, NH<sub>2</sub>, NHR, NRR', NO<sub>2</sub>, Me<sub>3</sub>Sn and halo;

Q is independently selected from O, S and NH;

R<sup>11</sup> is either H, or R or, where Q is O, SO<sub>3</sub>M, where M is a metal cation;

R and R' are each independently selected from optionally substituted C<sub>1-8</sub> alkyl,

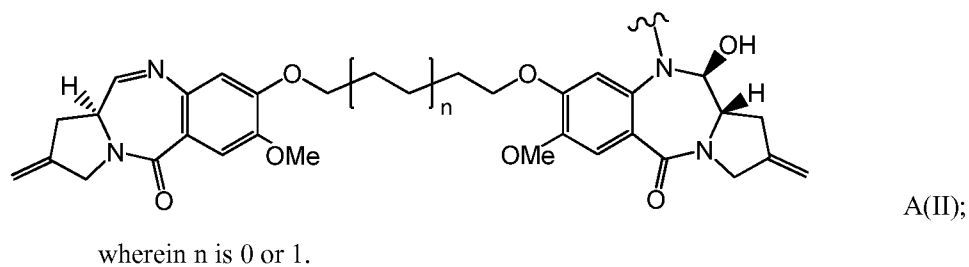
C<sub>3-8</sub> heterocyclyl and C<sub>5-20</sub> aryl groups, and optionally in relation to the group NRR', R and R' together with the nitrogen atom to which they are attached form an optionally substituted 4-, 5-, 6- or 7-membered heterocyclic ring;

R<sup>12</sup>, R<sup>16</sup>, R<sup>19</sup> and R<sup>17</sup> are as defined for R<sup>2</sup>, R<sup>6</sup>, R<sup>9</sup> and R<sup>7</sup> respectively;

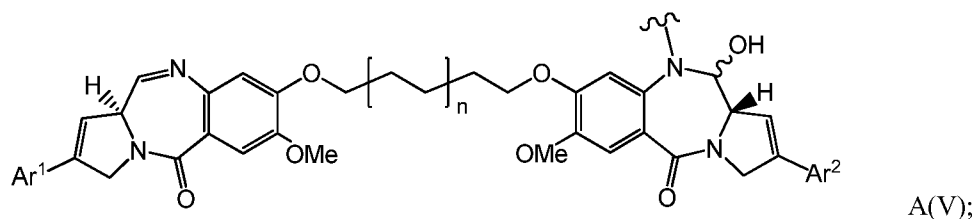
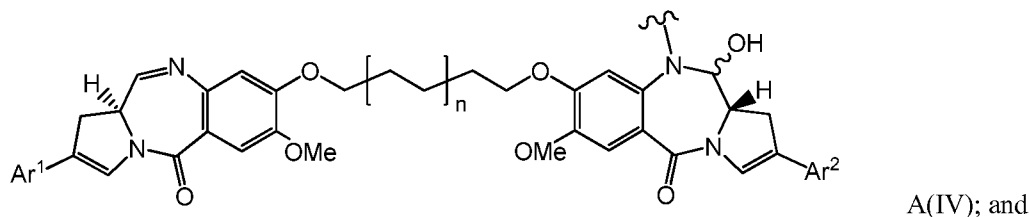
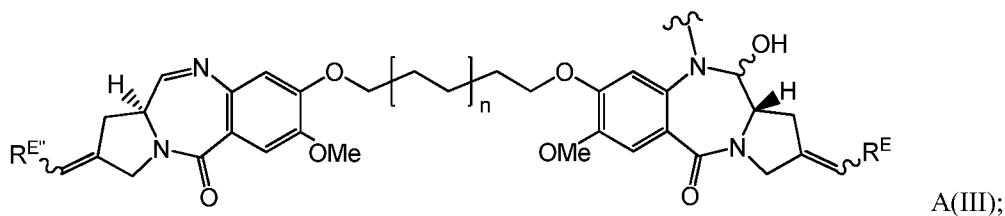
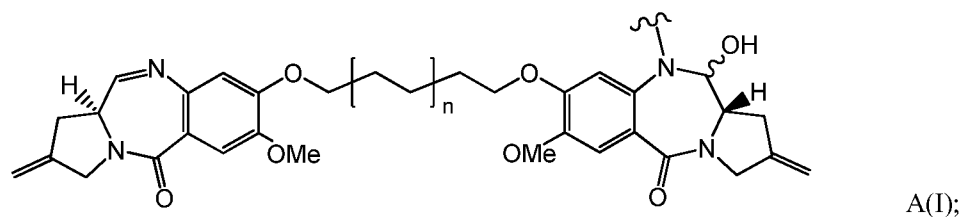
R'' is a C<sub>3-12</sub> alkylene group, which chain may be interrupted by one or more heteroatoms and/or aromatic rings that are optionally substituted; and

X and X' are independently selected from O, S and N(H).

- 23) The method of claim 18, wherein D has the structure:

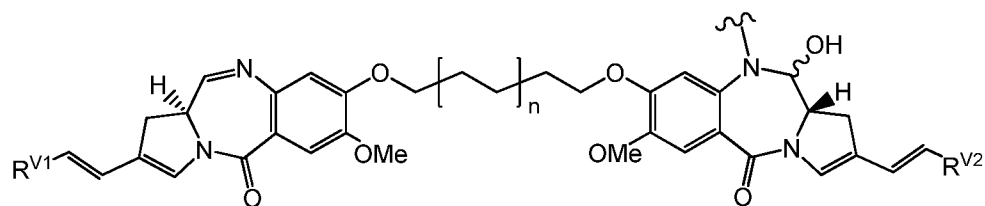


- 24) The method of claim 18, wherein D has a structure selected from:



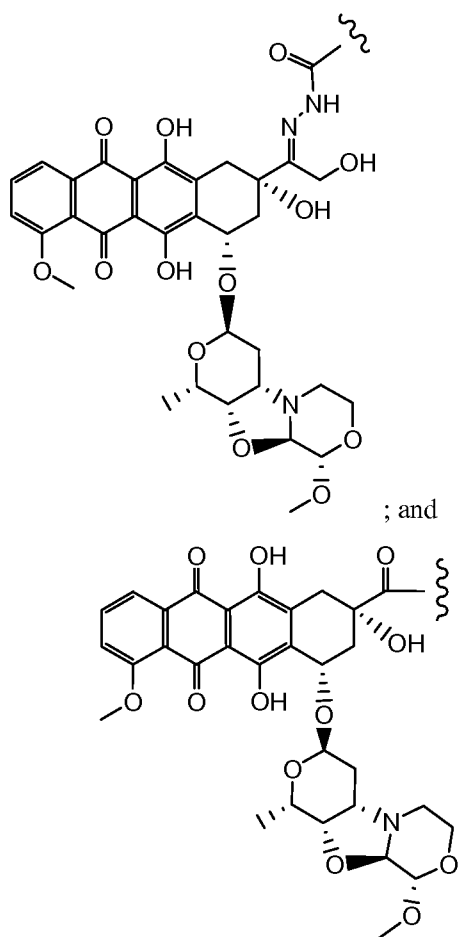
wherein  $R^E$  and  $R^{E'}$  are each independently selected from H or  $R^D$ , wherein  $R^D$  is independently selected from R,  $CO_2R$ , COR, CHO,  $CO_2H$ , and halo;  
 wherein  $Ar^1$  and  $Ar^2$  are each independently optionally substituted  $C_{5-20}$  aryl; and  
 wherein n is 0 or 1.

- 25) The method of claim 18, wherein D is a pyrrolobenzodiazepine of Formula B:



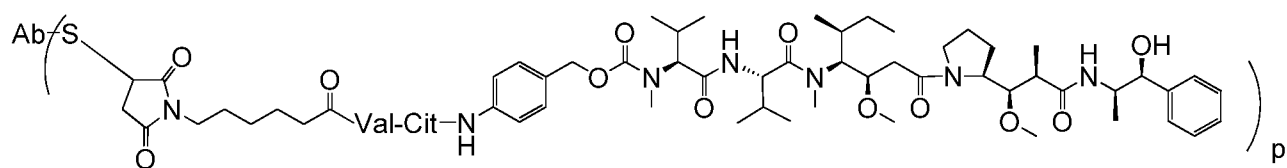
wherein the horizontal wavy line indicates the covalent attachment site to the linker;  
 $R^{V1}$  and  $R^{V2}$  are independently selected from H, methyl, ethyl, phenyl, fluoro-substituted phenyl, and  $C_{5-6}$  heterocyclyl; and  
 n is 0 or 1.

