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(71) Applicant: **GENENTECH, INC.** [US/US]; 1 DNA Way, South San Francisco, California 94080 (US).

(72) Inventors; and
(71) Applicants : **HONGO, Jo-Anne** [US/US]; 1 DNA Way, South San Francisco, California 94080 (US). **MAO, Weiguang** [US/US]; 1 DNA Way, South San Francisco, California 94080 (US). **POLAKIS, Paul** [US/US]; 1 DNA Way, South San Francisco, California 94080 (US). **POLSON, Andrew** [US/US]; 1 DNA Way, South San Francisco, CA 94080 (US). **VIJ, Rajesh** [CA/US]; 1 DNA Way, South San Francisco, California 94080 (US). **WU, Yan** [US/US]; 1 DNA Way, South San Francisco, California 94080 (US). **LIANG, Wei-Ching** [US/US]; 1 DNA Way, South San Francisco, California 94080 (US).

(74) Agent: **SCARR, Rebecca, B.**; Casimir Jones, S.C., 2275 Deming Way, Ste 310, Middleton, Wisconsin 53562 (US).

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(54) Title: ANTI-LGR5 ANTIBODIES AND IMMUNOCONJUGATES

(57) Abstract: The invention provides anti-LgR5 antibodies and immunoconjugates and methods of using the same.

ANTI-LGR5 ANTIBODIES AND IMMUNOCONJUGATES

FIELD OF THE INVENTION

[001] The present invention relates to anti-LgR5 antibodies and immunoconjugates and methods of using the same.

BACKGROUND

[002] Leucine-rich repeat-containing G protein-coupled receptor 5 (LgR5) is a seven-transmembrane protein found on the surface of actively cycling intestinal stem cells (ISCs). LgR5-expressing ISCs are sensitive to Wnt modulation and are primarily responsible for homeostatic regeneration of the intestinal epithelium. Elimination of LgR5-expressing cells in mice does not affect homeostasis of intestinal epithelium, however, suggesting that other cell types can compensate for loss of this cell population. Tian et al., *Nature* 478: 255-259 (2011). R-spondins enhance WNT signaling by WNT3A, and all four R-spondins, RSPO1, RSPO2, RSPO3, and RSPO4, are able to bind to LgR5. Lau et al., *Nature* 476: 293-297 (2011).

[003] 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.

[004] There is a need in the art for agents that target LgR5 for the diagnosis and treatment of LgR5-associated conditions, such as cancer. The invention fulfills that need and provides other benefits.

SUMMARY

[005] The invention provides anti-LgR5 antibodies and immunoconjugates and methods of using the same.

[006] In some embodiments, an isolated antibody that binds to LgR5 is provided. In some embodiments, the 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 and/or binds to an epitope within amino acids 22-123 of SEQ ID NO: 67 and/or binds to an epitope within amino acids 22-323 of SEQ ID NO: 67 and/or binds to an epitope within amino acids 22-424 of SEQ ID NO: 67 and/or binds to an epitope within amino acids 324-555 of SEQ ID NO: 67 and/or binds to an epitope within amino acids 324-424 of SEQ ID NO: 67; (b) binds LgR5 with an affinity of ≤ 5 nM, or ≤ 4 nM, or ≤ 3 nM, or ≤ 2 nM, or ≤ 1 nM, and optionally ≥ 0.0001 nM, or ≥ 0.001 nM, or ≥ 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.

[007] In some embodiments, the isolated anti-LgR5 antibody binds to an epitope within amino acids 22-323 of SEQ ID NO: 67 with an affinity of ≤ 5 nM, or ≤ 4 nM, or ≤ 3 nM, or ≤ 2 nM, or ≤ 1 nM, and optionally ≥ 0.0001 nM, or ≥ 0.001 nM, or ≥ 0.01 nM. .

[008] In some embodiments, the isolated anti-LgR5 antibody binds to an epitope within amino acids 22-123 of SEQ ID NO: 67 with an affinity of ≤ 5 nM, or ≤ 4 nM, or ≤ 3 nM, or ≤ 2 nM, or ≤ 1 nM, and optionally ≥ 0.0001 nM, or ≥ 0.001 nM, or ≥ 0.01 nM.

[009] In some embodiments, the isolated anti-LgR5 antibody binds to an epitope within amino acids 324-424 of SEQ ID NO: 67 with an affinity of ≤ 5 nM, or ≤ 4 nM, or ≤ 3 nM, or ≤ 2 nM, or ≤ 1 nM, and optionally ≥ 0.0001 nM, or ≥ 0.001 nM, or ≥ 0.01 nM.

[010] In some embodiments of any of the isolated anti-LgR5 antibodies, the anti-LgR5 antibody does not significantly disrupt the binding of R-spondin (RSPO) to LgR5. In some embodiments of any of the isolated anti-LgR5 antibodies, the anti-LgR5 antibody does not significantly disrupt wnt/beta-catenin signaling. In some embodiments of any of the isolated anti-LgR5 antibodies, the anti-LgR5 antibody does not significantly disrupt RSPO activation of LgR5 signaling. In some embodiments of any of the isolated anti-LgR5 antibodies, the anti-LgR5 antibody activates caspase 3 cleavage. In some embodiments of any of the isolated anti-LgR5 antibodies, the anti-LgR5 antibody recognizes both human and rodent LgR5. In some embodiments of any of the isolated anti-LgR5 antibodies, the anti-LgR5 antibody recognizes human LgR5 but not rodent LgR5. In some embodiments of any of the isolated anti-LgR5 antibodies, the anti-LgR5 antibody does not significantly inhibit tumor growth in its unconjugated form. In some embodiments of any of the isolated anti-LgR5 antibodies, the anti-LgR5 antibody does not induce stem cell differentiation.

[011] In some embodiments of any of the isolated anti-LgR5 antibodies, the anti-LgR5 antibody is a monoclonal antibody. In some embodiments of any of the isolated anti-LgR5 antibodies, the anti-LgR5 antibody is a human, humanized, or chimeric antibody. In some embodiments of any of the isolated anti-LgR5 antibodies, the anti-LgR5 antibody antibody is an IgG1, IgG2a or IgG2b antibody. In some embodiments of any of the isolated anti-LgR5 antibodies, the anti-LgR5 antibody is an antibody fragment that binds LgR5. In some embodiments of any of the isolated anti-LgR5 antibodies, LgR5 is human LgR5 of SEQ ID NO: 67.

[012] In some embodiments, an antibodythat binds LgR5 binds an epitope within amino acids 22-323 of SEQ ID NO: 67. In some embodiments, the antibody binds to LgR5 with an affinity of ≤ 5 nM. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a human, humanized, or chimeric antibody. In some embodiments, the antibody is an IgG1, IgG2a or IgG2b antibody. In some embodiments, the antibody is an antibody fragment that binds LgR5. In some embodiments, LgR5 is human LgR5 of SEQ ID NO: 67.

[013] In some embodiments, the antibody comprises (a)HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32, (b) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 29, and (c) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 31. In some embodiments, the antibody comprises (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, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32. In some embodiments, the antibody further comprises a heavy chain framework FR3 sequence of SEQ ID NO: 41. In some embodiments, the antibody further comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 27, (b) 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. In some embodiments, an isolated antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 27, (b) 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. In some embodiments, the antibody further comprises a light chain framework FR3 sequence of SEQ ID NO: 35.

[014] In some embodiments, an isolated antibody comprises (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 8; or (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:7; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some embodiments, the antibody comprises a VH sequence of SEQ ID NO: 8. In some embodiments, the antibody comprises a VL sequence of SEQ ID NO: 7. In some embodiments, and isolated antibody comprises a VH sequence of SEQ ID NO: 8 and a VL sequence of SEQ ID NO: 7.

[015] In some embodiments, an antibody that binds LgR5 comprises (a)HVR-H3 comprising the amino acid sequence of SEQ ID NO: 56, (b) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 53, and (c) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 55. In some embodiments, the antibody comprises (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, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 56. In some embodiments, the antibody further comprises (a) HVR-L1 comprising the amino acid sequence of

SEQ ID NO: 51, (b) 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. In some embodiments, an isolated antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 51, (b) 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.

[016] In some embodiments, an isolated antibody comprises (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 24; or (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:23; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some embodiments, the antibody comprises a VH sequence of SEQ ID NO: 24. In some embodiments, the antibody comprises a VL sequence of SEQ ID NO: 23. In some embodiments, and isolated antibody comprises a VH sequence of SEQ ID NO: 24 and a VL sequence of SEQ ID NO: 23.

[017] In some embodiments, an antibody that binds LgR5 comprises (a)HVR-H3 comprising the amino acid sequence of SEQ ID NO: 50, (b) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 47, and (c) HVR-H2 comprising the amino acid sequence of SEQ ID NO:495. In some embodiments, the antibody comprises (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, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 50. In some embodiments, the antibody further comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 45, (b) 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. In some embodiments, an isolated antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 45, (b) 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.

[018] In some embodiments, an isolated antibody comprises (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 22; or (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:21; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some embodiments, the antibody comprises a VH sequence of SEQ ID NO: 22. In some embodiments, the antibody comprises a VL sequence of SEQ ID NO: 21. In some embodiments, and isolated antibody comprises a VH sequence of SEQ ID NO: 22 and a VL sequence of SEQ ID NO: 21.

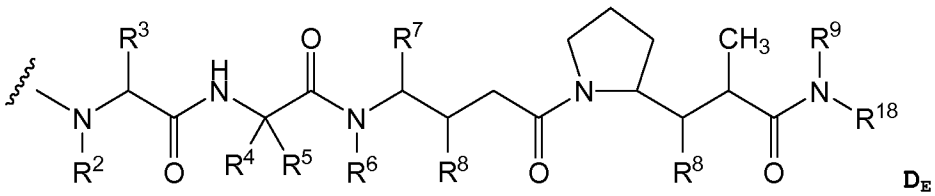
[019] In some embodiments, an antibody that binds LgR5 comprises (a)HVR-H3 comprising the amino acid sequence of SEQ ID NO: 62, (b) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 59, and (c) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 61. In some embodiments, the antibody comprises (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, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 62. In some embodiments, the antibody further comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 57, (b) 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. In some embodiments, an isolated antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 57, (b) 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.

[020] In some embodiments, an isolated antibody comprises (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 26; or (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:25; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some embodiments, the antibody comprises a VH sequence of SEQ ID NO: 26. In some embodiments, the antibody comprises a VL sequence of SEQ ID NO: 25. In some embodiments, and isolated antibody comprises a VH sequence of SEQ ID NO: 26 and a VL sequence of SEQ ID NO: 25.

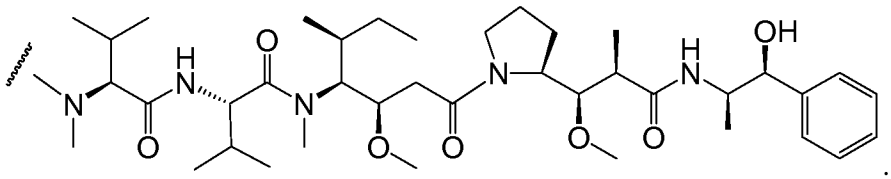
[021] In some embodiments, an isolated nucleic acid that encodes an antibody described herein is provided. In some embodiments, a host cell comprising the nucleic acid is provided. In some embodiments, a method of producing an antibody described herein is provided. In some embodiments, the method comprises culturing the host cell comprising the nucleic acid that encodes an antibody.

[022] In some embodiments, immunoconjugates are provided. In some embodiments, an immunoconjugate comprises an anti-LgR5 antibody and a cytotoxic agent. In some embodiments, an immunoconjugate has the formula Ab-(L-D)p, wherein: (a) Ab is an antibody described herein; (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. In some embodiments, D is an auristatin. In some such embodiments, D has formula D_E

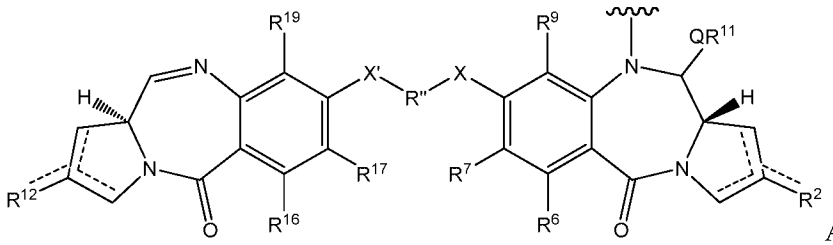


D_E

wherein R^2 and R^6 are each methyl, R^3 and R^4 are each isopropyl, R^5 is H, R^7 is sec-butyl, each R^8 is independently selected from CH_3, O-CH_3, OH, and H; R^9 is H; and R^{18} is $–C(R^8)_2–C(R^8)_2–$aryl. In some embodiments, D is MMAE having the structure:

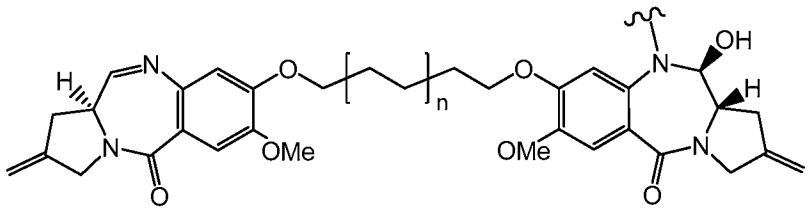


[023] In some embodiments, D is a pyrrolobenzodiazepine of Formula A:



A;

wherein 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_2, CN, R, OR, =CH-R^D, =$C(R^D)_2$, O-SO_2-R, CO_2R and COR, and optionally further selected from halo or dihalo, wherein R^D is independently selected from R, CO_2R, COR, CHO, CO_2H, and halo; R^6 and R^9 are independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', NO_2, Me_3Sn and halo; R^7 is independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', NO_2, Me_3Sn and halo; Q is independently selected from O, S and NH; R^{11} is either H, or R or, where Q is O, SO_3M, where M is a metal cation; R and R' are each independently selected from optionally substituted C_{1-8} alkyl, C_{1-12} alkyl, C_{3-8} heterocyclyl, C_{3-20} heterocyclyl, and C_{5-20} 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_{3-12} 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 such embodiments, D is



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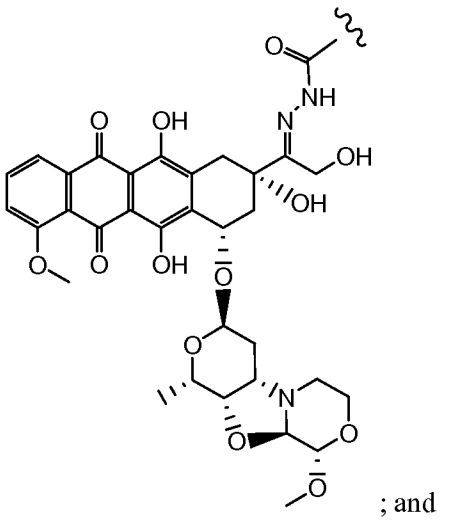
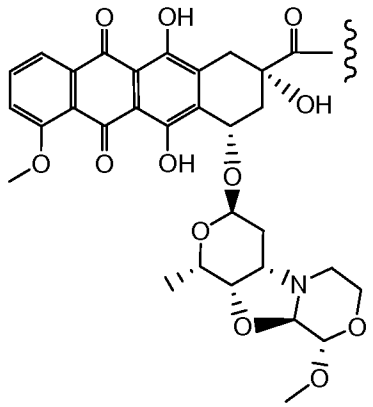
wherein n is 0 or 1.