- 26) The method of claim 18, wherein D is a nemorubicin derivative.  
 27) The method of claim 18, wherein D has a structure selected from:



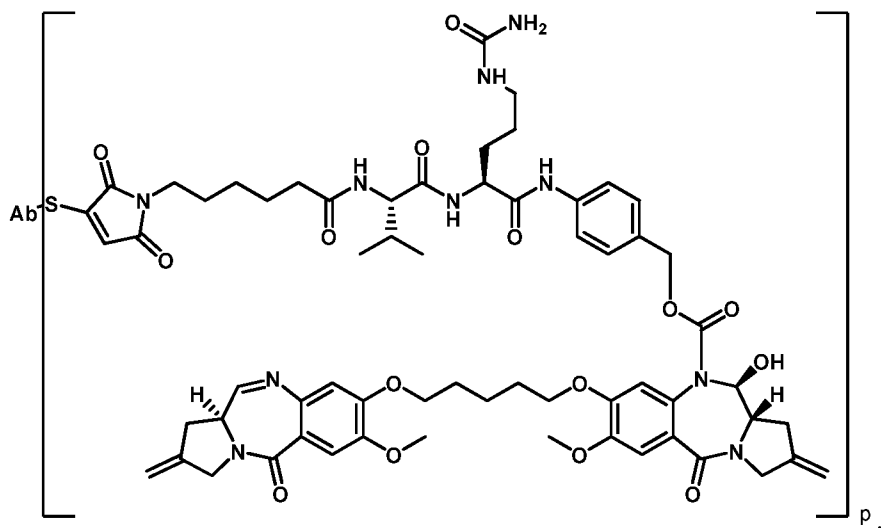
- 28) The method of any one of claims 18-27, wherein the linker is cleavable by a protease.  
 29) The method of claim 28, wherein the linker comprises a val-cit dipeptide or a Phe-Lys dipeptide.  
 30) The method of any one of claims 18-27, wherein the linker is acid-labile.  
 31) The method of claim 30, wherein the linker comprises hydrazone.  
 32) The method of claim 18 having the formula:



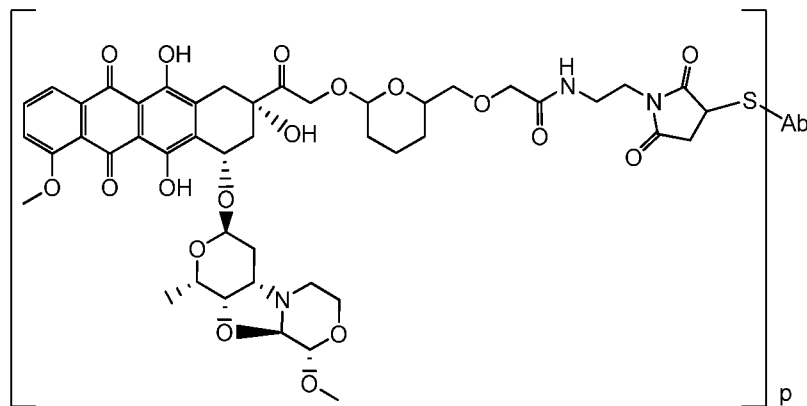


wherein S is a sulfur atom.

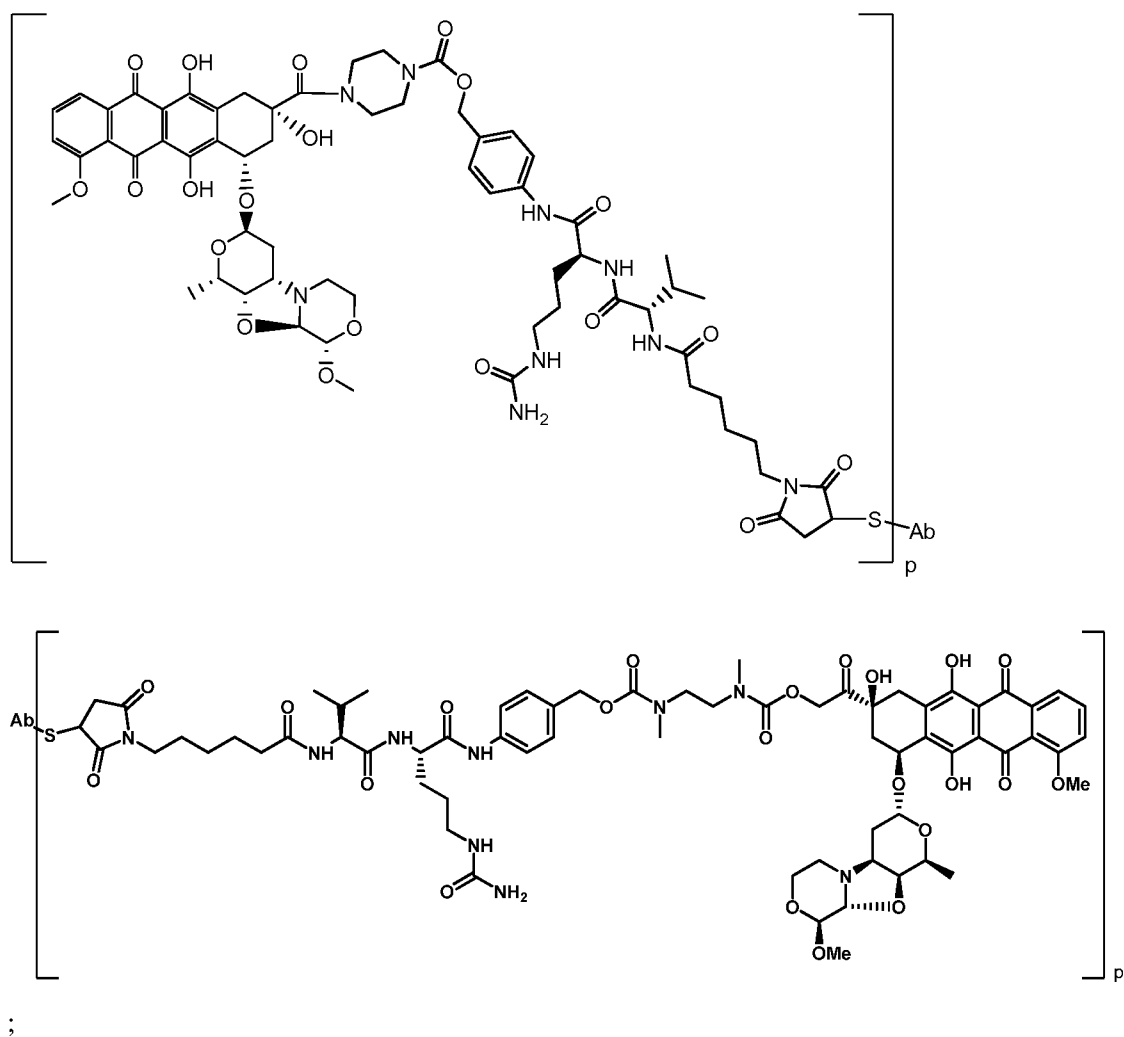
- 33) The method of claim 18 having the formula:

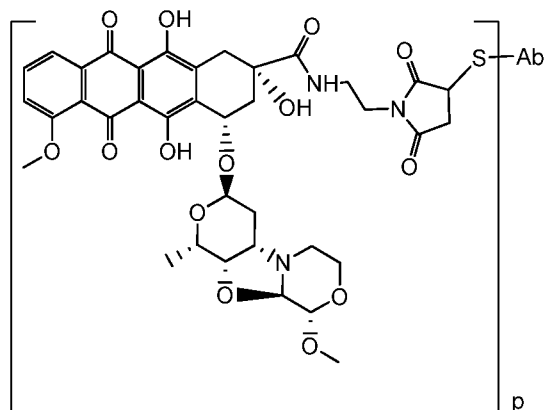
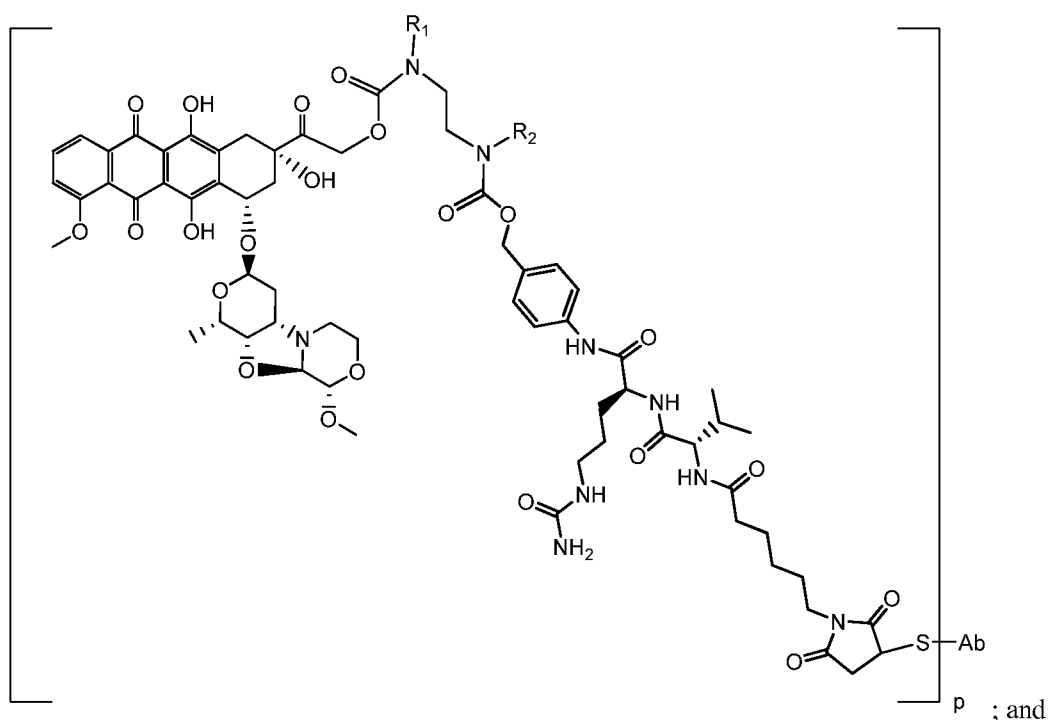


- 34) The method of claim 18 having a formula selected from:



;





- 35) The method of any one of claims 18-34, wherein p ranges from 2-5.
- 36) The method of any one of claims 1-35, wherein the inhibitor of the hedgehog pathway is an antagonist of the smoothened receptor.
- 37) The method of claim 36, wherein the antagonist of the smoothened receptor is 2-chloro-N-[4-chloro-3-(pyridin-2-yl)phenyl]-4-(methylsulfonyl)benzamide or a salt thereof.
- 38) The method of claim 37, wherein the antagonist of the smoothened receptor is 2-chloro-N-[4-chloro-3-(pyridin-2-yl)phenyl]-4-(methylsulfonyl)benzamide.
- 39) The method of claim 36, wherein the antagonist of the smoothened receptor is vismodegib.
- 40) The method of any one of claims 1-39, wherein the hedgehog-related disease is cancer.
- 41) The method of claim 40, wherein the cancer is basal cell carcinoma.

- 42) The method of claim 41, wherein the basal cell carcinoma is locally advanced or metastatic basal cell carcinoma.
- 43) The method of claim 40, wherein the cancer is medulloblastoma.
- 44) The method of any one of claims 40-43, wherein the hedgehog-related disease is LGR5-positive.
- 45) The method of any one of claims 1-44, wherein the inhibitor of the hedgehog pathway is administered concomitantly with the anti-LGR5 antibody.
- 46) The method of any one of claims 1-44, wherein the inhibitor of the hedgehog pathway is administered separately, sequentially, or simultaneously with the anti-LGR5 antibody.