[024] In some embodiments, D is a nemorubicin derivative. In some embodiments, D has a structure selected from:



; and



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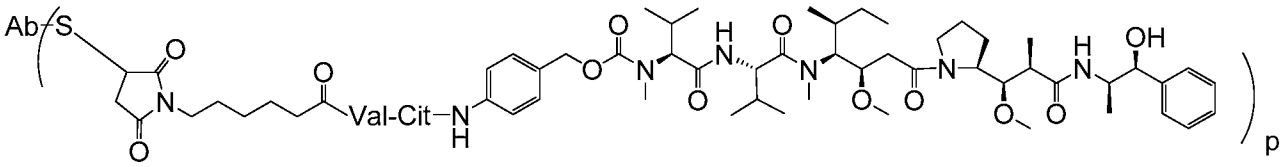
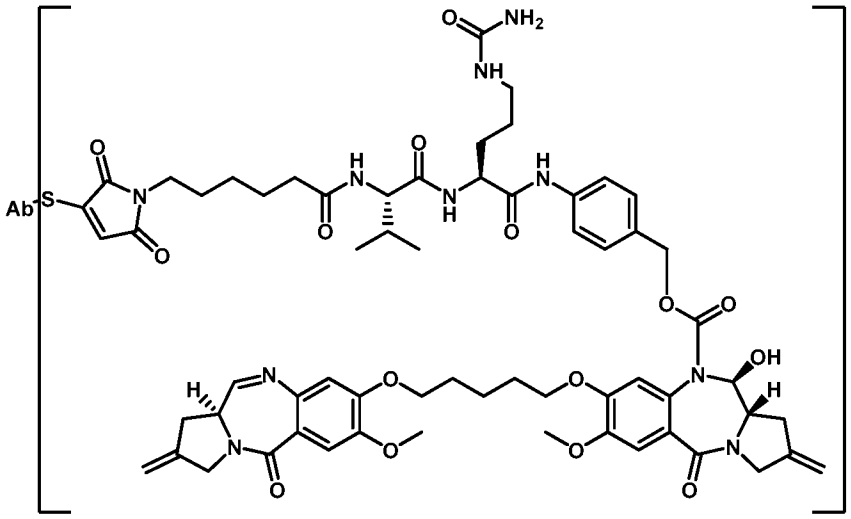
[025] In some embodiments, an immunoconjugate comprises a linker that is cleavable by a protease. In some embodiments, the linker comprises a val-cit dipeptide or a Phe-Lys dipeptide. In some embodiments, an immunoconjugate comprises a linker that is acid-labile. In some such embodiments, the linker comprises hydrazone.

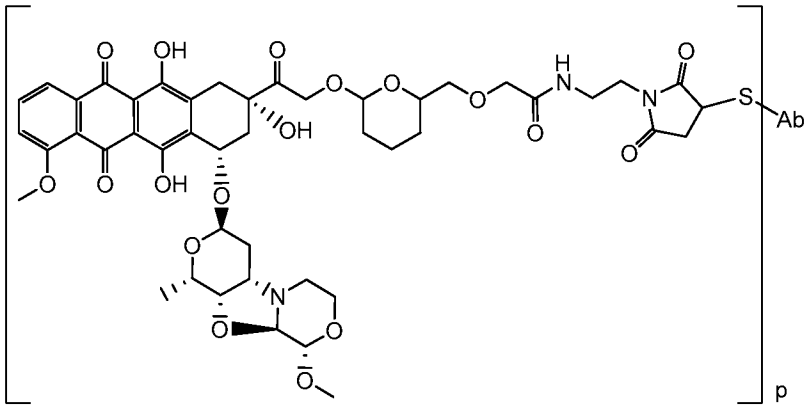
[026] In some embodiments, an immunoconjugate has a formula selected from:



wherein S is a sulfur atom;



p ;



p

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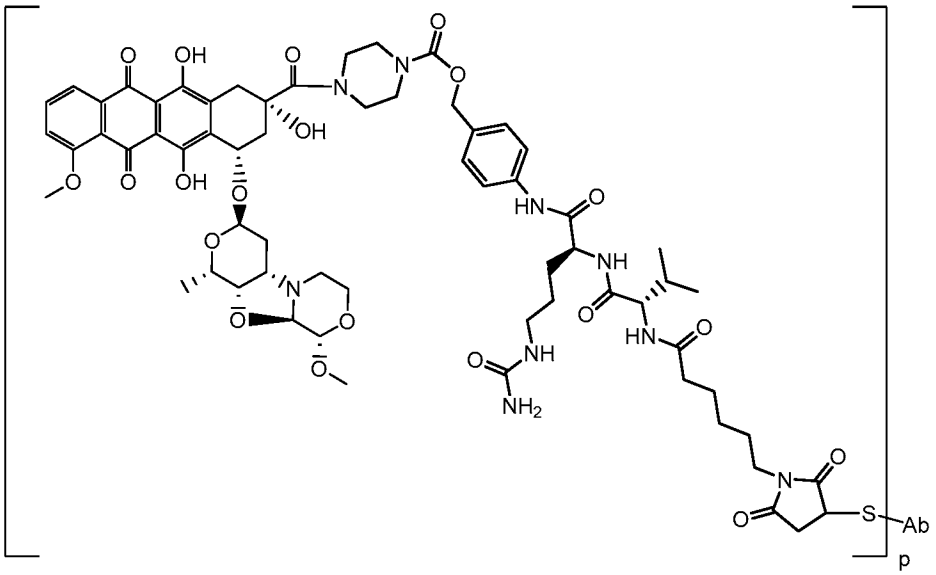
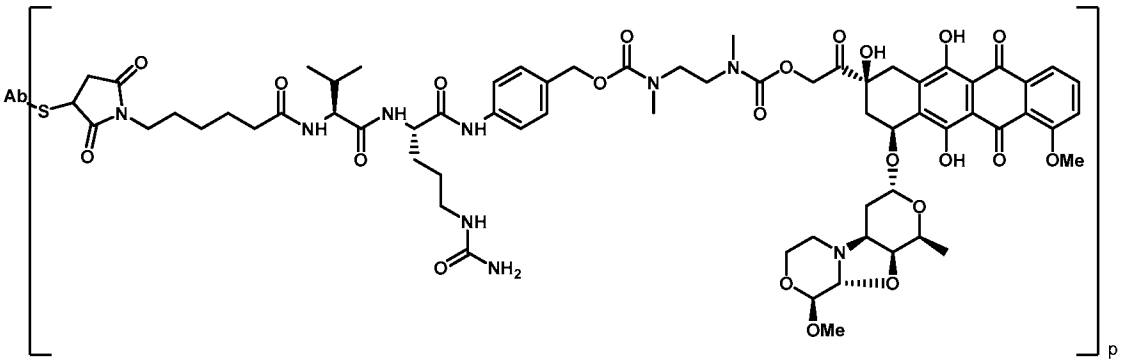


p ;



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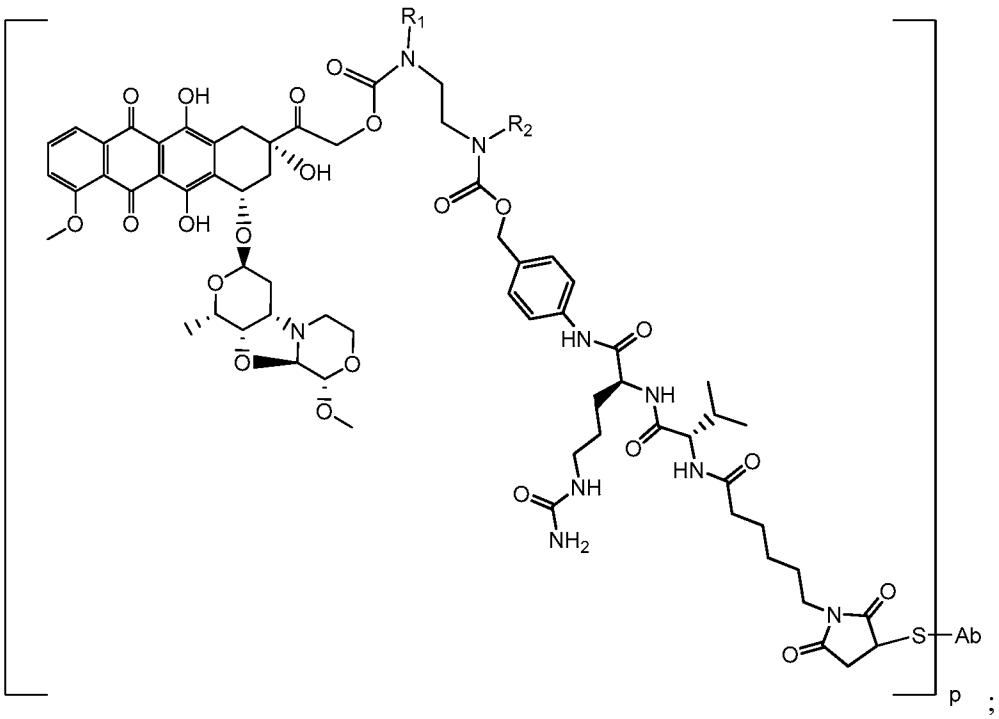
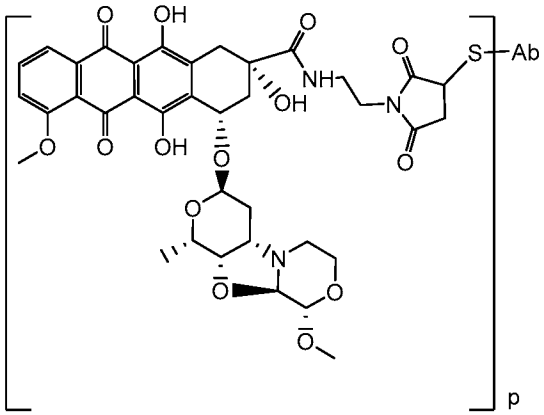


and



In some embodiments, p ranges from 2-5.

[027] In some embodiments, an immunoconjugate comprises an antibody that comprises (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, and (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. In some embodiments, an immunoconjugate comprises an antibody that comprises a VH sequence of SEQ ID NO: 8 and a VL sequence of SEQ ID NO: 7.

[028] In some embodiments, pharmaceutical formulations are provided. In some such embodiments, a pharmaceutical formulation comprises an immunoconjugate comprising an antibody that binds LgR5, *e.g.*, as described herein. In some embodiments, a pharmaceutical formulation further comprises an additional therapeutic agent. In some embodiments, the additional therapeutic agent is Avastin® (bevacizumab).

[029] In some embodiments, methods of treating individuals having LgR5 positive cancers are provided. In some such embodiments, a method comprises administering a pharmaceutical formulation comprising an immunoconjugate comprising an antibody that binds LgR5, *e.g.*, as described herein. In some embodiments, the LgR5-positive cancer is selected from colorectal cancer, pancreatic cancer, ovarian cancer, and endometrial cancer. In some embodiments, the LgR5-positive cancer is a small intestine cancer. In some embodiments, a small intestine cancer is a cancer of the duodenum, jejunum, and/or ilium. In some embodiments, a small intestine cancer is a cancer of the jejunum and/or ilium. In some embodiments, an LgR5-positive cancer comprises a Kras mutation, an APC mutation, or both a Kras mutation and an APC mutation (*e.g.*, in at least a portion of the cancer cells). In some embodiments, a method

comprises administering an additional therapeutic agent to the individual. In some such embodiments, the additional therapeutic agent is Avastin® (bevacizumab).

[030] In some embodiments, methods of inhibiting proliferation of an LgR5-positive cell are provided. In some embodiments, the method comprising exposing the cell to an immunoconjugate comprising an antibody that binds LgR5 under conditions permissive for binding of the immunoconjugate to LgR5 on the surface of the cell. In some embodiments, an antibody that binds LgR5 is an antibody described herein. In some embodiments, proliferation of the cell is thereby inhibited. In some embodiments, the cell is a colorectal, small intestine, pancreatic, ovarian, or endometrial cancer cell.

[031] In some embodiments, an antibody that binds LgR5 is conjugated to a label. In some embodiments, an antibody that binds LgR5 is an antibody described herein. In some embodiments, the label is a positron emitter. In some embodiments, the positron emitter is ^{89}Zr.

[032] In some embodiments, a method of detecting human LgR5 in a biological sample is provided. In some embodiments, a method comprises contacting the biological sample with an anti-LgR5 antibody under conditions permissive for binding of the anti-LgR5 antibody to a naturally occurring human LgR5, and detecting whether a complex is formed between the anti-LgR5 antibody and a naturally occurring human LgR5 in the biological sample. In some embodiments, an anti-LgR5 antibody is an antibody described herein. In some embodiments, the biological sample is a colorectal cancer sample, small intestine cancer sample, pancreatic cancer sample, ovarian cancer sample, or endometrial cancer sample.

[033] In some embodiments, a method for detecting an LgR5-positive cancer is provided. In some such embodiments, a method comprises (i) administering a labeled anti-LgR5 antibody to a subject having or suspected of having an LgR5-positive cancer, and (ii) detecting the labeled anti-LgR5 antibody in the subject, wherein detection of the labeled anti-LgR5 antibody indicates a LgR5-positive cancer in the subject. In some embodiments, an anti-LgR5 antibody is an antibody described herein.

BRIEF DESCRIPTION OF THE FIGURES

[034] **Figure 1** shows a graphic representation of the levels of human LgR5 gene expression in various tissues, as described in Example A. The inset in Figure 1 shows a graphic representation of the levels of human LgR5 gene expression in normal colon tissues and colon tumors, as described in Example A.

[035] **Figure 2** shows expression of LgR5 in colon tumors by in situ hybridization, as described in Example B.

[036] **Figure 3** shows (A) the prevalence of various levels of LgR5 expression in a colon tumor tissue microarray, and (B) the heterogeneity of LgR5 expression in three cores from each colorectal adenocarcinoma sample, both determined by in situ hybridization, as described in Example B.

[037] **Figure 4** shows the properties of certain anti-LgR5 monoclonal antibodies developed as described in Examples C through F.

[038] **Figure 5** shows an alignment of the light chain variable region sequences of murine antibody mu8E11 and humanized variants thereof (hu8E11.v1 to hu8E11.v8). The three hypervariable regions (HVRs: HVR1, HVR2, HVR3) are indicated by boxes in the sequences.

[039] **Figure 6** shows an alignment of the heavy chain variable region sequences of murine antibody mu8E11 and humanized variants thereof (hu8E11.v1 to hu8E11.v8). The three hypervariable regions (HVRs: HVR1, HVR2, HVR3) are indicated by boxes in the sequences.