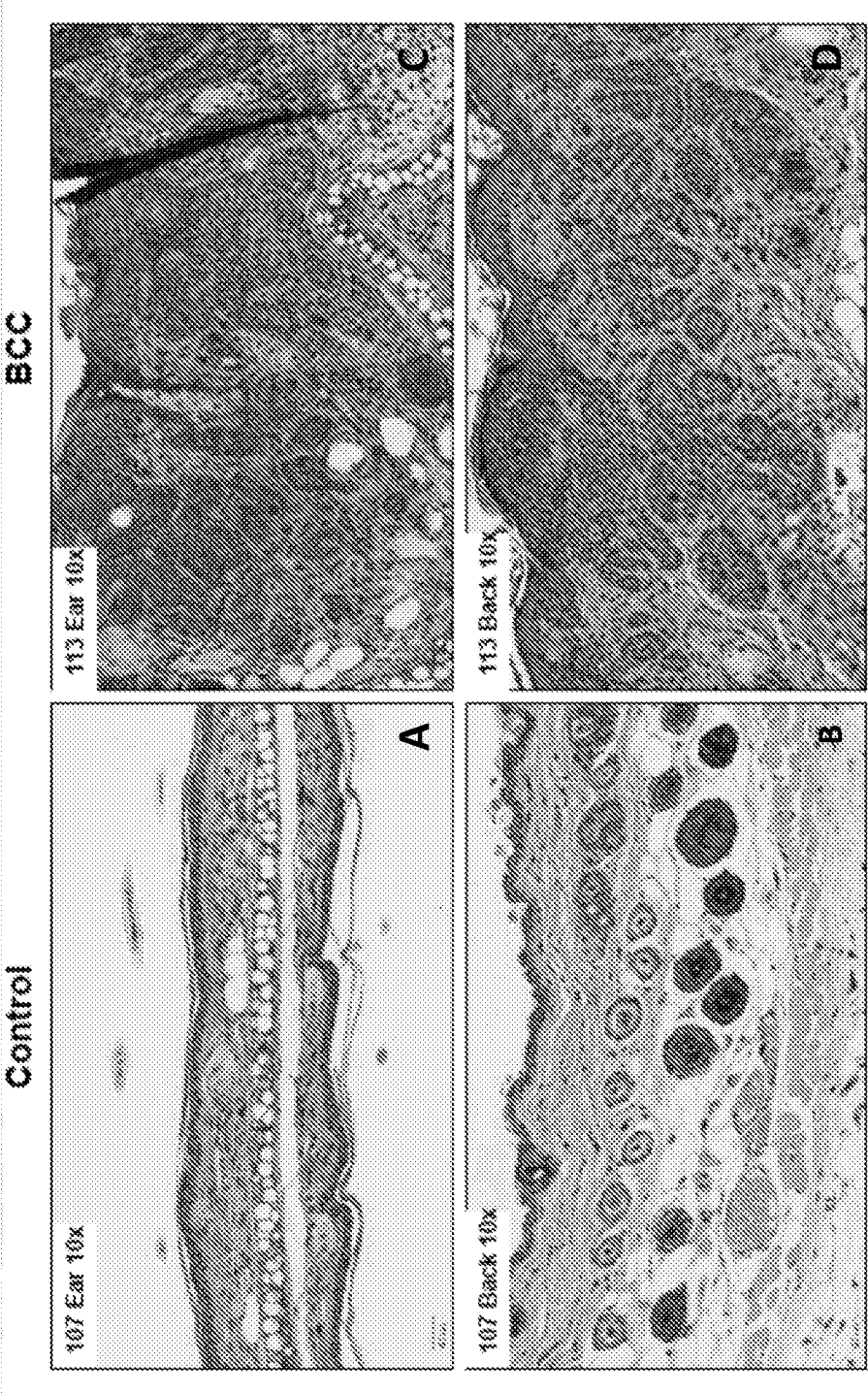


Fig. 1

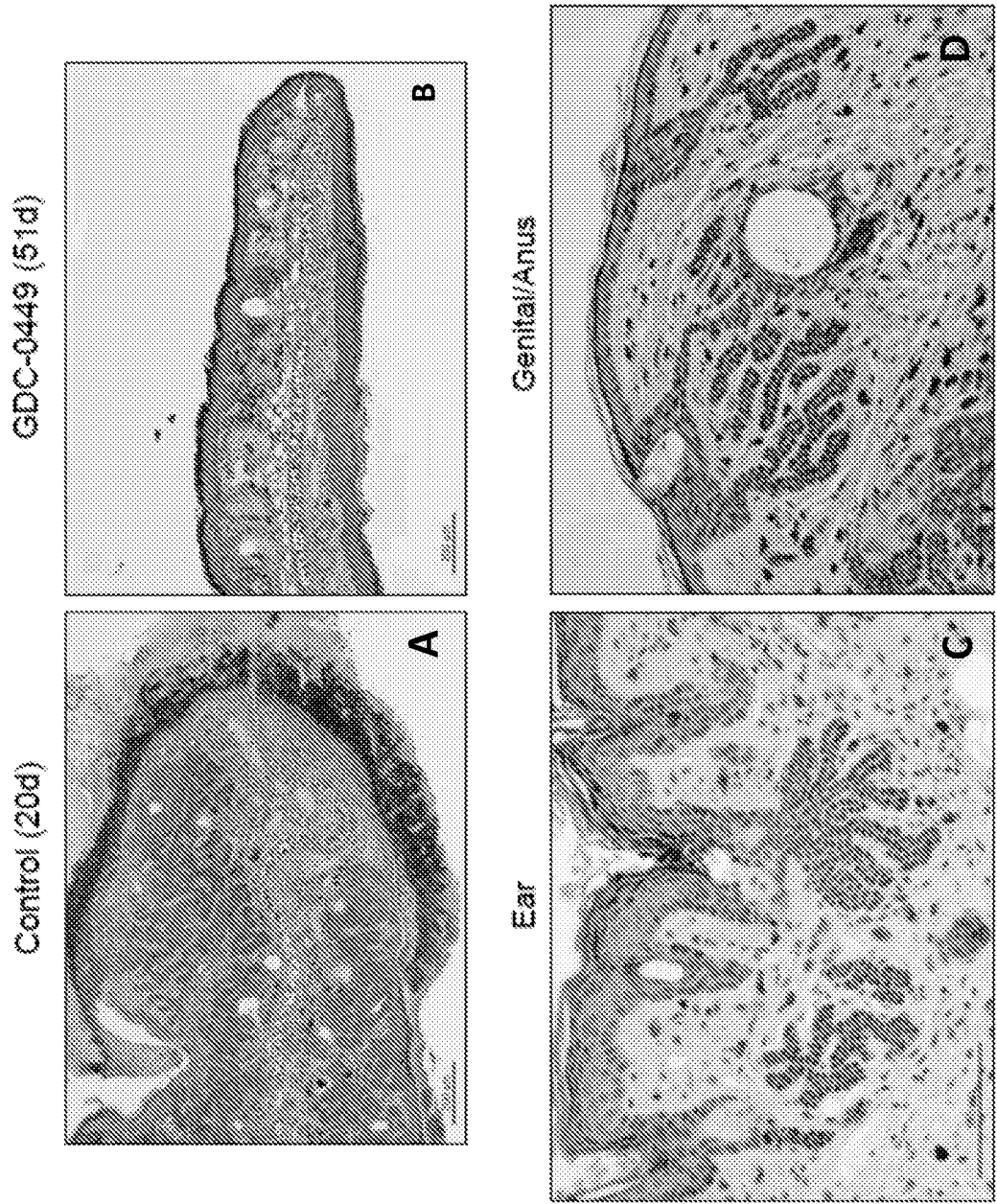


Fig. 2

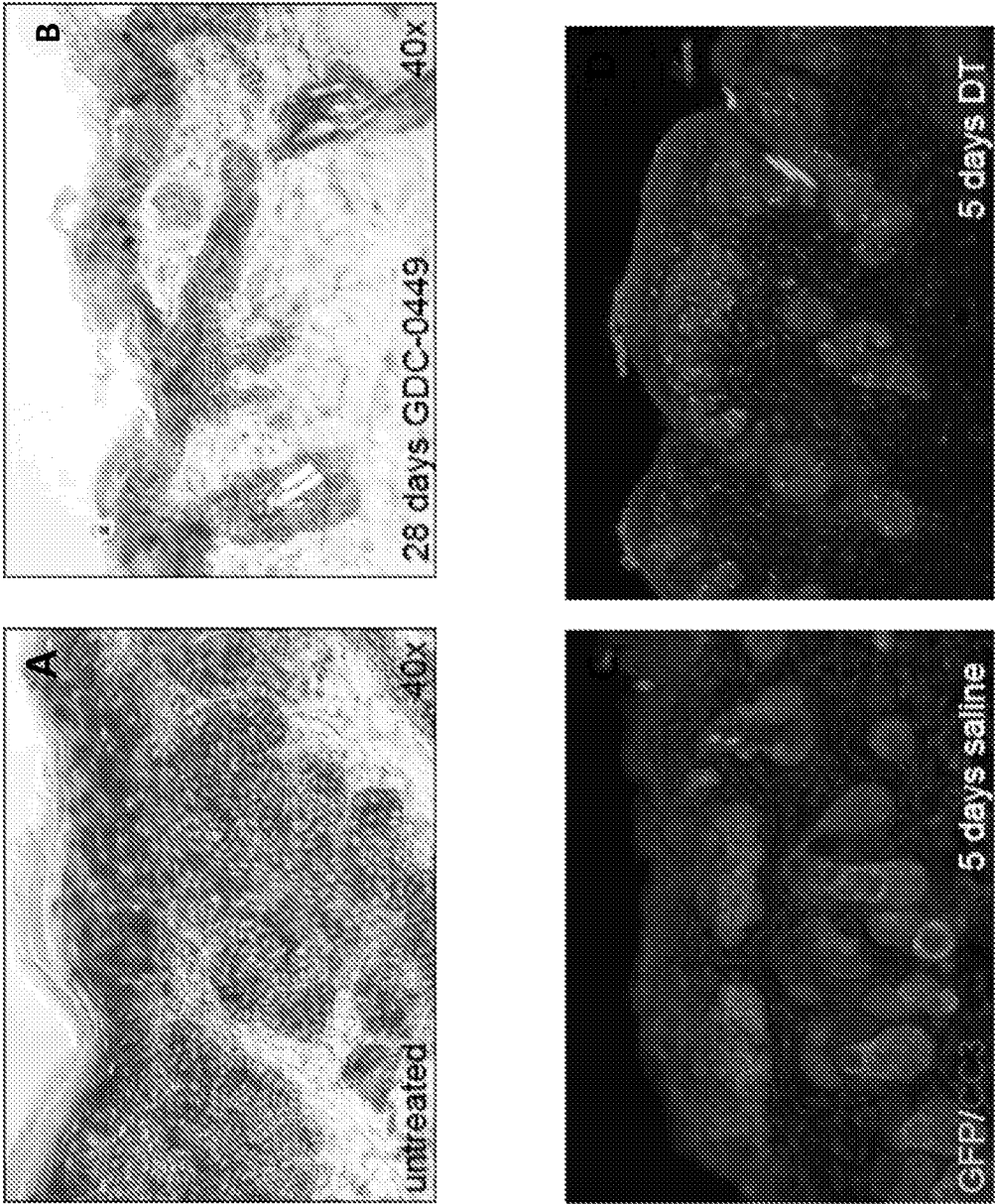


Fig. 3

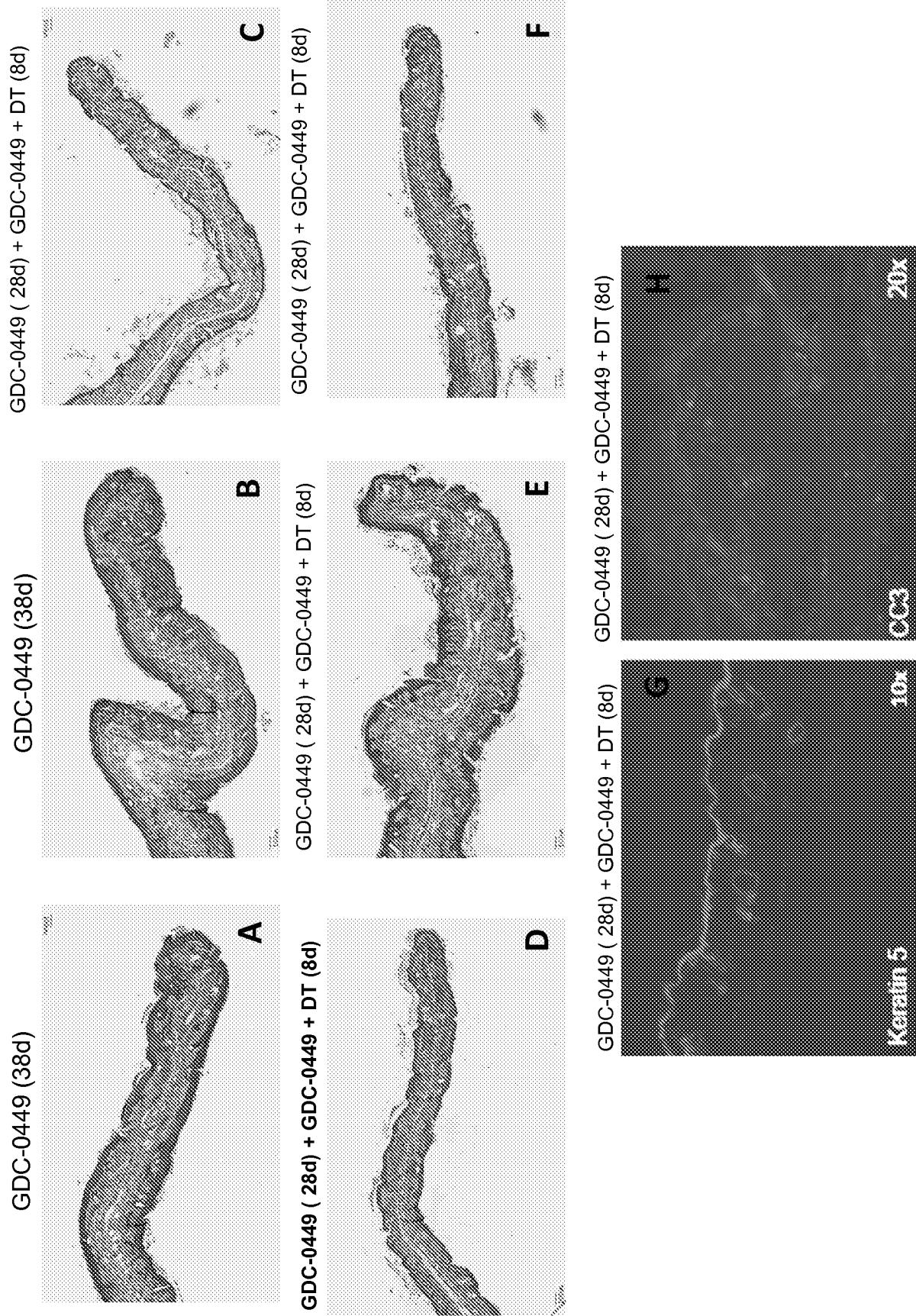


Fig. 4



# INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2014/056017

## A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K16/30 A61K39/395 A61K31/015 A61P35/00  
ADD.

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

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K 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, BIOSIS, EMBASE, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|-----------|---|-----------------------|
| X         | WO 2013/067054 A1 (BIONOMICS INC [US])<br>10 May 2013 (2013-05-10)<br>examples 20,21,24,25<br>-----   | 1                     |
| X         | DATABASE WPI<br>Week 200844<br>Thomson Scientific, London, GB;<br>AN 2008-G94600<br>XP002733926,<br>& JP 2008 125491 A (GH KEIO GIJUKU)<br>5 June 2008 (2008-06-05)<br>abstract<br>-----<br>-/- | 1                     |

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

\* Special categories of cited documents :

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

"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

19 December 2014

Date of mailing of the international search report

19/01/2015

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Authorized officer

Covone-van Hees, M

## INTERNATIONAL SEARCH REPORT

International application No

PCT/US2014/056017

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|-----------|---|-----------------------|
| Y         | SASAKI Y ET AL: "Establishment of a novel monoclonal antibody against LGR5", BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, ACADEMIC PRESS INC, ORLANDO, FL, US, vol. 394, no. 3, 9 April 2010 (2010-04-09), pages 498-502, XP002685669, ISSN: 0006-291X, DOI: 10.1016/J.BBRC.2010.02.166 [retrieved on 2010-03-01] the whole document                                  | 2-46                  |
| Y         | -----<br>R N Amaria ET AL: "Vismodegib in basal cell carcinoma", Drugs of today (Barcelona, Spain : 1998), 1 July 2012 (2012-07-01), 12 December 2014 (2014-12-12), page 459, XP55156203, United States<br>DOI: 10.1358/dot.2012.48.7.1808490<br>Retrieved from the Internet:<br>URL:http://www.ncbi.nlm.nih.gov/pubmed/22844657<br>[retrieved on 2014-12-12]<br>examples 4,5 | 2-46                  |
| X         | -----<br>WO 2009/005809 A2 (ONCOMED PHARM INC [US]; GURNEY AUSTIN [US])<br>8 January 2009 (2009-01-08)<br>examples 4,5  | 1                     |
| A         | -----<br>TANESE KEIJI ET AL: "G-protein-coupled receptor GPR49 is up-regulated in basal cell carcinoma and promotes cell proliferation and tumor formation", AMERICAN JOURNAL OF PATHOLOGY; [10640], ELSEVIER INC, US, vol. 173, no. 3, 1 September 2008 (2008-09-01), pages 835-843, XP009166443, ISSN: 0002-9440, DOI: 10.2353/AJPATH.2008.071091 the whole document        | 1-46                  |
| A         | -----<br>KATOH Y ET AL: "Hedgehog target genes: mechanisms of carcinogenesis induced by aberrant hedgehog signaling activation", CURRENT MOLECULAR MEDICINE, BENTHAM SCIENCE PUBLISHERS, NL, vol. 9, no. 7, 1 September 2009 (2009-09-01), pages 873-886, XP009157188, ISSN: 1566-5240<br>page 879, right-hand column - page 880, right-hand column<br>-----<br>-/--          | 1-46                  |

## INTERNATIONAL SEARCH REPORT

International application No

PCT/US2014/056017

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|-----------|---|-----------------------|
| A         | TEGLUND S ET AL: "Hedgehog beyond medulloblastoma and basal cell carcinoma", BBA - REVIEWS ON CANCER, ELSEVIER SCIENCE BV, AMSTERDAM, NL, vol. 1805, no. 2, 1 April 2010 (2010-04-01), pages 181-208, XP026953172, ISSN: 0304-419X [retrieved on 2010-01-18] page 185, left-hand column - page 186, left-hand column<br>----- | 1-46                  |
| A         | WO 2010/037715 A1 (NOVARTIS AG [CH]; DORSCH MARION [US]; MONAHAN JOHN E [US]; MORRISSEY M) 8 April 2010 (2010-04-08) claims example 1<br>-----  | 1-46                  |
| A         | WO 2011/025838 A1 (ABRAXIS BIOSCIENCE LLC [US]; TAO CHUNLIN [US]; DESAI NEIL P [US]; SOON) 3 March 2011 (2011-03-03) examples<br>-----  | 1-46                  |
| A         | WO 2013/037043 A1 (UNIV MONTREAL [CA]; BORDEN KATHERINE [CA]; ZAHREDDINE HIBA [CA]; CULJK) 21 March 2013 (2013-03-21) examples<br>-----   | 1-46                  |
| A         | WO 2007/008603 A1 (SEATTLE GENETICS INC [US]; DORONINA SVETLANA O [US]; KLINE TONI BETH []) 18 January 2007 (2007-01-18) claims<br>-----  | 1-46                  |
| A,P       | WO 2013/149159 A1 (GENENTECH INC [US]; HONGO JO-ANNE [US]; MAO WEIGUANG [US]; POLAKIS PAU) 3 October 2013 (2013-10-03) sequences 5,6,8,9,14,16,25,26<br>-----   | 1-46                  |
| A,P       | NAKATA SUSUMU ET AL: "Emerging role for leucine-rich repeat-containing G-protein-coupled receptors LGR5 and LGR4 in cancer stem cells.", CANCER MANAGEMENT AND RESEARCH 2014, vol. 6, 2014, pages 171-180, XP002733920, ISSN: 1179-1322 the whole document<br>-----   | 1-46                  |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2014/056017

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s)  | Publication<br>date         |
|---|---------------------|-----------------------------|-----------------------------|
| WO 2013067054                             | A1                  | 10-05-2013                  | AU 2012332587 A1 22-05-2014 |
|   |                     | CA 2853951 A1 10-05-2013    |                             |
|   |                     | CN 104053671 A 17-09-2014   |                             |
|   |                     | EP 2773665 A1 10-09-2014    |                             |
|   |                     | WO 2013067054 A1 10-05-2013 |                             |
| JP 2008125491                             | A                   | 05-06-2008                  | NONE                        |
| WO 2009005809                             | A2                  | 08-01-2009                  | AU 2008270972 A1 08-01-2009 |
|   |                     | CA 2691378 A1 08-01-2009    |                             |
|   |                     | EP 2173379 A2 14-04-2010    |                             |
|   |                     | JP 2010532169 A 07-10-2010  |                             |
|   |                     | JP 2014144016 A 14-08-2014  |                             |
|   |                     | US 2009074782 A1 19-03-2009 |                             |
|   |                     | US 2009191205 A1 30-07-2009 |                             |
|   |                     | US 2013115206 A1 09-05-2013 |                             |
|   |                     | US 2013121993 A1 16-05-2013 |                             |
|   |                     | US 2013336970 A1 19-12-2013 |                             |
|   |                     | US 2014134177 A1 15-05-2014 |                             |
|   |                     | WO 2009005809 A2 08-01-2009 |                             |
| WO 2010037715                             | A1                  | 08-04-2010                  | AU 2009299927 A1 08-04-2010 |
|   |                     | CA 2738868 A1 08-04-2010    |                             |
|   |                     | CN 102170873 A 31-08-2011   |                             |
|   |                     | CN 103784457 A 14-05-2014   |                             |
|   |                     | EP 2346499 A1 27-07-2011    |                             |
|   |                     | EP 2617414 A2 24-07-2013    |                             |
|   |                     | JP 2012504573 A 23-02-2012  |                             |
|   |                     | KR 20110066200 A 16-06-2011 |                             |
|   |                     | MA 32734 B1 02-10-2011      |                             |
|   |                     | RU 2011117059 A 10-11-2012  |                             |
|   |                     | TW 201018672 A 16-05-2010   |                             |
|   |                     | US 2011183962 A1 28-07-2011 |                             |
|   |                     | US 2014200217 A1 17-07-2014 |                             |
|   |                     | WO 2010037715 A1 08-04-2010 |                             |
| WO 2011025838                             | A1                  | 03-03-2011                  | AU 2010286670 A1 29-03-2012 |
|   |                     | CA 2772301 A1 03-03-2011    |                             |
|   |                     | CN 102573832 A 11-07-2012   |                             |
|   |                     | CR 20120115 A 04-09-2012    |                             |
|   |                     | EP 2470173 A1 04-07-2012    |                             |
|   |                     | JP 2013503174 A 31-01-2013  |                             |
|   |                     | KR 20120053052 A 24-05-2012 |                             |
|   |                     | NZ 598588 A 30-05-2014      |                             |
|   |                     | RU 2012111235 A 10-10-2013  |                             |
|   |                     | SG 178873 A1 27-04-2012     |                             |
|   |                     | US 2013045240 A1 21-02-2013 |                             |
|   |                     | US 2014072630 A1 13-03-2014 |                             |
|   |                     | WO 2011025838 A1 03-03-2011 |                             |
| WO 2013037043                             | A1                  | 21-03-2013                  | AU 2012308057 A1 24-04-2014 |
|   |                     | CA 2752008 A1 13-03-2013    |                             |
|   |                     | CA 2848453 A1 21-03-2013    |                             |
|   |                     | EP 2755662 A1 23-07-2014    |                             |
|   |                     | US 2014343007 A1 20-11-2014 |                             |
|   |                     | WO 2013037043 A1 21-03-2013 |                             |
| WO 2007008603                             | A1                  | 18-01-2007                  | AU 2006269422 A1 18-01-2007 |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2014/056017