[040] **Figure 7** shows the light chain variable region sequences of murine antibodies 3G12 and 2H6. The three hypervariable regions (HVRs: HVR1, HVR2, HVR3) are indicated by boxes in the sequences.

[041] **Figure 8** shows the heavy chain variable region sequences of murine antibodies 3G12 and 2H6. The three hypervariable regions (HVRs: HVR1, HVR2, HVR3) are indicated by boxes in the sequences.

[042] **Figure 9** shows affinity measurements of chimeric antibody ch8E11 and various humanized variants, as described in Example E.

[043] **Figure 10** shows the light chain variable region sequence of human antibody YW353. The three hypervariable regions (HVRs: HVR1, HVR2, HVR3) are indicated by boxes in the sequences.

[044] **Figure 11** shows the heavy chain variable region sequence of human antibody YW353. The three hypervariable regions (HVRs: HVR1, HVR2, HVR3) are indicated by boxes in the sequences.

[045] **Figure 12A-C** show an alignment of LgR5 from human, cynomolgus monkey, rat, and mouse.

[046] **Figure 13** shows that anti-LgR5 immunoconjugates demonstrate efficacy in LoVo colon cancer xenografts, as described in Example L.

[047] **Figure 14** shows that anti-LgR5 immunoconjugates demonstrate efficacy in D5124 pancreatic cancer xenografts, as described in Example M.

[048] **Figure 15** shows that huYW353-vcMMAE immunoconjugate demonstrates efficacy at 3, 6, and 12 mg/kg in D5124 pancreatic cancer xenografts, as described in Example M.

[049] **Figure 16** shows LgR5 mRNA expression in normal tissue and polyps from colons of AV and AKV mice, as described in Example N.

[050] **Figure 17** shows survival of AKV mice administered anti-LgR5 antibody and anti-LgR5 antibody-drug conjugate have longer survival times than control AKV mice, as described in Example N.

[051] **Figure 18** shows percentage of tumor area that is positive for cleaved caspase 3 in AKV mice administered a control ADC, an anti-LgR5 ADC, or an anti-LgR5 antibody, as described in Example N.

[052] **Figure 19** shows AKV mice administered anti-LgR5 antibody-drug conjugate have longer survival times than untreated AKV mice and AKV mice administered gp120-ADC or anti-LgR5, as described in Example N.

[053] **Figure 20** shows LgR5+ area in small intestine polyps and colon polyps in AKV *LgR5*$^{DTR/+}$ mice, as described in Example N.

[054] **Figure 21** shows (A) CC3+GFP+ area per cellular area in control ADC and anti-LgR5-ADC treated AKV *LgR5*$^{DTR/+}$ mice, and (B) exemplary immunohistochemistry staining in the control ADC and anti-LgR5-ADC treated AKV *LgR5*$^{DTR/+}$ mice, as described in Example N.

[055] **Figure 22** shows Ki67+ area per cellular area (either GFP+ cells or GFP- cells) in control ADC and anti-LgR5-ADC treated AKV *LgR5*$^{DTR/+}$ mice, as described in Example N.

[056] **Figure 23** shows the ratio of GFP intensity to GFP+ area in crypts and tumors of AKV *LgR5*$^{DTR/+}$ mice, as described in Example N.

[057] **Figure 24** shows that huYW353-vcMMAE, hu8E11v2-vcMMAE, and ch8E11-vcMMAE immunoconjugate demonstrates efficacy in D5124 pancreatic cancer xenografts, as described in Example O.

[058] **Figure 25** shows that hu8E11v2-vcMMAE immunoconjugate demonstrates efficacy in D5124 pancreatic cancer xenografts, as described in Example O.

[059] **Figure 26** shows that huYW353-vcMMAE and hu8E11v2-vcMMAE immunoconjugates demonstrate efficacy in LoVoX1.1 colon cancer xenografts, as described in Example P.

[060] **Figure 27** shows that hu8E11v2-vcMMAE immunoconjugate demonstrates efficacy in LoVoX1.1 colon cancer xenografts, as described in Example P.

[061] **Figure 28** shows that huYW353-vcMMAE, huYW353-acetal-PNU, and huYW353-vcPNU immunoconjugates demonstrate efficacy in D5124 pancreatic cancer xenografts, as described in Example Q.

[062] **Figure 29** shows that hu8E11v2-acetal-PNU, hu8E11v2-vcPNU, and hu8E11v2-PNU immunoconjugates demonstrate in D5124 pancreatic cancer xenografts, as described in Example R.

[063] **Figure 30** shows the results of administering certain hu8E11v2 immunoconjugates and control antibody immunoconjugates in LoVoX1.1 colon cancer xenografts, as described in Example S.

[064] **Figure 31** that hu8E11v2-acetal-PNU immunoconjugate demonstrates efficacy in LoVoX1.1 colon cancer xenografts in mice coadminstered excess control antibody, as described in Example S.

[065] **Figure 32** shows that an anti-LgR5 huYW353 PBD immunoconjugate demonstrates efficacy in D5124 pancreatic cancer xenografts, as described in Example T.

[066] **Figure 33** shows that an anti-LgR5 hu8E11v2 PBD immunoconjugate demonstrate efficacy in D5124 pancreatic cancer xenografts, as described in Example T.

[067] **Figure 34** shows that an anti-LgR5 hu8E11v2 PBD immunoconjugate demonstrates efficacy in a LoVoX1.1 colong cancer cancer xenograft, as described in Example U.

[068] **Figure 35** shows the structures of (A) an antibody-vcMMAE immunoconjugate, (B) an antibody-acetal-PNU immunoconjugate, (C) an antibody-acetal-PNU immunoconjugate, (D) an antibody-PNU immunoconjugate, and (E) an antibody-vcPBD immunoconjugate.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS OF THE INVENTION

I. DEFINITIONS

[069] 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.

[070] "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.

[071] 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.

[072] 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 (Kd) of $\leq$ 1μM, $\leq$ 100 nM, $\leq$ 10 nM, , $\leq$ 5 Nm, , $\leq$ 4 nM, , $\leq$ 3 nM, , $\leq$ 2 nM, $\leq$ 1 nM, $\leq$ 0.1 nM, $\leq$ 0.01 nM, or $\leq$ 0.001 nM (*e.g.*, 10^{-8} M or less, *e.g.* from 10^{-8} M to 10^{-13} M, *e.g.*, from 10^{-9} M to 10^{-13} M). In certain embodiments, an anti-LgR5 antibody binds to an epitope of LgR5 that is conserved among LgR5 from different species.

[073] 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.

[074] 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, $F(ab')_2$; diabodies; linear antibodies; single-chain antibody molecules (*e.g.* scFv); and multispecific antibodies formed from antibody fragments.

[075] 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.

[076] 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.

[077] 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.

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

[079] 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^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu); chemotherapeutic agents or drugs (*e.g.*, methotrexate, adriamicin, 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.

[080] "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.

[081] 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.

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

[083] 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.

[084] “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.

[085] 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.

[086] The term “glycosylated forms of LgR5” refers to naturally occurring forms of LgR5 that are post-translationally modified by the addition of carbohydrate residues.

[087] 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.

[088] 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.

[089] 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*.

[090] 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.

[091] 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*.

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

[093] 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.

[094] 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).

[095] 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.

[096] "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.

[097] 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 is shown in SEQ ID NO: 68. The predicted sequence for amino acids 33 to 907 of an exemplary cynomolgus monkey LgR5 is shown in SEQ ID NO: 69. The amino acid sequences for exemplary rat LgR5 precursor (with signal sequence, amino acids 1-21) and mature sequences are shown in SEQ ID NOs: 70 and 71, respectively. The amino acid sequences for exemplary mouse LgR5 precursor (with signal sequence, amino acids 1-21) and mature sequences are shown in SEQ ID NOs: 72 and 73, respectively.

[098] 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.

[099] The term "LgR5-positive cell" refers to a cell that expresses LgR5 on its surface.

[0100] 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.

[0101] 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.

[0102] "Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a

constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

[0103] 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.

[0104] "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.

[0105] In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid

sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

[0106] 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.

[0107] 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.

[0108] 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.

[0109] The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (*See*, *e.g.*, Kindt et al. *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. *See*, *e.g.*, Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

[0110] 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."

[0111] "Alkyl" is C_1-C_{18} hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms. Examples are methyl (Me, -CH_3), ethyl (Et, -CH_2CH_3), 1-propyl (n-Pr, n-propyl, -$CH_2CH_2CH_3$), 2-propyl (i-Pr, i-propyl, -$CH(CH_3)_2$), 1-butyl (n-Bu, n-butyl, -$CH_2CH_2CH_2CH_3$), 2-methyl-1-propyl (i-Bu, i-butyl, -$CH_2CH(CH_3)_2$), 2-butyl (s-Bu, s-butyl, -$CH(CH_3)CH_2CH_3$), 2-methyl-2-propyl (t-Bu, t-butyl, -$C(CH_3)_3$), 1-pentyl (n-pentyl, -$CH_2CH_2CH_2CH_2CH_3$), 2-pentyl (-$CH(CH_3)CH_2CH_2CH_3$), 3-pentyl (-$CH(CH_2CH_3)_2$), 2-methyl-2-butyl (-$C(CH_3)_2CH_2CH_3$), 3-methyl-2-butyl (-$CH(CH_3)CH(CH_3)_2$), 3-methyl-1-butyl (-$CH_2CH_2CH(CH_3)_2$), 2-methyl-1-butyl (-$CH_2CH(CH_3)CH_2CH_3$), 1-hexyl (-$CH_2CH_2CH_2CH_2CH_2CH_3$), 2-hexyl (-$CH(CH_3)CH_2CH_2CH_2CH_3$), 3-hexyl (-$CH(CH_2CH_3)(CH_2CH_2CH_3)$), 2-methyl-2-pentyl (-$C(CH_3)_2CH_2CH_2CH_3$), 3-methyl-2-pentyl (-$CH(CH_3)CH(CH_3)CH_2CH_3$), 4-methyl-2-pentyl (-$CH(CH_3)CH_2CH(CH_3)_2$), 3-methyl-3-pentyl (-$C(CH_3)(CH_2CH_3)_2$), 2-methyl-3-pentyl (-$CH(CH_2CH_3)CH(CH_3)_2$), 2,3-dimethyl-2-butyl (-$C(CH_3)_2CH(CH_3)_2$), 3,3-dimethyl-2-butyl (-$CH(CH_3)C(CH_3)_3$.

[0112] The term "C_1-C_8 alkyl," as used herein refers to a straight chain or branched, saturated or unsaturated hydrocarbon having from 1 to 8 carbon atoms. Representative "C_1-C_8 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_1-C_8 alkyls include, but are not limited to, -isopropyl, -*sec*-butyl, -isobutyl, -*tert*-butyl, -isopentyl, 2-methylbutyl, unsaturated C_1-C_8 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-butynyl, -2-butynyl, -1-pentynyl, -2-pentynyl, -3-methyl-1 butynyl. A C_1-C_8 alkyl group can be unsubstituted or substituted with one or more groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -$C(O)NH_2$, -C(O)NHR', -$C(O)N(R')_2$ -NHC(O)R', -SO_3R', -$S(O)_2$R', -S(O)R', -OH, -halogen, -N_3 , -NH_2, -NH(R'), -$N(R')_2$ and -CN; where each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

[0113] The term "C_1-C_{12} alkyl," as used herein refers to a straight chain or branched, saturated or unsaturated hydrocarbon having from 1 to 12 carbon atoms. A C_1-C_{12} alkyl group can be unsubstituted or substituted with one or more groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -$C(O)NH_2$, -C(O)NHR', -$C(O)N(R')_2$ -NHC(O)R', -SO_3R', -$S(O)_2$R', -S(O)R', -OH, -halogen, -N_3 , -NH_2, -NH(R'), -$N(R')_2$ and -CN; where each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

[0114] The term "C_1-C_6 alkyl," as used herein refers to a straight chain or branched, saturated or unsaturated hydrocarbon having from 1 to 6 carbon atoms. Representative "C_1-C_6 alkyl" groups include, but are not limited to, -methyl, -ethyl, -n-propyl, -n-butyl, -n-pentyl, -and n-hexyl; while branched C_1-C_6 alkyls include, but are not limited to, -isopropyl, -*sec*-butyl, -isobutyl, -*tert*-butyl, -isopentyl, and 2-methylbutyl; unsaturated C_1-C_6 alkyls include, but are not limited to, -vinyl, -allyl, -1-butenyl, -2-butenyl, and -isobutylenyl, -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_1-C_6 alkyl group can be unsubstituted or substituted with one or more groups, as described above for C_1-C_8 alkyl group.

[0115] The term "C_1-C_4 alkyl," as used herein refers to a straight chain or branched, saturated or unsaturated hydrocarbon having from 1 to 4 carbon atoms. Representative "C_1-C_4 alkyl" groups include, but are not limited to, -methyl, -ethyl, -n-propyl, -n-butyl; while branched C_1-C_4 alkyls include, but are not limited to, -isopropyl, -*sec*-butyl, -isobutyl, -*tert*-butyl; unsaturated C_1-C_4 alkyls include, but are not limited to, -vinyl, -allyl, -1-butenyl, -2-butenyl, and -isobutylenyl. A C_1-C_4 alkyl group can be unsubstituted or substituted with one or more groups, as described above for C_1-C_8 alkyl group.

[0116] "Alkoxy" is an alkyl group singly bonded to an oxygen. Exemplary alkoxy groups include, but are not limited to, methoxy (-OCH_3) and ethoxy (-OCH_2CH_3). A "C_1-C_5 alkoxy" is an alkoxy group with 1 to 5 carbon atoms. Alkoxy groups may can be unsubstituted or substituted with one or more groups, as described above for alkyl groups.

[0117] "Alkenyl" is C_2-C_{18} hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, i.e. a carbon-carbon, sp^2 double bond. Examples include, but are not limited to: ethylene or vinyl (-$CH{=}CH_2$), allyl (-$CH_2CH{=}CH_2$), cyclopentenyl (-C_5H_7), and 5-hexenyl (-CH_2 $CH_2CH_2CH_2CH{=}CH_2$). A "C_2-C_8 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^2 double bond.

[0118] "Alkynyl" is C_2-C_{18} 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_2C≡CH). A "C_2-C_8 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.

[0119] "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 (-CH_2-) 1,2-ethyl (-CH_2CH_2-), 1,3-propyl (-$CH_2CH_2CH_2$-), 1,4-butyl (-$CH_2CH_2CH_2CH_2$-), and the like.

[0120] A "C_1-C_{10} alkylene" is a straight chain, saturated hydrocarbon group of the formula -$(CH_2)_{1-10}$-. Examples of a C_1-C_{10} alkylene include methylene, ethylene, propylene, butylene, pentylene, hexylene, heptylene, ocytylene, nonylene and decalene.

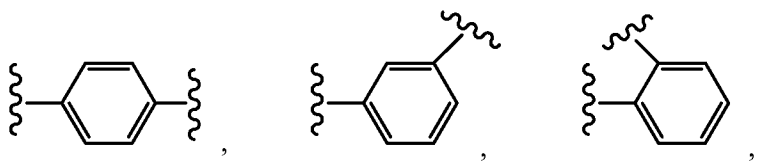
[0121] "Alkenylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkene. Typical alkenylene radicals include, but are not limited to: 1,2-ethylene (-CH=CH-).