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
|---|---------------------|----------------------------|---------------------|
|   |                     | CA 2614436 A1              | 18-01-2007          |
|   |                     | EP 1917020 A1              | 07-05-2008          |
|   |                     | EP 2722051 A1              | 23-04-2014          |
|   |                     | JP 5171621 B2              | 27-03-2013          |
|   |                     | JP 2009500424 A            | 08-01-2009          |
|   |                     | JP 2012144576 A            | 02-08-2012          |
|   |                     | US 2009018086 A1           | 15-01-2009          |
|   |                     | US 2013123465 A1           | 16-05-2013          |
|   |                     | WO 2007008603 A1           | 18-01-2007          |
| -----                                     |                     |                            |                     |
| WO 2013149159                             | A1                  | 03-10-2013                 |                     |
|   |                     | AU 2013237892 A1           | 28-08-2014          |
|   |                     | CA 2864311 A1              | 03-10-2013          |
|   |                     | CO 7071096 A2              | 30-09-2014          |
|   |                     | TW 201343674 A             | 01-11-2013          |
|   |                     | US 2013336885 A1           | 19-12-2013          |
|   |                     | WO 2013149159 A1           | 03-10-2013          |
| -----                                     |                     |                            |                     |

# SEQUENCE LISTING

<110> GENENTECH, INC. ET AL.

<120> METHODS OF USING ANTI-LGR5 ANTIBODIES

<130> P5706R1-WO

<140> Frederic J. De Sauvage

<141> Brian Biehs

<150> 61/879,089

<151> 2013-09-17

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| Asp | Ile | Val | Met | Thr | Gln | Ser | Pro | Asp | Ser | Leu | Ala | Val | Ser | Leu | Gly |
| 1   |     |     |     | 5   |     |     |     | 10  |     |     |     |     |     | 15  |     |

Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Glu Ser Val Asp Asn Tyr

20

25

30

Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro  
 35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Asp  
 50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser  
 65 70 75 80

Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Asn Tyr  
 85 90 95

Glu Asp Pro Phe Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg  
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Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Ala Tyr  
 20 25 30

Trp Ile Glu Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile  
 35 40 45

Gly Glu Ile Leu Pro Gly Ser Asp Ser Thr Asp Tyr Asn Glu Lys Phe  
 50 55 60

Lys Val Arg Val Thr Ile Thr Ser Asp Thr Ser Thr Ser Thr Val Tyr  
 65 70 75 80

Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

Ala Arg Gly Gly His Tyr Gly Ser Leu Asp Tyr Trp Gly Gln Gly Thr  
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Leu Val Thr Val Ser Ser  
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Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Glu Ser Val Asp Asn Tyr  
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Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro  
35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Asp  
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser  
65 70 75 80

Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Asn Tyr  
85 90 95

Glu Asp Pro Phe Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg  
100 105 110

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Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
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Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Ala Tyr  
20 25 30

Trp Ile Glu Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile  
35 40 45

Gly Glu Ile Leu Pro Gly Ser Asp Ser Thr Asp Tyr Asn Glu Lys Phe  
50 55 60

Lys Val Arg Ala Thr Phe Thr Ser Asp Thr Ser Thr Ser Thr Val Tyr  
65 70 75 80

Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Gly Gly His Tyr Gly Ser Leu Asp Tyr Trp Gly Gln Gly Thr  
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35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Asp  
50 55 60

Arg Phe Ser Gly Ser Gly Ser Arg Thr Asp Phe Thr Leu Thr Ile Ser  
65 70 75 80

Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Asn Tyr  
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Gly Glu Ile Leu Pro Gly Ser Asp Ser Thr Asp Tyr Asn Glu Lys Phe  
50 55 60

Lys Val Arg Val Thr Ile Thr Ser Asp Thr Ser Thr Ser Thr Val Tyr  
65 70 75 80

Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
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20 25 30

Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro  
35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Asp  
50 55 60

Arg Phe Ser Gly Ser Gly Ser Arg Thr Asp Phe Thr Leu Thr Ile Ser  
65 70 75 80

Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Asn Tyr  
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Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Ala Tyr  
20 25 30

Trp Ile Glu Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile  
35 40 45

Gly Glu Ile Leu Pro Gly Ser Asp Ser Thr Asp Tyr Asn Glu Lys Phe  
50 55 60

Lys Val Arg Ala Thr Phe Thr Ser Asp Thr Ser Thr Ser Thr Val Tyr  
65 70 75 80

Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
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Ala Arg Gly Gly His Tyr Gly Ser Leu Asp Tyr Trp Gly Gln Gly Thr  
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Leu Val Thr Val Ser Ser  
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Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Glu Ser Val Asp Asn Tyr  
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Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro  
35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Asp  
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser  
65 70 75 80

Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Asn Tyr  
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Glu Asp Pro Phe Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg

100

105

110

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1 5 10 15Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Ala Tyr  
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35 40 45Gly Glu Ile Leu Pro Gly Ser Asp Ser Thr Asp Tyr Asn Glu Lys Phe  
50 55 60Lys Val Arg Val Thr Ile Thr Arg Asp Thr Ser Thr Ser Thr Ala Tyr  
65 70 75 80Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95Ala Arg Gly Gly His Tyr Gly Ser Leu Asp Tyr Trp Gly Gln Gly Thr  
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&lt;400&gt; 15

Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly

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|---|-----|-----|-----|
| 1   | 5   | 10  | 15  |
| Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Glu Ser Val Asp Asn Tyr | 20  | 25  | 30  |
| Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro | 35  | 40  | 45  |
| Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Asp | 50  | 55  | 60  |
| Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser | 65  | 70  | 75  |
| Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Asn Tyr | 85  | 90  | 95  |
| Glu Asp Pro Phe Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg | 100 | 105 | 110 |

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| 1 5 10 15   |

|   |    |    |    |
|---|----|----|----|
| Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Ala Tyr | 20 | 25 | 30 |
|---|----|----|----|

|   |    |    |    |
|---|----|----|----|
| Trp Ile Glu Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile | 35 | 40 | 45 |
|---|----|----|----|

|   |    |    |    |
|---|----|----|----|
| Gly Glu Ile Leu Pro Gly Ser Asp Ser Thr Asp Tyr Asn Glu Lys Phe | 50 | 55 | 60 |
|---|----|----|----|

|   |    |    |    |    |
|---|----|----|----|----|
| Lys Val Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr | 65 | 70 | 75 | 80 |
|---|----|----|----|----|

Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
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Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro  
35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Asp  
50 55 60

Arg Phe Ser Gly Ser Gly Ser Arg Thr Asp Phe Thr Leu Thr Ile Ser  
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35 40 45