[0122] "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 (-C≡C-), propargyl (-CH_2C≡C-), and 4-pentynyl (-$CH_2CH_2CH_2$C≡C-).

[0123] "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, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2 , -C(O)NHR', -C(O)N(R')$_2$ -NHC(O)R', -S(O)$_2$R', -S(O)R', -OH, -halogen, -N_3 , -NH_2, -NH(R'), -N(R')$_2$ and -CN; wherein each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

[0124] A "C_5-C_{20} aryl" is an aryl group with 5 to 20 carbon atoms in the carbocyclic aromatic rings. Examples of C_5-C_{20} aryl groups include, but are not limited to, phenyl, naphthyl and anthracenyl. A C_5-C_{20} aryl group can be substituted or unsubstituted as described above for aryl groups. A "C_5-C_{14} aryl" is an aryl group with 5 to 14 carbon atoms in the carbocyclic aromatic rings. Examples of C_5-C_{14} aryl groups include, but are not limited to, phenyl, naphthyl and anthracenyl. A C_5-C_{14} aryl group can be substituted or unsubstituted as described above for aryl groups.

[0125] 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, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -$C(O)NH_2$, -C(O)NHR', -$C(O)N(R')_2$ -NHC(O)R', -$S(O)_2$R', -S(O)R', -OH, -halogen, -N_3 , -NH_2, -NH(R'), -$N(R')_2$ and -CN; wherein each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

[0126] "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.

[0127] "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.

[0128] "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^- , -OR, -SR, -S^-, -NR_2, -NR_3, =NR, -CX_3, -CN, -OCN, -SCN, -N=C=O, -NCS, -NO, -NO_2, =N_2, -N_3, NC(=O)R, -C(=O)R, -$C(=O)NR_2$, -SO_3^- , -SO_3H, -$S(=O)_2$R, -$OS(=O)_2$OR, -$S(=O)_2$NR, -S(=O)R, -$OP(=O)(OR)_2$, -$P(=O)(OR)_2$, -PO_3, -PO_3H_2, -C(=O)R, -C(=O)X, -C(=S)R, -CO_2R, -CO_2, -C(=S)OR, -C(=O)SR, -C(=S)SR, -$C(=O)NR_2$, -$C(=S)NR_2$, -$C(=NR)NR_2$, where each X is independently a halogen: F, Cl, Br, or I; and each R is independently -H, C_2-C_{18} alkyl, C_6-C_{20} aryl, C_3-C_{14} heterocycle, protecting group or prodrug moiety. Alkylene, alkenylene, and alkynylene groups as described above may also be similarly substituted.

[0129] "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.

[0130] 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.

[0131] Examples of heterocycles include by way of example and not limitation pyridyl, dihydroypyridyl, 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, indolizinyl, isoindolyl, 3H-indolyl, 1H-indazolyl, purinyl, 4H-quinolizinyl, phthalazinyl, naphthyridinyl, quinoxalinyl, quinazolinyl, cinnolinyl, pteridinyl, 4aH-carbazolyl, carbazolyl, β-carbolinyl, phenanthridinyl, acridinyl, pyrimidinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, furazanyl, phenoxazinyl, isochromanyl, chromanyl, imidazolidinyl, imidazolinyl, pyrazolidinyl, pyrazolinyl, piperazinyl, indolinyl, isoindolinyl, quinuclidinyl, morpholinyl, oxazolidinyl, benzotriazolyl, benzisoxazolyl, oxindolyl, benzoxazolinyl, and isatinoyl.

[0132] 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.

[0133] 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 β-carboline. Still more typically, nitrogen bonded heterocycles include 1-aziridyl, 1-azetedyl, 1-pyrrolyl, 1-imidazolyl, 1-pyrazolyl, and 1-piperidinyl.

[0134] A "C_3-C_8 heterocycle" refers to an aromatic or non-aromatic C_3-C_8 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_3-C_8 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_3-C_8 heterocycle can be unsubstituted or substituted with up to seven groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2 , -C(O)NHR', -C(O)N(R')$_2$ -NHC(O)R', -S(O)$_2$R', -S(O)R', -OH, -halogen, -N_3 , -NH_2, -NH(R'), -N(R')$_2$ and -CN; wherein each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

[0135] "C_3-C_8 heterocyclo" refers to a C_3-C_8 heterocycle group defined above wherein one of the heterocycle group's hydrogen atoms is replaced with a bond. A C_3-C_8 heterocyclo can be unsubstituted or substituted with up to six groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2 , -C(O)NHR', -C(O)N(R')$_2$ -NHC(O)R', -S(O)$_2$R', -S(O)R', -OH, -halogen, -N_3 , -NH_2, -NH(R'), -N(R')$_2$ and -CN; wherein each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

[0136] A "C_3-C_{20} heterocycle" refers to an aromatic or non-aromatic C_3-C_8 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_3-C_{20} heterocycle can be unsubstituted or substituted with up to seven groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2 , -C(O)NHR', -C(O)N(R')$_2$ -NHC(O)R', -S(O)$_2$R', -S(O)R', -OH, -halogen, -N_3 , -NH_2, -NH(R'), -N(R')$_2$ and -CN; wherein each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

[0137] "C_3-C_{20} heterocyclo" refers to a C_3-C_{20} heterocycle group defined above wherein one of the heterocycle group's hydrogen atoms is replaced with a bond.

[0138] "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.

[0139] A "C_3-C_8 carbocycle" is a 3-, 4-, 5-, 6-, 7- or 8-membered saturated or unsaturated non-aromatic carbocyclic ring. Representative C_3-C_8 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_3-C_8 carbocycle group can be unsubstituted or substituted with one or more groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2 , -C(O)NHR', -C(O)N(R')$_2$ -NHC(O)R', -S(O)$_2$R', -S(O)R', -OH, -halogen, -N_3 , -NH_2, -NH(R'), -N(R')$_2$ and -CN; where each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

[0140] A "C_3-C_8 carbocyclo" refers to a C_3-C_8 carbocycle group defined above wherein one of the carbocycle groups' hydrogen atoms is replaced with a bond.

[0141] "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 alkyldiyl, an aryldiyl, a heteroaryldiyl, 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.

[0142] 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.

[0143] 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.

[0144] "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.

[0145] "Enantiomers" refer to two stereoisomers of a compound which are non-superimposable mirror images of one another.

[0146] 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 1 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.

[0147] "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.

[0148] 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. COMPOSITIONS AND METHODS

[0149] In one aspect, the invention is based, in part, on antibodies that bind to LgR5 and immunoconjugates comprising such antibodies. Antibodies and immunoconjugates of the invention are useful, *e.g.*, for the diagnosis or treatment of LgR5-positive cancers.

A. Exemplary Anti-LgR5 Antibodies

[0150] In some embodiments, the invention provides isolated antibodies that bind to LgR5. LgR5 is is a seven-transmembrane protein found, for example, on the surface of actively

cycling intestinal stem cells. As demonstrated herein, LgR5 is expressed in about 77% of colon tumor sections examined.

[0151] 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 is shown in SEQ ID NO: 68 (corresponding to amino acids 22-907 of SEQ ID NO: 67).

[0152] 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

[0153] 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 as described in Example I 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.

[0154] 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.

[0155] 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.

[0156] It is understood that aspect and embodiments described herein include "consisting" and/or "consisting effectially of" aspects and embodiments.

(b) binds LgR5 with an affinity of ≤ 5 nM, or ≤ 4 nM, or ≤ 3 nM, or ≤ 2 nM, or ≤ 1 nM, and optionally ≥ 0.0001 nM, or ≥ 0.001 nM, or ≥ 0.01 nM

[0157] Methods of determining binding affinity are known in the art. In some embodiments, the binding affinity may be determined according to a BIAcore® assay as described herein in Example E. Specifically, in some embodiments, Kd 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 µl/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 (Kd) may be calculated as the ratio k_{off}/k_{on}. If the on-rate exceeds 10^6 M^{-1} 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.

[0158] In some embodiments, the anti-LgR5 antibody binds LgR5 with an affinity of about any of ≤ 5 nM, or ≤ 4 nM, or ≤ 3 nM, or ≤ 2 nM, or ≤ 1 nM. In some embodiments, the anti-LgR5 antibody binds LgR5 with an affinity of about ≤ 5. In some embodiments, the anti-LgR5 antibody binds LgR5 with an affinity of about ≤ 4 nM. In some embodiments, the anti-LgR5 antibody binds LgR5 with an affinity of about ≤ 3 nM. In some embodiments, the anti-LgR5 antibody binds LgR5 with an affinity of about ≤2 nM. In some embodiments, LgR5 is human LgR5. In some embodiments, LgR5 is human LgR5 or cynomolgus monkey LgR5.

[0159] 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

[0160] 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.

[0161] 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.

[0162] 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

[0163] 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.

[0164] 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

[0165] 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.

[0166] 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

[0167] 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, *e.g.*, as described in Example N. 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) small intestine and colon tissue collected from intestinal tumorogenesis 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, *e.g.*, as shown in Example N and Figure 18.

[0168] In some embodiments, an anti-LgR5 antibody increases the percentage of caspase 3 positive tumor area according to the assay described in Example N 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

[0169] 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-espressing 293 cells is tested by FACS as described in Example G. 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

[0170] 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-espressing 293 cells is tested by FACS as described in Example G. 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

[0171] Methods of determining the ability of an anti-LgR5 antibody to inhibit tumor growth in its unconjugated form are known in the art. In some embodiments, a rodent xenograft model such as the D5124 pancreatic cancer xenograft model described in Example M is used. In some embodiments, an anti-LgR5 antibody does not significantly inhibit tumor growth in its unconjugated form in a LoVo colon cancer cell line xenograft model, for example, as described in Example L. In some embodiments, an anti-LgR5 antibody does not significantly inhibit tumor growth in its unconjugated form in a murine intestinal tumorigenesis model, for example, as described in Example N. Inhibition of tumor growth in a xenograft model or murine intestinal tumorigenesis model is determined relative to a vehicle control or control antibody.

[0172] 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

[0173] Methods of determining the ability of an anti-LgR5 antibody to induce stem cell differntiation 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.

[0174] 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.

Antibody 8E11 and other embodiments

[0175] In some embodiments, the invention provides an anti-LgR5 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 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.

[0176] In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 30; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 31; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32 and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 29. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 29, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 31. In a further embodiment, the antibody comprises (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; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32.

[0177] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 27; (b) 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. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 27; (b) 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.

[0178] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 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 (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 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.

[0179] In another aspect, the invention provides an antibody comprising (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.

[0180] 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_{KIV}) framework and/or the VH framework VH_1. In certain embodiments, the human acceptor framework is the human VL kappa IV consensus (VL_{KIV}) framework and/or the VH framework VH_1 comprising an R71S mutation and an A78V mutation in heavy chain framework region FR3.

[0181] 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_1 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_1 framework having an FR3 sequence of SEQ ID NO: 41.

[0182] 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_{KIV}) framework having an FR3 sequence of SEQ ID NO: 36.

[0183] In another aspect, an anti-LgR5 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID NOs: 6, 8, 10, 12, 14, 16, 18, and 20. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to an amino acid sequence selected from SEQ ID NOs: 6, 8, 10, 12, 14, 16, 18, and 20 contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-LgR5 antibody comprising that sequence retains the ability to bind to LgR5. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in a sequence selected from SEQ ID NOs: 6, 8, 10, 12, 14, 16, 18, and 20. In certain embodiments, a total of 1 to 5 amino acids have been substituted, inserted and/or deleted in a sequence selected from SEQ ID NOs: 6, 8, 10, 12, 14, 16, 18, and 20. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs).

[0184] In some embodiments, an anti-LgR5 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 6. In some embodiments, an anti-LgR5 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 8. In some embodiments, an anti-LgR5 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 10. In some embodiments, an anti-LgR5 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 12. In some embodiments, an anti-LgR5 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 14. In some embodiments, an anti-LgR5 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 16. In some embodiments, an anti-LgR5 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%

sequence identity to the amino acid sequence of SEQ ID NO: 18. In some embodiments, an anti-LgR5 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 20.

[0185] Optionally, the anti-LgR5 antibody comprises the VH sequence selected from SEQ ID NOs: 6, 8, 10, 12, 14, 16, 18, and 20, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three 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, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32.

[0186] In another aspect, an anti-LgR5 antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID NOs: 5, 7, 9, 11, 13, 15, 17, and 19. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to an amino acid sequence selected from SEQ ID NOs: 5, 7, 9, 11, 13, 15, 17, and 19 contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-LgR5 antibody comprising that sequence retains the ability to bind to LgR5. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in an amino acid sequence selected from SEQ ID NOs: 5, 7, 9, 11, 13, 15, 17, and 19. In certain embodiments, a total of 1 to 5 amino acids have been substituted, inserted and/or deleted in an amino acid sequence selected from SEQ ID NOs: 5, 7, 9, 11, 13, 15, 17, and 19. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs).

[0187] In some embodiments, an anti-LgR5 antibody comprises a light chain variable domain (VL) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 5. In some embodiments, an anti-LgR5 antibody comprises a light chain variable domain (VL) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 7. In some embodiments, an anti-LgR5 antibody comprises a light chain variable domain (VL) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 9. In some embodiments, an anti-LgR5 antibody comprises a light chain variable domain (VL) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 11. In some embodiments, an anti-

LgR5 antibody comprises a light chain variable domain (VL) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 13. In some embodiments, an anti-LgR5 antibody comprises a light chain variable domain (VL) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 15. In some embodiments, an anti-LgR5 antibody comprises a light chain variable domain (VL) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 17. In some embodiments, an anti-LgR5 antibody comprises a light chain variable domain (VL) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 19.

[0188] Optionally, the anti-LgR5 antibody comprises the VL sequence of an amino acid sequence selected from SEQ ID NOs: 5, 7, 9, 11, 13, 15, 17, and 19, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 27; (b) 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.

[0189] 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: 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.

[0190] In a further aspect, the invention provides an antibody that 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: 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 of SEQ ID NO: 68 from, within, or overlapping amino acids 1-312.

[0191] In a further aspect of the invention, 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 $F(ab')_2$ 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.

[0192] 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.

Antibody YW353 and other embodiments

[0193] In one aspect, the invention provides an anti-LgR5 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 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.

[0194] In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 60; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 61; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 62. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 62. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 62, and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 59. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 62, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 59,

and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 61. In a further embodiment, the 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; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:62.

[0195] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 57; (b) 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. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 57; (b) 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.

[0196] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 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 (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 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.

[0197] In another aspect, the invention provides an antibody comprising (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.

[0198] In any of the above embodiments, an anti-LgR5 antibody is a human antibody.

[0199] In another aspect, an anti-LgR5 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 26. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 26 contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-LgR5 antibody comprising that sequence retains the ability to bind to LgR5. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 26. In certain embodiments, a total of 1 to 5 amino acids have been substituted, inserted

and/or deleted in SEQ ID NO: 26. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-LgR5 antibody comprises the VH sequence of SEQ ID NO: 26, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three 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, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 62.

[0200] In another aspect, an anti-LgR5 antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 25. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 25 contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-LgR5 antibody comprising that sequence retains the ability to bind to LgR5. In certain embodiments, a total of 1 to 5 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 25. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 25. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-LgR5 antibody comprises the VL sequence of SEQ ID NO: 25, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 57; (b) 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.

[0201] 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.

[0202] In a further aspect, the invention provides an antibody that 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: 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 of SEQ ID NO: 68 from, within, or overlapping amino acids 1-102.

[0203] In a further aspect of the invention, an anti-LgR5 antibody according to any of the above embodiments is a monoclonal antibody, including a human antibody. In one embodiment, an anti-LgR5 antibody is an antibody fragment, *e.g.*, a Fv, Fab, Fab', scFv, diabody, or $F(ab')_2$ fragment. In another embodiment, the antibody is a substantially full length antibody, *e.g.*, an IgG2a antibody or other antibody class or isotype as defined herein.

[0204] 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.

Antibody 3G12 and other embodiments

[0205] In some embodiments, the invention provides an anti-LgR5 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 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.

[0206] In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 48; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 49; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 50. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 50. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 50 and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 47. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 50, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 47, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 49. In a further embodiment, the antibody comprises (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; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 50.

[0207] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 45; (b) 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. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 45; (b)

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.

[0208] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 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 (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 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.

[0209] In another aspect, the invention provides an antibody comprising (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.

[0210] 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 consensus (VL_K) framework and/or the human VH subgroup 3 consensus (VH_3) framework.

[0211] In another aspect, an anti-LgR5 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 22. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 22 contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-LgR5 antibody comprising that sequence retains the ability to bind to LgR5. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in the amino acid sequence of SEQ ID NO: 22. In certain embodiments, a total of 1 to 5 amino acids have been substituted, inserted and/or deleted in the amino acid sequence of SEQ ID NO: 22. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs).

[0212] Optionally, the anti-LgR5 antibody comprises the VH sequence of SEQ ID NO: 22, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three 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, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 50.

[0213] In another aspect, an anti-LgR5 antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 21. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 21 contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-LgR5 antibody comprising that sequence retains the ability to bind to LgR5. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in the amino acid sequence of SEQ ID NO: 21. In certain embodiments, a total of 1 to 5 amino acids have been substituted, inserted and/or deleted in the amino acid sequence of SEQ ID NO: 21. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs).

[0214] Optionally, the anti-LgR5 antibody comprises the VL sequence of SEQ ID NO: 21, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 45; (b) 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.

[0215] 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: 22 and SEQ ID NO: 21, respectively, including post-translational modifications of those sequences.

[0216] In a further aspect, the invention provides an antibody that 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: 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 of SEQ ID NO: 68 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 of SEQ ID NO: 68 from, within, or overlapping amino acids 303-534.

[0217] In a further aspect of the invention, 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 $F(ab')_2$ 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.

[0218] 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.

Antibody 2H6 and other embodiments

[0219] In some embodiments, the invention provides an anti-LgR5 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 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.

[0220] In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 54; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 55; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 56. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 56. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 56 and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 53. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 56, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 53, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 55. In a further embodiment, the antibody comprises (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; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 56.

[0221] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 51; (b) 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. In one embodiment,

the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 51; (b) 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.

[0222] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 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 (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 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.

[0223] In another aspect, the invention provides an antibody comprising (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.

[0224] 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 consensus (VL_K) framework and/or the human VH subgroup 3 (VH_3) framework.

[0225] In another aspect, an anti-LgR5 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 24. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 24 contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-LgR5 antibody comprising that sequence retains the ability to bind to LgR5. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in the amino acid sequence of SEQ ID NO: 24. In certain embodiments, a total of 1 to 5 amino acids have been substituted, inserted and/or deleted in the amino acid sequence of SEQ ID NO: 24. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs).

[0226] Optionally, the anti-LgR5 antibody comprises the VH sequence of SEQ ID NO: 24, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three 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, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 56.

[0227] In another aspect, an anti-LgR5 antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 23. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 23 contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-LgR5 antibody comprising that sequence retains the ability to bind to LgR5. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in the amino acid sequence of SEQ ID NO: 23. In certain embodiments, a total of 1 to 5 amino acids have been substituted, inserted and/or deleted in the amino acid sequence of SEQ ID NO: 23. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs).

[0228] Optionally, the anti-LgR5 antibody comprises the VL sequence of the amino acid sequence of SEQ ID NO: 23, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 51; (b) 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.

[0229] 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.

[0230] In a further aspect, the invention provides an antibody that 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 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 of SEQ ID NO: 68 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 of SEQ ID NO: 68 from, within, or overlapping amino acids 303-534.

[0231] In a further aspect of the invention, 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 $F(ab')_2$ 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.

[0232] 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

[0233] In certain embodiments, an antibody provided herein has a dissociation constant (Kd) of $\leq 1\mu M$, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM, and optionally is $\geq 10^{-13}$ M. (*e.g.* 10^{-8} M or less, *e.g.* from 10^{-8} M to 10^{-13} M, *e.g.*, from 10^{-9} M to 10^{-13} M).

[0234] In one embodiment, Kd is measured by a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen as described by the following assay. Solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (*see*, *e.g.*, Chen et al., *J. Mol. Biol.* 293:865-881(1999)). To establish conditions for the assay, MICROTITER® multi-well plates (Thermo Scientific) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (*e.g.*, consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., *Cancer Res.* 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (*e.g.*, about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (*e.g.*, for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, 150 μl/well of scintillant (MICROSCINT-20™; Packard) is added, and the plates are counted on a TOPCOUNT™ gamma counter (Packard) for

ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

[0235] According to another embodiment, Kd is measured using surface plasmon resonance assays using a BIACORE®-2000 or a BIACORE®-3000 (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with *N*-ethyl-*N'*- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 µg/ml (~0.2 µM) before injection at a flow rate of 5 µl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25°C at a flow rate of approximately 25 µl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio k_{off}/k_{on}. *See, e.g.*, Chen et al., *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds 10^6 M^{-1} s^{-1} by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

2. Antibody Fragments

[0236] In certain embodiments, an antibody provided herein is an antibody fragment. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, $F(ab')_2$, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, *see* Hudson et al. *Nat. Med.* 9:129-134 (2003). For a review of scFv fragments, *see, e.g.*, Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); *see also* WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and $F(ab')_2$ fragments comprising salvage receptor binding epitope residues and having increased *in vivo* half-life, *see* U.S. Patent No. 5,869,046.

[0237] 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).

[0238] 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).

[0239] 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

[0240] 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.

[0241] 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.

[0242] 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).

[0243] 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

[0244] 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).

[0245] 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.

[0246] 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).

[0247] 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

[0248] Antibodies of the invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, *e.g.*, in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, *e.g.*, in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132(2004).

[0249] 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.

[0250] Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

6. Multispecific Antibodies

[0251] 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.

[0252] 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).

[0253] Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (*see*, *e.g.* US 2006/0025576A1).

[0254] 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

[0255] 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

[0256] 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

Original Residue	Exemplary Substitutions	Preferred Substitutions
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

Original Residue	Exemplary Substitutions	Preferred Substitutions
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.

[0257] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0258] 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).

[0259] 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.

[0260] 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.

[0261] 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.

[0262] 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

[0263] 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.

[0264] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. *See*, *e.g.*, Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, *e.g.*, mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

[0265] In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. *See*, *e.g.*, US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as

alpha-1,6-fucosyltransferase gene, *FUT8,* knockout CHO cells (*see*, *e.g.*, Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

[0266] 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

[0267] 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.

[0268] In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express Fc(RIII only, whereas monocytes express Fc(RI, Fc(RII and Fc(RIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (*see*, *e.g.* Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (*see* Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (*see*, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive

cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in a animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. *See*, *e.g.*, C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (*see*, for example, Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996); Cragg, M.S. et al., *Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half life determinations can also be performed using methods known in the art (*see*, *e.g.*, Petkova, S.B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006)).

[0269] 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).

[0270] 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).)

[0271] 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).

[0272] 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).

[0273] 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).

[0274] *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

[0275] 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.

[0276] An exemplary hu8E11.v2 light chain (LC) V205C thiomab has the heavy chain and light chain sequences of SEQ ID NOs: 64 and 74, respectively. An exemplary hu8E11.v2 heavy chain (HC) A118C thiomab has the heavy chain and light chain sequences of SEQ ID NOs: 75 and 63, respectively. An exemplary hu8E11.v2 heavy chain (HC) S400C thiomab has the heavy chain and light chain sequences of SEQ ID NOs: 76 and 63, respectively.

[0277] An exemplary YW353 light chain (LC) V205C thiomab has the heavy chain and light chain sequences of SEQ ID NOs: 66 and 77, respectively. An exemplary YW353 heavy chain (HC) A118C thiomab has the heavy chain and light chain sequences of SEQ ID NOs: 78 and 65, respectively. An exemplary YW353 heavy chain (HC) S400C thiomab has the heavy chain and light chain sequences of SEQ ID NOs: 79 and 65, respectively.

[0278] 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 of SEQ ID NO: 81. 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 of SEQ ID NO: 82.

e) Antibody Derivatives

[0279] In certain embodiments, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

[0280] In another embodiment, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam et al., *Proc. Natl. Acad. Sci. USA* 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

B. Recombinant Methods and Compositions

[0281] 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).

[0282] 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).

[0283] 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.

[0284] 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).

[0285] 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.

[0286] 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).

[0287] Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, *e.g.*, in Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, *e.g.*, in Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, *e.g.*, in Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including $DHFR^-$ CHO cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, *see*, *e.g.*, Yazaki and Wu, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003).

C. Assays

[0288] 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.

[0289] In one aspect, an antibody of the invention is tested for its antigen binding activity, *e.g.*, by known methods such as ELISA, BIACore®, FACS, or Western blot.

[0290] 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).

[0291] 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).

D. Immunoconjugates

[0292] The invention also provides immunoconjugates comprising an anti-LgR5 antibody herein conjugated to one or more cytotoxic agents, 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).

[0293] 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).

[0294] 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 .

[0295] The ADC compounds of the invention 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) of the invention 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").

[0296] 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

[0297] 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.

[0298] An exemplary ADC has Formula I:

$$Ab-(L-D)_p \qquad \mathbf{I}$$

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

[0299] 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.

[0300] 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, α-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.

[0301] 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.

[0302] 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-aminobenzyloxycarbonyl (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.

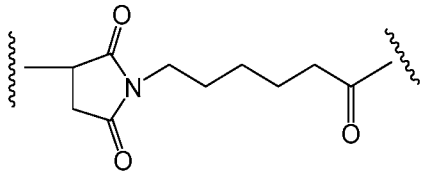
[0303] A linker may be a "cleavable linker," facilitating release of a drug. Nonlimiting exemplary cleavable linkers include acid-labile linkers (*e.g.*, comprising hydrazone), protease-sensitive (*e.g.*, peptidase-sensitive) linkers, photolabile linkers, or disulfide-containing linkers (Chari et al., Cancer Research 52:127-131 (1992); US 5208020).

[0304] In certain embodiments, a linker has the following Formula II:

$$—A_a—W_w—Y_y— \qquad \text{II}$$

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_a-W_w-Y_y-D)p, 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.

[0305] 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):



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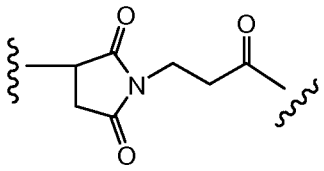

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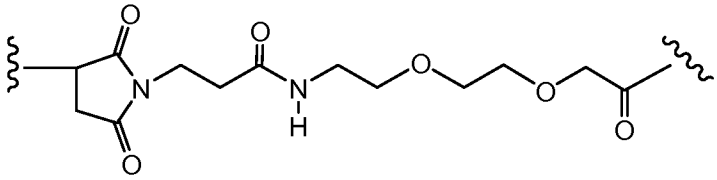
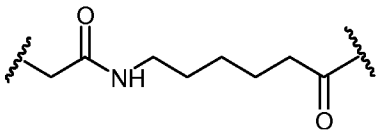


mPEG



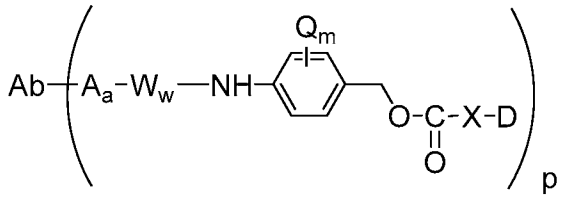
[0306] In some embodiments, a linker component comprises an "amino acid unit" (W). In some such embodiments, the amino acid unit allows for cleavage of the linker by a protease, thereby facilitating release of the drug from the immunoconjugate upon exposure to intracellular proteases, such as lysosomal 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.

[0307] 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,

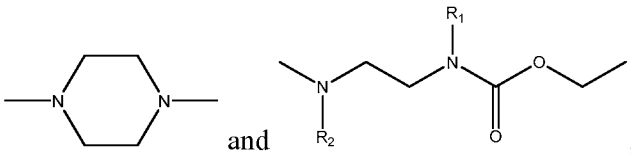
for example, according to a liquid phase synthesis method (*e.g.*, E. Schröder and K. Lübke (1965) "The Peptides", volume 1, pp 76-136, Academic Press).

[0308] 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.

[0309] 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-aminobenzyloxycarbonyl (PAB). In some embodiments, an ADC comprising a self-immolative linker has the structure:



wherein Q is -C_1-C_8 alkyl, -O-(C_1-C_8 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:



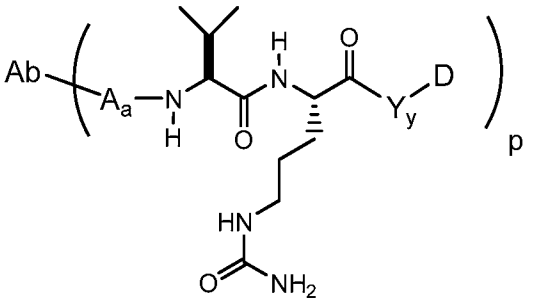
; wherein R_1 and R_2 are independently selected from H and C_1-C_6 alkyl. In some embodiments, R1 and R2 are each $-CH_3$.

[0310] 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.*

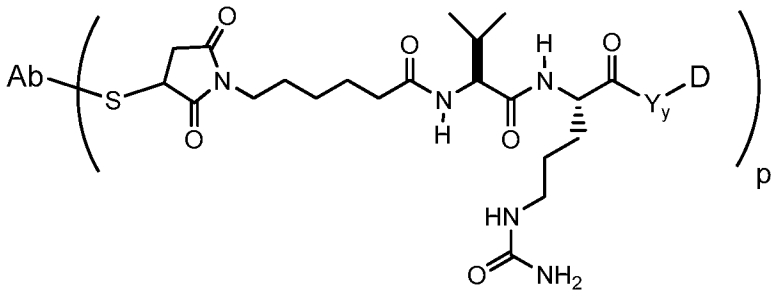
9:2237) and ortho- or para-aminobenzylacetals. In some embodiments, spacers can be used that undergo cyclization upon amide bond hydrolysis, such as substituted and unsubstituted 4-aminobutyric acid amides (Rodrigues et al (1995) *Chemistry Biology* 2:223), appropriately substituted bicyclo[2.2.1] and 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 α-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).