Gly Glu Ile Leu Pro Gly Ser Asp Ser Thr Asp Tyr Asn Glu Lys Phe  
50 55 60

Lys Val Arg Val Thr Ile Thr Arg Asp Thr Ser Thr Ser Thr Ala Tyr  
65 70 75 80

Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
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Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Glu Ser Val Asp Asn Tyr  
20 25 30

Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro  
35 40 45



Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Asp  
50 55 60

Arg Phe Ser Gly Ser Gly Ser Arg Thr Asp Phe Thr Leu Thr Ile Ser  
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Glu Asp Pro Phe Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg  
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Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Ala Tyr  
20 25 30

Trp Ile Glu Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile  
35 40 45

Gly Glu Ile Leu Pro Gly Ser Asp Ser Thr Asp Tyr Asn Glu Lys Phe  
50 55 60

Lys Val Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr  
65 70 75 80

Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
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<210> 22

<400> 22  
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<210> 23

<400> 23  
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<210> 24

<400> 24  
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<210> 25

<211> 108

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic  
polypeptide"

<400> 25

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala  
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile  
35 40 45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly  
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Thr Thr Pro Pro

85

90

95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg  
 100 105

&lt;210&gt; 26

&lt;211&gt; 116

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Description of Artificial Sequence: Synthetic polypeptide"

&lt;400&gt; 26

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Thr Ser Tyr  
 20 25 30

Ser Ile Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45

Ala Glu Ile Tyr Pro Pro Gly Gly Tyr Thr Asp Tyr Ala Asp Ser Val  
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr  
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

Ala Lys Ala Arg Leu Phe Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val  
 100 105 110

Thr Val Ser Ser  
 115

&lt;210&gt; 27

&lt;211&gt; 15

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Description of Artificial Sequence: Synthetic

peptide"

<400> 27

Arg Ala Ser Glu Ser Val Asp Asn Tyr Gly Asn Ser Phe Met His  
1 5 10 15

<210> 28

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic  
peptide"

<400> 28

Leu Ala Ser Asn Leu Glu Ser  
1 5

<210> 29

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic  
peptide"

<400> 29

Gln Gln Asn Tyr Glu Asp Pro Phe Thr  
1 5

<210> 30

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic  
peptide"

<400> 30

Gly Tyr Thr Phe Ser Ala Tyr Trp Ile Glu  
1 5 10

<210> 31

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<221> source  
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 31  
Glu Ile Leu Pro Gly Ser Asp Ser Thr Asp Tyr Asn Glu Lys Phe Lys  
1 5 10 15

Val

<210> 32  
<211> 9  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 32  
Gly Gly His Tyr Gly Ser Leu Asp Tyr  
1 5

<210> 33  
<211> 23  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 33  
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly  
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys  
20

<210> 34  
<211> 15  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 34

Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr  
1 5 10 15

<210> 35  
<211> 32  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic  
polypeptide"

<400> 35  
Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr  
1 5 10 15

Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys  
20 25 30

<210> 36  
<211> 32  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic  
polypeptide"

<400> 36  
Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Arg Thr Asp Phe Thr  
1 5 10 15

Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys  
20 25 30

<210> 37  
<211> 11  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic  
peptide"

<400> 37  
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg  
1 5 10

<210> 38

<211> 25  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 38  
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser  
20 25

<210> 39  
<211> 14  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 39  
Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile Gly  
1 5 10

<210> 40  
<211> 32  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 40  
Arg Val Thr Ile Thr Ser Asp Thr Ser Thr Ser Thr Val Tyr Leu Glu  
1 5 10 15

Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg  
20 25 30

<210> 41  
<211> 32  
<212> PRT  
<213> Artificial Sequence

<220>

<221> source  
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 41  
Arg Ala Thr Phe Thr Ser Asp Thr Ser Thr Ser Thr Val Tyr Leu Glu  
1 5 10 15

Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg  
20 25 30

<210> 42  
<211> 32  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 42  
Arg Val Thr Ile Thr Arg Asp Thr Ser Thr Ser Thr Ala Tyr Leu Glu  
1 5 10 15

Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg  
20 25 30

<210> 43  
<211> 32  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 43  
Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr Leu Glu  
1 5 10 15

Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg  
20 25 30

<210> 44  
<211> 11  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source



<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 44

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser  
1 5 10

<210> 45

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 45

Arg Ser Ser Gln Ser Leu Val His Ser Asn Gly Asn Thr Tyr Leu Gln  
1 5 10 15

<210> 46

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 46

Lys Val Ser Asn Arg Phe Ser  
1 5

<210> 47

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 47

Ser Gln Ser Thr His Phe Pro Tyr Thr  
1 5

<210> 48

<211> 10

<212> PRT

<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 48  
Val Asp Thr Phe Asn Ser Tyr Trp Met His  
1 5 10

<210> 49  
<211> 17  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 49  
Glu Ile Asn Pro Ser Asn Gly Arg Thr Asn Tyr Ile Glu Lys Phe Lys  
1 5 10 15

Asn

<210> 50  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 50  
Gly Trp Tyr Phe Asp Val  
1 5

<210> 51  
<211> 17  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 51  
Lys Ser Ser Gln Ser Leu Leu Asn Ser Gly Asn Gln Lys Asn Tyr Leu  
1 5 10 15

Thr

<210> 52

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 52

Trp Ala Ser Thr Arg Glu Ser  
1 5

<210> 53

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 53

Gln Asn Asp Tyr Ser Phe Pro Phe Thr  
1 5

<210> 54

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 54

Gly Tyr Ser Phe Thr Gly Tyr Thr Met Asn  
1 5 10

<210> 55

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

peptide"

<400> 55

Leu Ile Asn Cys Tyr Asn Gly Gly Thr Asn Tyr Asn Gln Lys Phe Lys  
1 5 10 15

Gly

<210> 56

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic  
peptide"

<400> 56

Gly Gly Ser Thr Met Ile Thr Pro Arg Phe Ala Tyr  
1 5 10

<210> 57

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic  
peptide"

<400> 57

Arg Ala Ser Gln Asp Val Ser Thr Ala Val Ala  
1 5 10

<210> 58

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic  
peptide"

<400> 58

Ser Ala Ser Phe Leu Tyr Ser  
1 5

<210> 59

<211> 9

<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 59  
Gln Gln Ser Tyr Thr Thr Pro Pro Thr  
1 5

<210> 60  
<211> 10  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 60  
Gly Phe Thr Phe Thr Ser Tyr Ser Ile Ser  
1 5 10

<210> 61  
<211> 17  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 61  
Glu Ile Tyr Pro Pro Gly Gly Tyr Thr Asp Tyr Ala Asp Ser Val Lys  
1 5 10 15

Gly

<210> 62  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 62

Ala Arg Leu Phe Phe Asp Tyr  
1 5

<210> 63  
<211> 218  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 63  
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly  
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Glu Ser Val Asp Asn Tyr  
20 25 30

Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro  
35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Asp  
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser  
65 70 75 80

Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Asn Tyr  
85 90 95

Glu Asp Pro Phe Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg  
100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln  
115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr  
130 135 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser  
145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr  
165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys  
180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro  
195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
210 215

<210> 64

<211> 448

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic  
polypeptide"

<400> 64

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Ala Tyr  
20 25 30

Trp Ile Glu Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile  
35 40 45

Gly Glu Ile Leu Pro Gly Ser Asp Ser Thr Asp Tyr Asn Glu Lys Phe  
50 55 60

Lys Val Arg Ala Thr Phe Thr Ser Asp Thr Ser Thr Ser Thr Val Tyr  
65 70 75 80

Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Gly Gly His Tyr Gly Ser Leu Asp Tyr Trp Gly Gln Gly Thr  
100 105 110

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro  
115 120 125

Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly  
130 135 140

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn  
145 150 155 160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln  
165 170 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser  
180 185 190

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser  
195 200 205

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr  
210 215 220

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser  
225 230 235 240

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg  
245 250 255

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro  
260 265 270

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala  
275 280 285

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val  
290 295 300

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr  
305 310 315 320

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr  
325 330 335

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu  
340 345 350

Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys  
355 360 365



Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser  
370 375 380

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp  
385 390 395 400

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser  
405 410 415

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala  
420 425 430

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
435 440 445

<210> 65  
<211> 214  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic  
polypeptide"

<400> 65  
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala  
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile  
35 40 45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly  
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Thr Thr Pro Pro  
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala  
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly  
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala  
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln  
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser  
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr  
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser  
195 200 205

Phe Asn Arg Gly Glu Cys  
210

<210> 66  
<211> 446  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic  
polypeptide"

<400> 66  
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Thr Ser Tyr  
20 25 30