[0311] 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.

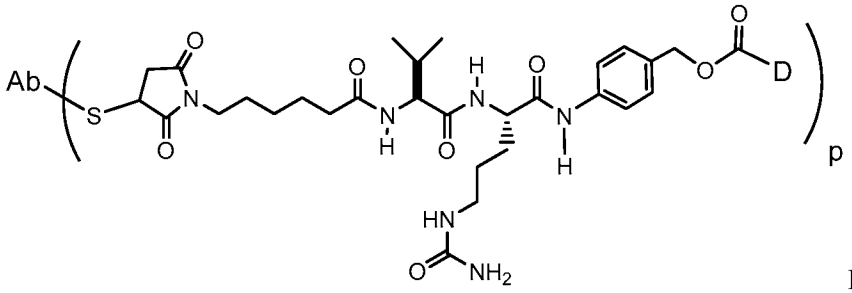
[0312] Nonlimiting exemplary linkers are shown below in the context of an ADC of Formula I:



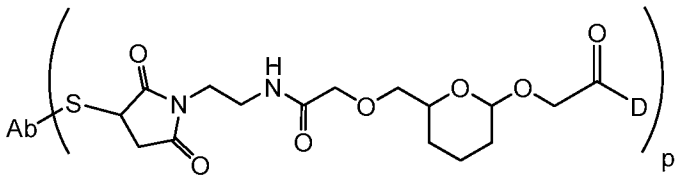
val-cit

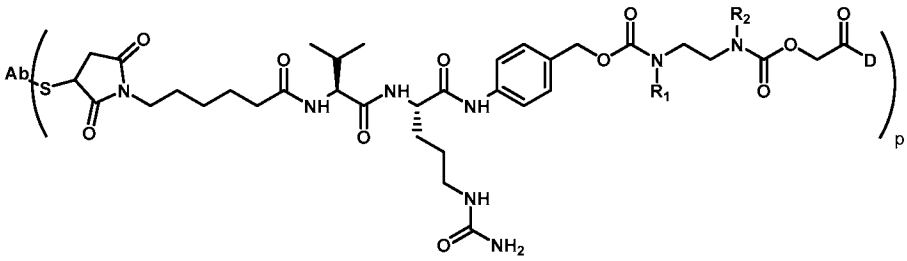


MC-val-cit

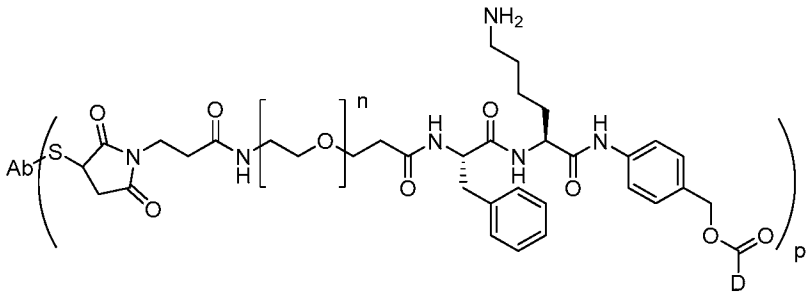


MC-val-cit-PAB





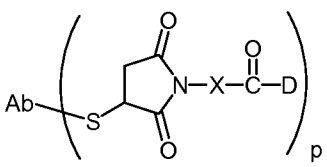
; wherein R_1 and R_2 are independently selected from H and C_1-C_6 alkyl. In some embodiments, R1 and R2 are each $-CH_3$.



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.

[0313] Further nonlimiting exemplary ADCs include the structures:



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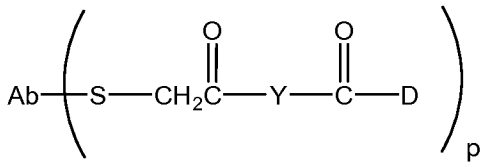

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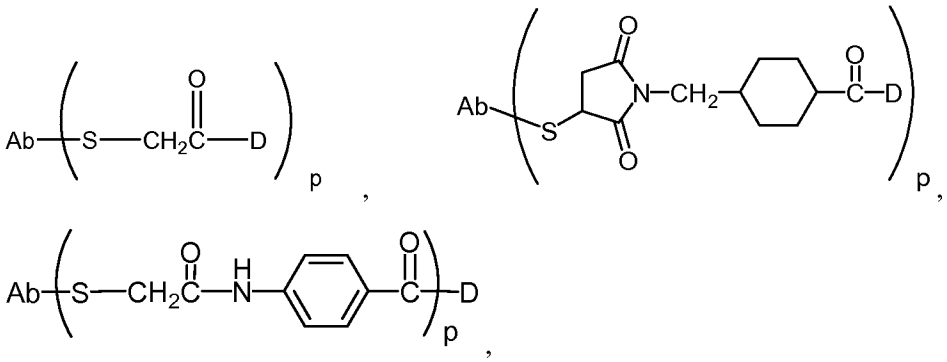
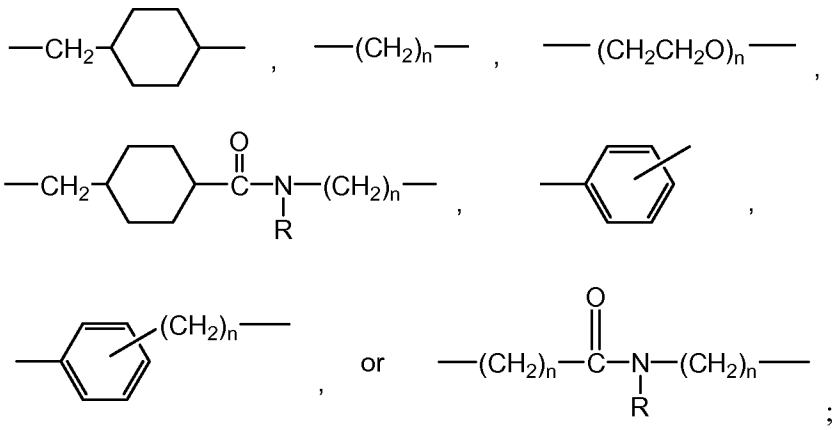
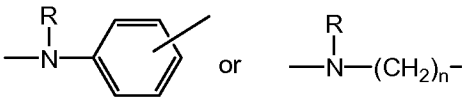


where X is:



Y is:

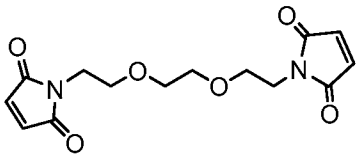


each R is independently H or C_1–C_6 alkyl; and n is 1 to 12.

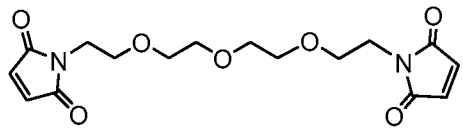
[0314] In some embodiments, a linker is substituted with groups that modulate solubility and/or reactivity. As a nonlimiting example, a charged substituent such as sulfonate ($-SO_3^-$) or ammonium may increase water solubility of the linker reagent and facilitate the coupling reaction of the linker 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)^a is coupled to drug-(linker portion)^b to form the ADC of Formula I.

[0315] The compounds of the invention expressly contemplate, but are not limited to, ADC prepared with the following linker reagents: bis-maleimido-trioxyethylene glycol (BMPEO), N-(β-maleimidopropyloxy)-N-hydroxy succinimide ester (BMPS), N-(ε-maleimidocaproyloxy) succinimide ester (EMCS), N-[γ-maleimidobutyryloxy]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-[(beta-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), bismaleimidohexane (BMH), bismaleimidoethane (BMOE), $BM(PEG)_2$ (shown below), and $BM(PEG)_3$ (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.



$BM(PEG)_2$



$BM(PEG)_3$

[0316] 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.

[0317] 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

[0318] 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.

[0319] 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.

[0320] 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.

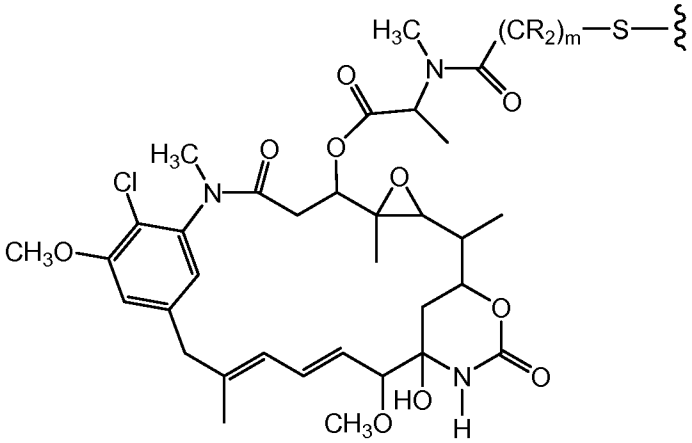
[0321] Exemplary maytansinoid drug moieties include, but are not limited to, those having a modified aromatic ring, such as: C-19-dechloro (US Pat. No. 4256746) (prepared, for example, by lithium aluminum hydride reduction of ansamytocin P2); C-20-hydroxy (or C-20-demethyl) +/-C-19-dechloro (US Pat. 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.

[0322] 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_2S or P_2S_5); C-14-alkoxymethyl(demethoxy/CH_2OR)(US 4331598); C-14-hydroxymethyl

or acyloxymethyl (CH_2OH or CH_2OAc) (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 nudlflora); 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).

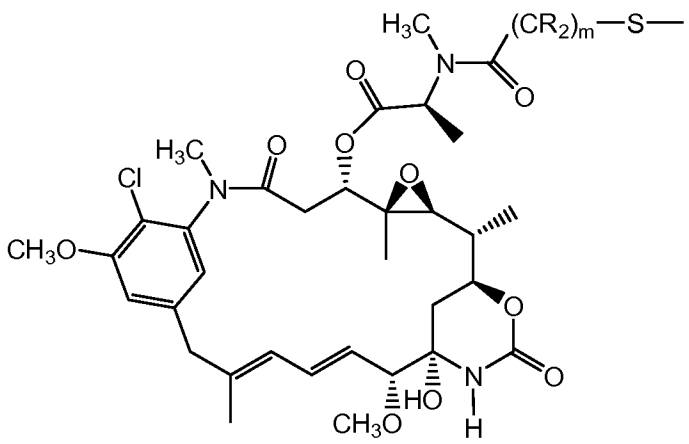
[0323] Many positions on maytansinoid compounds are useful as the linkage position. For example, an ester linkage may be formed by reaction with a hydroxyl group using conventional coupling techniques. In some embodiments, the reaction may occur at the C-3 position having a hydroxyl group, the C-14 position 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.

[0324] Maytansinoid drug moieties include those having the structure:

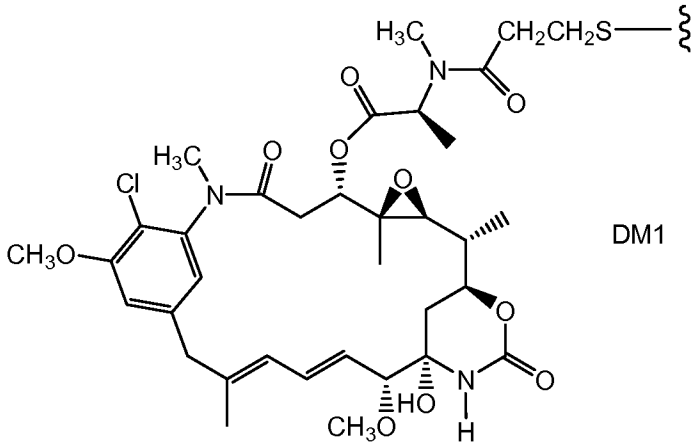


where the wavy line indicates the covalent attachment of the sulfur atom of the maytansinoid drug moiety to a linker of an ADC. Each R may independently be H or a C_1–C_6 alkyl. The alkylene chain attaching the amide group to the sulfur atom may be methanyl, ethanyl, or propyl, i.e., m is 1, 2, or 3 (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).

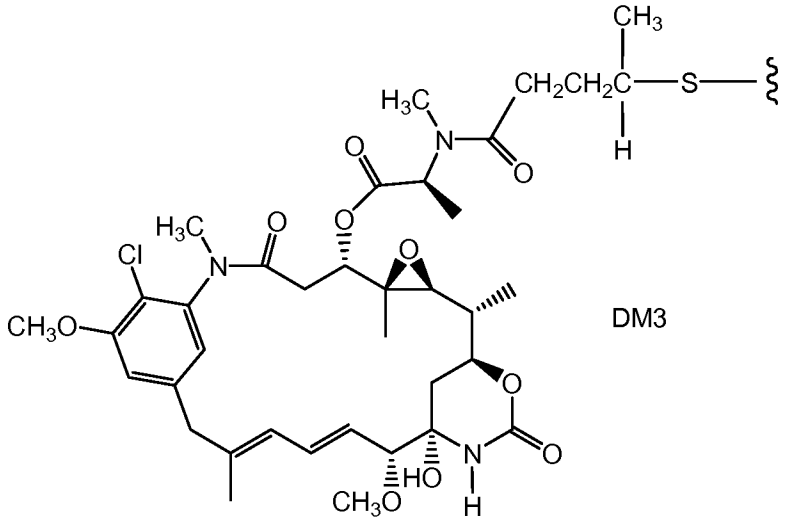
[0325] All stereoisomers of the maytansinoid drug moiety are contemplated for the ADC of the invention, i.e. any combination of *R* and *S* configurations at the chiral carbons (US 7276497; US 6913748; US 6441163; US 633410 (RE39151); US 5208020; Widdison et al (2006) J. Med. Chem. 49:4392-4408, which are incorporated by reference in their entirety). In some embodiments, the maytansinoid drug moiety has the following stereochemistry:



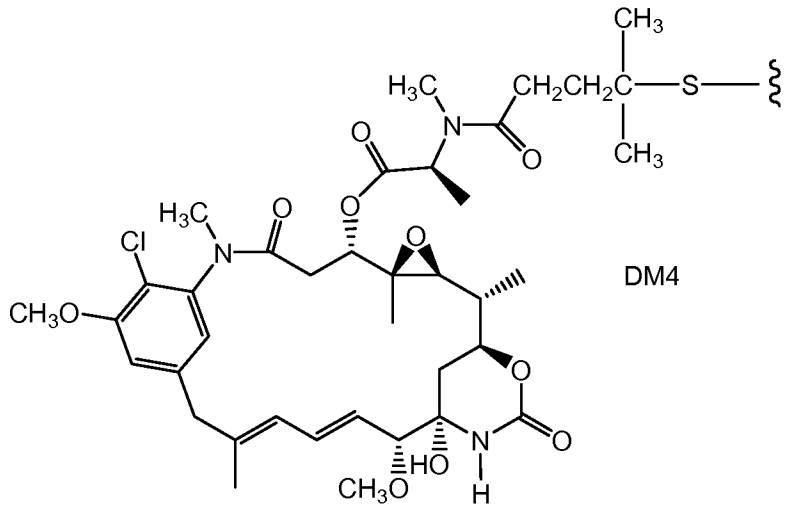
[0326] Exemplary embodiments of maytansinoid drug moieties include, but are not limited to, DM1; DM3; and DM4, having the structures:



DM1



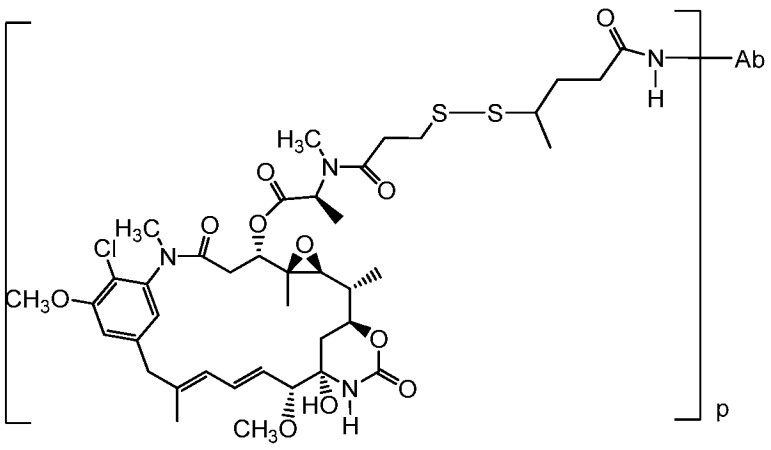
DM3



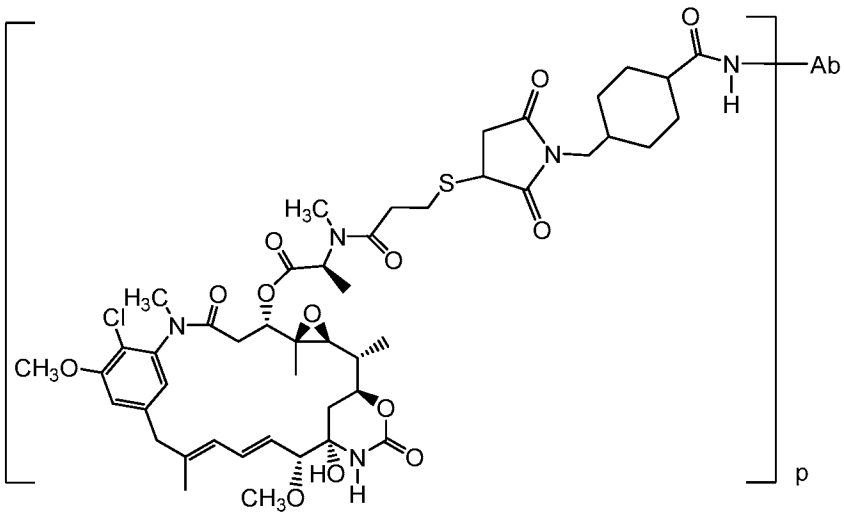
DM4

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

[0327] 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):

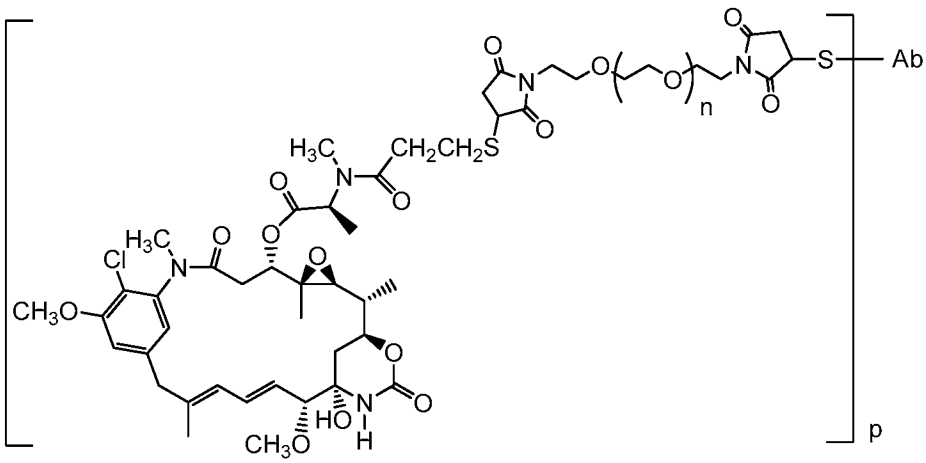


Ab -SPP-DM1



Ab-SMCC-DM1

[0328] 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.

[0329] 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).

[0330] 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

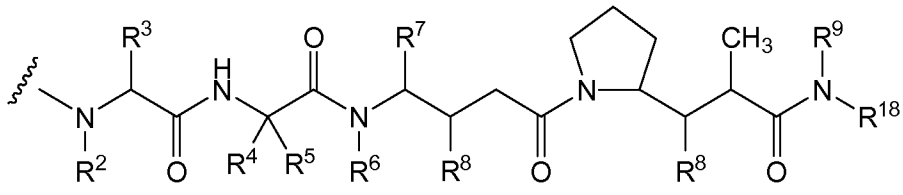
embodiments, ADC with an average of 3-4 maytansinoid molecules conjugated per antibody molecule has shown efficacy in enhancing cytotoxicity of target cells without negatively affecting the function or solubility of the antibody. In some instances, even one molecule of toxin/antibody is expected to enhance cytotoxicity over the use of naked antibody.

[0331] Exemplary linking groups for making antibody-maytansinoid conjugates include, for example, those described herein and those disclosed in U.S. Patent No. 5208020; 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.

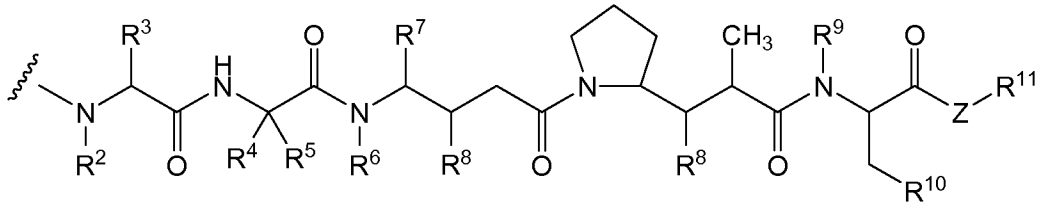
(2) Auristatins and dolastatins

[0332] 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).

[0333] Exemplary auristatin embodiments include the N-terminus linked monomethylauristatin drug moieties D_E and D_F, disclosed in US 7498298 and US 7659241, the disclosures of which are expressly incorporated by reference in their entirety:



D_E



D_F

wherein the wavy line of D_E and D_F indicates the covalent attachment site to an antibody or antibody-linker component, and independently at each location:

R^2 is selected from H and C_1-C_8 alkyl;

R^3 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-aryl, C_1-C_8 alkyl-(C_3-C_8 carbocycle), C_3-C_8 heterocycle and C_1-C_8 alkyl-(C_3-C_8 heterocycle);

R^4 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-aryl, C_1-C_8 alkyl-(C_3-C_8 carbocycle), C_3-C_8 heterocycle and C_1-C_8 alkyl-(C_3-C_8 heterocycle);

R^5 is selected from H and methyl;

or R^4 and R^5 jointly form a carbocyclic ring and have the formula $-(CR^aR^b)_n-$ wherein R^a and R^b are independently selected from H, C_1-C_8 alkyl and C_3-C_8 carbocycle and n is selected from 2, 3, 4, 5 and 6;

R^6 is selected from H and C_1-C_8 alkyl;

R^7 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-aryl, C_1-C_8 alkyl-(C_3-C_8 carbocycle), C_3-C_8 heterocycle and C_1-C_8 alkyl-(C_3-C_8 heterocycle);

each R^8 is independently selected from H, OH, C_1-C_8 alkyl, C_3-C_8 carbocycle and O-(C_1-C_8 alkyl);

R^9 is selected from H and C_1-C_8 alkyl;

R^{10} is selected from aryl or C_3-C_8 heterocycle;

Z is O, S, NH, or NR^{12}, wherein R^{12} is C_1-C_8 alkyl;

R^{11} is selected from H, C_1-C_{20} alkyl, aryl, C_3-C_8 heterocycle, $-(R^{13}O)_m-R^{14}$, or $-(R^{13}O)_m-CH(R^{15})_2$;

m is an integer ranging from 1-1000;

R^{13} is C_2-C_8 alkyl;

R^{14} is H or C_1-C_8 alkyl;

each occurrence of R^{15} is independently H, COOH, $-(CH_2)_n-N(R^{16})_2$, $-(CH_2)_n-SO_3H$, or $-(CH_2)_n-SO_3-C_1$-C_8 alkyl;

each occurrence of R^{16} is independently H, C_1-C_8 alkyl, or $-(CH_2)_n-COOH$;

R^{18} is selected from $-C(R^8)_2-C(R^8)_2-$aryl, $-C(R^8)_2-C(R^8)_2-(C_3$-C_8 heterocycle), and $-C(R^8)_2-C(R^8)_2-(C_3$-C_8 carbocycle); and

n is an integer ranging from 0 to 6.

[0334] 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.

[0335] In yet another embodiment, R^2 and R^6 are each methyl, and R^9 is -H.

[0336] In still another embodiment, each occurrence of R^8 is $-OCH_3$.

[0337] 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 $-OCH_3$, and R^9 is -H.

[0338] In one embodiment, Z is -O- or -NH-.

[0339] In one embodiment, R^{10} is aryl.

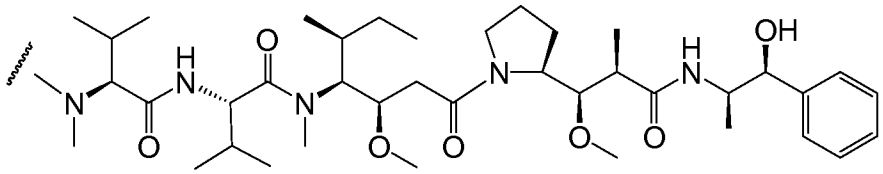
[0340] In an exemplary embodiment, R^{10} is -phenyl.

[0341] In an exemplary embodiment, when Z is -O-, R^{11} is –H, methyl or t-butyl.

[0342] In one embodiment, when Z is -NH, R^{11} is $-CH(R^{15})_2$, wherein R^{15} is $-(CH_2)_n-N(R^{16})_2$, and R^{16} is $-C_1-C_8$ alkyl or $-(CH_2)_n-COOH$.

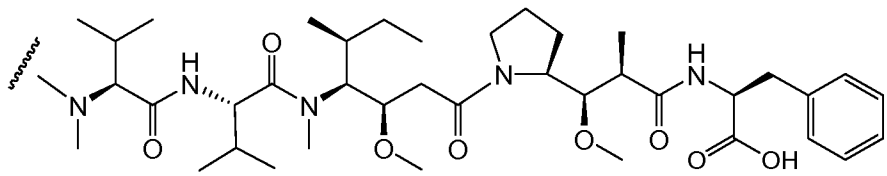
[0343] In another embodiment, when Z is -NH, R^{11} is $-CH(R^{15})_2$, wherein R^{15} is $-(CH_2)_n-SO_3H$.

[0344] An exemplary auristatin embodiment of formula D_E is MMAE, wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody-drug conjugate:



MMAE

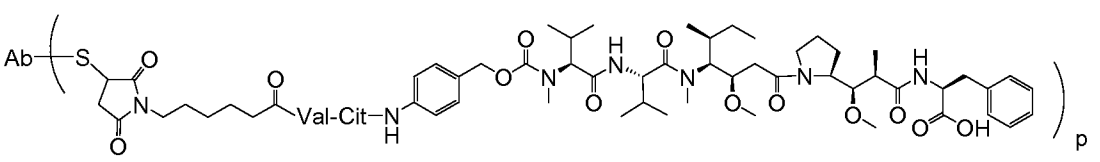
[0345] An exemplary auristatin embodiment of formula D_F is MMAF, wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody-drug conjugate:



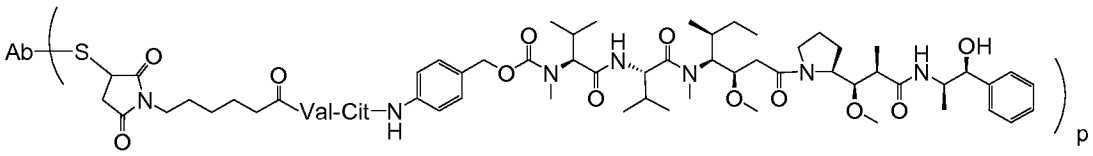
MMAF

[0346] 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).

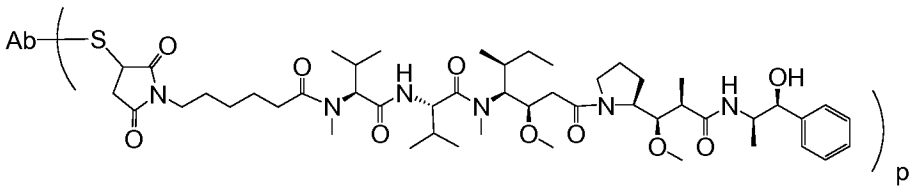
[0347] Nonlimiting exemplary embodiments of ADC of Formula I comprising MMAE or MMAF and various linker components have the following structures and abbreviations (wherein "Ab" is an antibody; p is 1 to about 8, "Val-Cit" is a valine-citrulline dipeptide; and "S" is a sulfur atom:



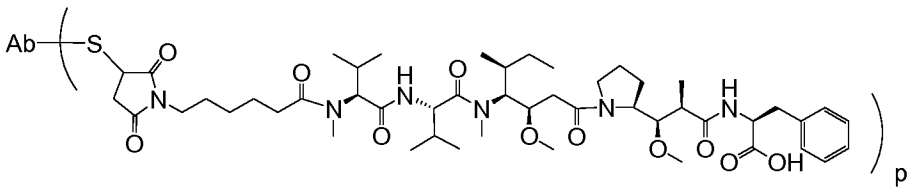
Ab-MC-vc-PAB-MMAF



Ab-MC-vc-PAB-MMAE



Ab-MC-MMAE



Ab-MC-MMAF

[0348] 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.

[0349] 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.

[0350] In some embodiments, auristatin/dolastatin drug moieties of formulas D_E such as MMAE, and D_F, 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.* 21:778-784and then conjugated to an antibody of interest.

(3) Calicheamicin

[0351] 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 enhances 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.