Ser Ile Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
35 40 45

Ala Glu Ile Tyr Pro Pro Gly Gly Tyr Thr Asp Tyr Ala Asp Ser Val  
50 55 60

Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr  
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Lys Ala Arg Leu Phe Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val  
100 105 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala  
115 120 125

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu  
130 135 140

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly  
145 150 155 160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser  
165 170 175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu  
180 185 190

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr  
195 200 205

Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr  
210 215 220

Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe  
225 230 235 240

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro  
245 250 255

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val  
260 265 270

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr  
275 280 285

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val  
290 295 300

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys  
305 310 315 320

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser  
325 330 335

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro  
340 345 350

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val  
355 360 365

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly  
370 375 380

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp  
385 390 395 400

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp  
405 410 415

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His  
420 425 430

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
435 440 445

<210> 67  
<211> 907  
<212> PRT  
<213> Homo sapiens

<400> 67  
Met Asp Thr Ser Arg Leu Gly Val Leu Leu Ser Leu Pro Val Leu Leu  
1 5 10 15

Gln Leu Ala Thr Gly Gly Ser Ser Pro Arg Ser Gly Val Leu Leu Arg  
20 25 30

Gly Cys Pro Thr His Cys His Cys Glu Pro Asp Gly Arg Met Leu Leu  
35 40 45

Arg Val Asp Cys Ser Asp Leu Gly Leu Ser Glu Leu Pro Ser Asn Leu  
50 55 60

Ser Val Phe Thr Ser Tyr Leu Asp Leu Ser Met Asn Asn Ile Ser Gln  
65 70 75 80

Leu Leu Pro Asn Pro Leu Pro Ser Leu Arg Phe Leu Glu Glu Leu Arg  
85 90 95

Leu Ala Gly Asn Ala Leu Thr Tyr Ile Pro Lys Gly Ala Phe Thr Gly  
100 105 110

Leu Tyr Ser Leu Lys Val Leu Met Leu Gln Asn Asn Gln Leu Arg His  
115 120 125

Val Pro Thr Glu Ala Leu Gln Asn Leu Arg Ser Leu Gln Ser Leu Arg  
130 135 140

Leu Asp Ala Asn His Ile Ser Tyr Val Pro Pro Ser Cys Phe Ser Gly  
145 150 155 160

Leu His Ser Leu Arg His Leu Trp Leu Asp Asp Asn Ala Leu Thr Glu  
165 170 175

Ile Pro Val Gln Ala Phe Arg Ser Leu Ser Ala Leu Gln Ala Met Thr  
180 185 190

Leu Ala Leu Asn Lys Ile His His Ile Pro Asp Tyr Ala Phe Gly Asn  
195 200 205

Leu Ser Ser Leu Val Val Leu His Leu His Asn Asn Arg Ile His Ser  
210 215 220

Leu Gly Lys Lys Cys Phe Asp Gly Leu His Ser Leu Glu Thr Leu Asp  
225 230 235 240

Leu Asn Tyr Asn Asn Leu Asp Glu Phe Pro Thr Ala Ile Arg Thr Leu  
245 250 255

Ser Asn Leu Lys Glu Leu Gly Phe His Ser Asn Asn Ile Arg Ser Ile  
260 265 270

Pro Glu Lys Ala Phe Val Gly Asn Pro Ser Leu Ile Thr Ile His Phe  
275 280 285

Tyr Asp Asn Pro Ile Gln Phe Val Gly Arg Ser Ala Phe Gln His Leu  
290 295 300

Pro Glu Leu Arg Thr Leu Thr Leu Asn Gly Ala Ser Gln Ile Thr Glu  
305 310 315 320

Phe Pro Asp Leu Thr Gly Thr Ala Asn Leu Glu Ser Leu Thr Leu Thr  
325 330 335

Gly Ala Gln Ile Ser Ser Leu Pro Gln Thr Val Cys Asn Gln Leu Pro  
340 345 350

Asn Leu Gln Val Leu Asp Leu Ser Tyr Asn Leu Leu Glu Asp Leu Pro  
355 360 365

Ser Phe Ser Val Cys Gln Lys Leu Gln Lys Ile Asp Leu Arg His Asn  
370 375 380

Glu Ile Tyr Glu Ile Lys Val Asp Thr Phe Gln Gln Leu Leu Ser Leu  
385 390 395 400

Arg Ser Leu Asn Leu Ala Trp Asn Lys Ile Ala Ile Ile His Pro Asn  
405 410 415

Ala Phe Ser Thr Leu Pro Ser Leu Ile Lys Leu Asp Leu Ser Ser Asn  
420 425 430

Leu Leu Ser Ser Phe Pro Ile Thr Gly Leu His Gly Leu Thr His Leu  
435 440 445

Lys Leu Thr Gly Asn His Ala Leu Gln Ser Leu Ile Ser Ser Glu Asn  
450 455 460

Phe Pro Glu Leu Lys Val Ile Glu Met Pro Tyr Ala Tyr Gln Cys Cys  
465 470 475 480

Ala Phe Gly Val Cys Glu Asn Ala Tyr Lys Ile Ser Asn Gln Trp Asn  
485 490 495

Lys Gly Asp Asn Ser Ser Met Asp Asp Leu His Lys Lys Asp Ala Gly  
500 505 510

Met Phe Gln Ala Gln Asp Glu Arg Asp Leu Glu Asp Phe Leu Leu Asp  
515 520 525

Phe Glu Glu Asp Leu Lys Ala Leu His Ser Val Gln Cys Ser Pro Ser  
530 535 540

Pro Gly Pro Phe Lys Pro Cys Glu His Leu Leu Asp Gly Trp Leu Ile  
545 550 555 560

Arg Ile Gly Val Trp Thr Ile Ala Val Leu Ala Leu Thr Cys Asn Ala  
565 570 575

Leu Val Thr Ser Thr Val Phe Arg Ser Pro Leu Tyr Ile Ser Pro Ile  
580 585 590

Lys Leu Leu Ile Gly Val Ile Ala Ala Val Asn Met Leu Thr Gly Val  
595 600 605

Ser Ser Ala Val Leu Ala Gly Val Asp Ala Phe Thr Phe Gly Ser Phe  
610 615 620

Ala Arg His Gly Ala Trp Trp Glu Asn Gly Val Gly Cys His Val Ile  
625 630 635 640

Gly Phe Leu Ser Ile Phe Ala Ser Glu Ser Ser Val Phe Leu Leu Thr  
645 650 655

Leu Ala Ala Leu Glu Arg Gly Phe Ser Val Lys Tyr Ser Ala Lys Phe  
660 665 670

Glu Thr Lys Ala Pro Phe Ser Ser Leu Lys Val Ile Ile Leu Leu Cys  
675 680 685

Ala Leu Leu Ala Leu Thr Met Ala Ala Val Pro Leu Leu Gly Gly Ser  
690 695 700

Lys Tyr Gly Ala Ser Pro Leu Cys Leu Pro Leu Pro Phe Gly Glu Pro  
705 710 715 720

Ser Thr Met Gly Tyr Met Val Ala Leu Ile Leu Leu Asn Ser Leu Cys  
725 730 735

Phe Leu Met Met Thr Ile Ala Tyr Thr Lys Leu Tyr Cys Asn Leu Asp  
740 745 750

Lys Gly Asp Leu Glu Asn Ile Trp Asp Cys Ser Met Val Lys His Ile  
755 760 765

Ala Leu Leu Leu Phe Thr Asn Cys Ile Leu Asn Cys Pro Val Ala Phe  
770 775 780

Leu Ser Phe Ser Ser Leu Ile Asn Leu Thr Phe Ile Ser Pro Glu Val  
785 790 795 800

Ile Lys Phe Ile Leu Leu Val Val Val Pro Leu Pro Ala Cys Leu Asn  
805 810 815

Pro Leu Leu Tyr Ile Leu Phe Asn Pro His Phe Lys Glu Asp Leu Val  
820 825 830

Ser Leu Arg Lys Gln Thr Tyr Val Trp Thr Arg Ser Lys His Pro Ser  
835 840 845

Leu Met Ser Ile Asn Ser Asp Asp Val Glu Lys Gln Ser Cys Asp Ser  
850 855 860

Thr Gln Ala Leu Val Thr Phe Thr Ser Ser Ser Ile Thr Tyr Asp Leu  
865 870 875 880

Pro Pro Ser Ser Val Pro Ser Pro Ala Tyr Pro Val Thr Glu Ser Cys  
885 890 895

His Leu Ser Ser Val Ala Phe Val Pro Cys Leu  
900 905



## 摘要

本文中提供的是使用抗 LGR5 抗体的方法,例如用于治疗 Hedgehog 相关疾病,包括基底细胞癌。