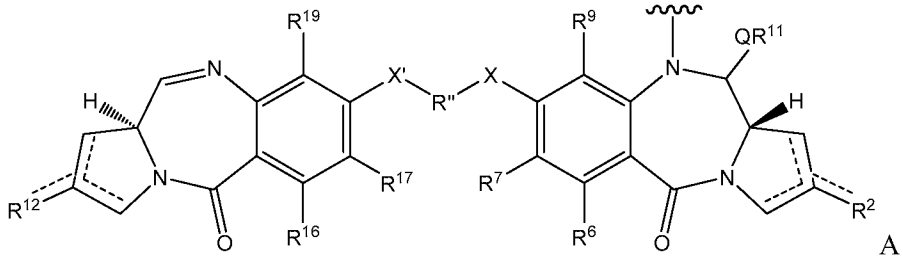
(4) Pyrrolobenzodiazepines

[0352] In some embodiments, an ADC comprises a pyrrolobenzodiazepine (PBD). In some embodiments, PDB 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).

[0353] 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).

[0354] Nonlimiting exemplary PBD dimer components of ADCs are of Formula A:



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, $=CH_2$, CN, R, OR, $=CH\text{-}R^D$, $=C(R^D)_2$, $O\text{-}SO_2\text{-}R$, CO_2R and COR, and optionally further selected from halo or dihalo, wherein R^D is independently selected from R, CO_2R, COR, CHO, CO_2H, and halo;

R^6 and R^9 are independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', NO_2, Me_3Sn and halo;

R^7 is independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', NO_2, Me_3Sn and halo;

Q is independently selected from O, S and NH;

R^{11} is either H, or R or, where Q is O, SO_3M, where M is a metal cation;

R and R' are each independently selected from optionally substituted C_{1-8} alkyl, C_{1-12} alkyl, C_{3-8} heterocyclyl, C_{3-20} heterocycle, and C_{5-20} 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_{3-12} 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).

[0355] In some embodiments, R and R' are each independently selected from optionally substituted C_{1-12} alkyl, C_{3-20} heterocycle, and C_{5-20} 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.

[0356] In some embodiments, R^9 and R^{19} are H.

[0357] In some embodiments, R^6 and R^{16} are H.

[0358] In some embodiments, R^7 are 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 is Ch_2Ph, where Ph is a phenyl group.

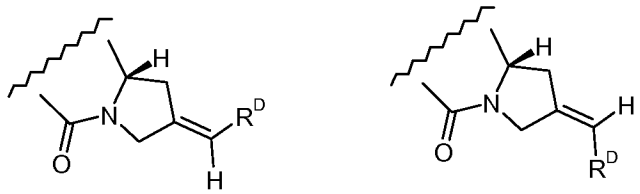
[0359] In some embodiments, X is O.

[0360] In some embodiments, R^{11} is H.

[0361] In some embodiments, there is a double bond between C2 and C3 in each monomer unit.

[0362] 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, napthyl, pyridyl, quinolinyl, or isoquinolinyl. In some embodiments, R^2 and R^{12} are independently selected from =O, $=CH_2$, $=CH\text{-}R^D$, 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 $=CH\text{-}R^D$.

[0363] In some embodiments, when R^2 and/or R^{12} is $=CH\text{-}R^D$, each group may independently have either configuration shown below:

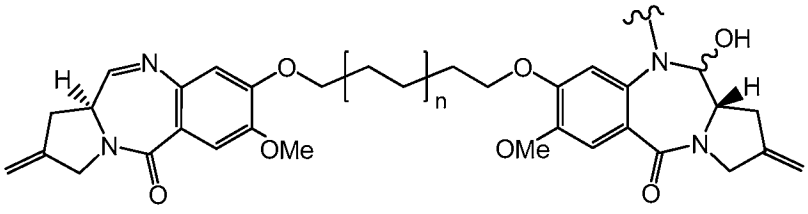


(I) **(II)**

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

[0364] In some embodiments, R″ is a C_3 alkylene group or a C_5 alkylene group.

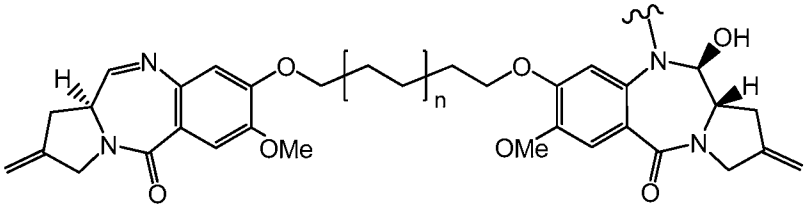
[0365] In some embodiments, an exemplary PBD dimer component of an ADC has the structure of Formula A(I):



A(I);

wherein n is 0 or 1.

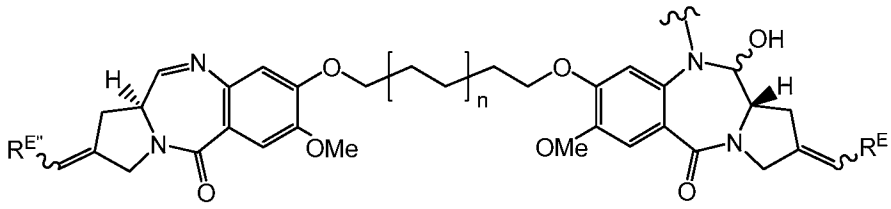
[0366] In some embodiments, an exemplary PBD dimer component of an ADC has the structure of Formula A(II):



A(II);

wherein n is 0 or 1.

[0367] In some embodiments, an exemplary PBD dimer component of an ADC has the structure of Formula A(III):



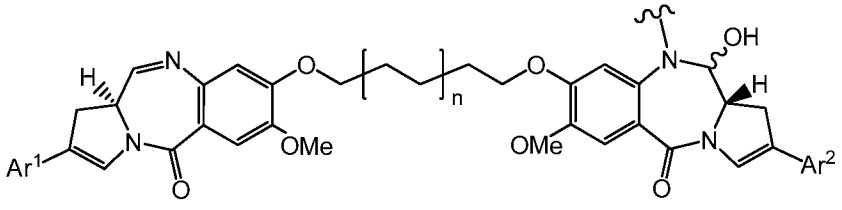
A(III);

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.

[0368] 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.

[0369] In some embodiments, an exemplary PBD dimer component of an ADC has the structure of Formula A(IV):

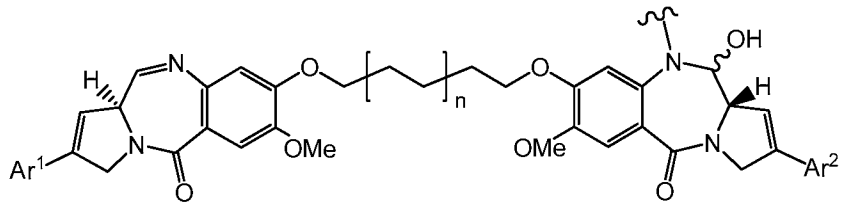


A(IV);

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.

[0370] In some embodiments, an exemplary PBD dimer component of an ADC has the structure of Formula A(V):



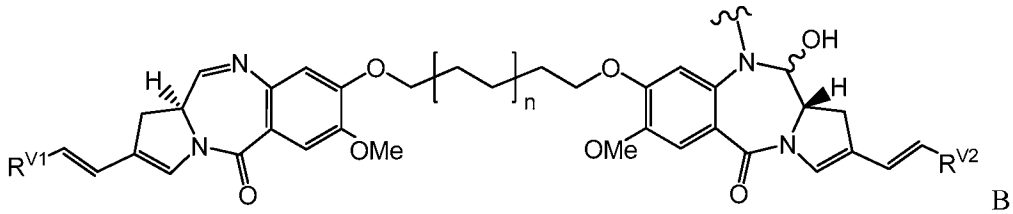
A(V);

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.

[0371] In some embodiments, Ar^1 and Ar^2 are each independently selected from optionally substituted phenyl, furanyl, thiophenyl and pyridyl. In some embodiments, Ar^1 and Ar^2 are each independently optionally substituted phenyl. In some embodiments, Ar^1 and Ar^2 are each independently optionally substituted thien-2-yl or thien-3-yl. In some embodiments, Ar^1 and Ar^2 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.

[0372] Further nonlimiting exemplary PBD dimer components of ADCs are of Formula B:



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;

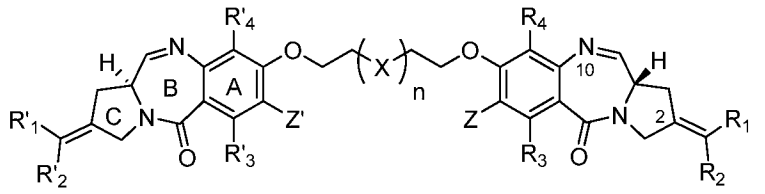
R^{V1} and R^{V2} are independently selected from H, methyl, ethyl and phenyl (which phenyl may be optionally substituted with fluoro, particularly in the 4 position) and C_{5-6} heterocyclyl; wherein R^{V1} and R^{V2} may be the same or different; and

n is 0 or 1.

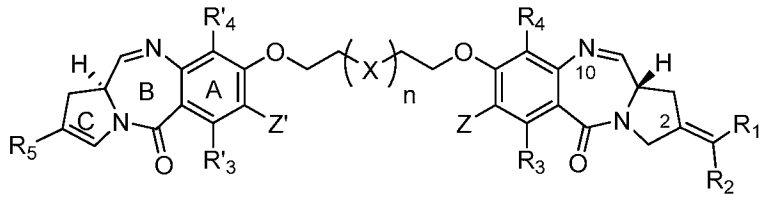
[0373] In some embodiments, R^{V1} and R^{V2} are independently selected from H, phenyl, and 4-fluorophenyl.

[0374] 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).

[0375] Nonlimiting exemplary PBD dimer components of ADCs include Formulas C(I) and C(II):

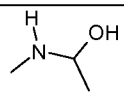
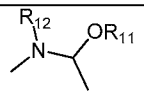


C(I)



C(II)

[0376] 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_2 (n = 1 to 5), N, or O;

Z and Z' are independently selected from OR and NR_2, where R is a primary, secondary or tertiary alkyl chain containing 1 to 5 carbon atoms;

R_1, R'_1, R_2 and R'_2 are each independently selected from H, C_1-C_8 alkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_{5-20} aryl (including substituted aryls), C_{5-20} heteroaryl groups, $–NH_2$, -NHMe, -OH, and -SH, where, in some embodiments, alkyl, alkenyl and alkynyl chains comprise up to 5 carbon atoms;

R_3 and R'_3 are independently selected from H, OR, NHR, and NR_2, where R is a primary, secondary or tertiary alkyl chain containing 1 to 5 carbon atoms;

R_4 and R'_4 are independently selected from H, Me, and OMe;

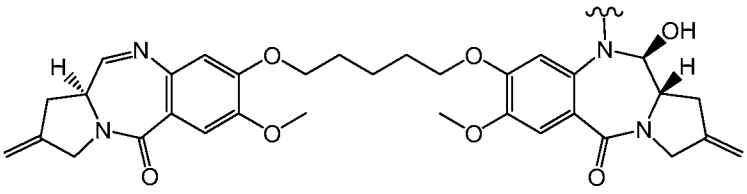
R_5 is selected from C_1-C_8 alkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_{5-20} aryl (including aryls substituted by halo, nitro, cyano, alkoxy, alkyl, heterocyclyl) and C_{5-20} heteroaryl groups, where, in some embodiments, alkyl, alkenyl and alkynyl chains comprise up to 5 carbon atoms;

R_{11} is H, C_1-C_8 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_{12} is is H, C_1-C_8 alkyl, or a protecting group;

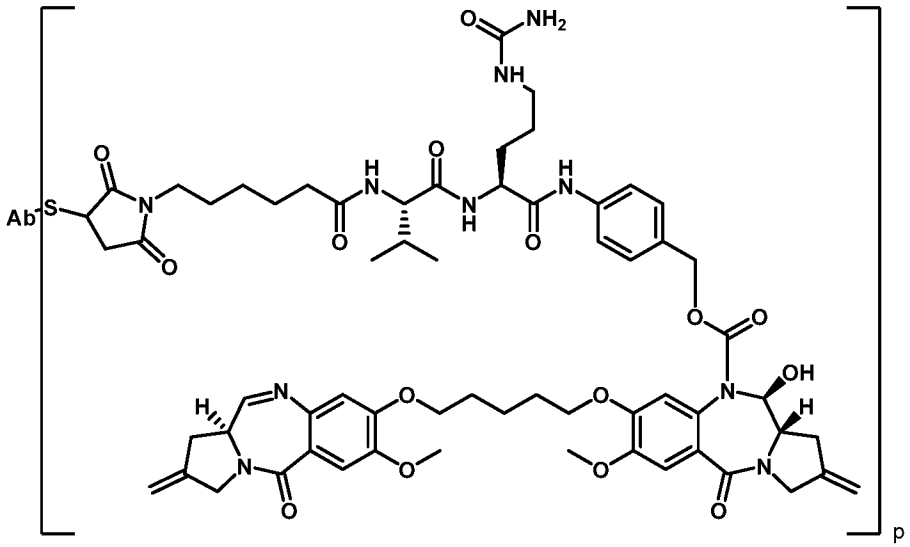
wherein a hydrogen of one of R_1, R'_1, R_2, R'_2, R_5, or R_{12} or a hydrogen of the $-OCH_2CH_2(X)_nCH_2CH_2O-$ spacer between the A rings is replaced with a bond connected to the linker of the ADC.

[0377] Exemplary PDB dimer portions of ADC include, but are not limited to (the wavy line indicates the site of covalent attachment to the linker):

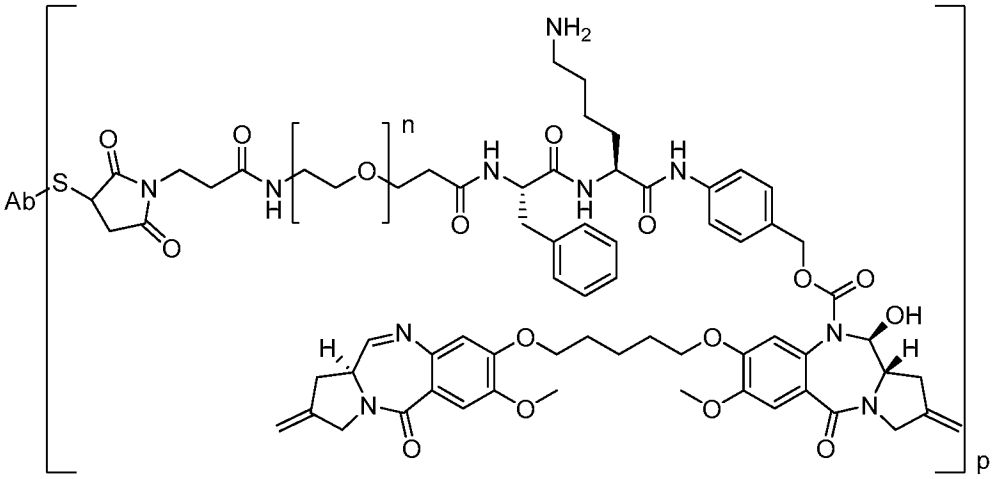


PBD dimer;

[0378] Nonlimiting exemplary embodiments of ADCs comprising PBD dimers have the following structures:



PBD dimer-val-cit-PAB-Ab;



PBD dimer-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. In some embodiments, n is selected from 4, 5, 6, 7, and 8.

[0379] 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.

[0380] 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

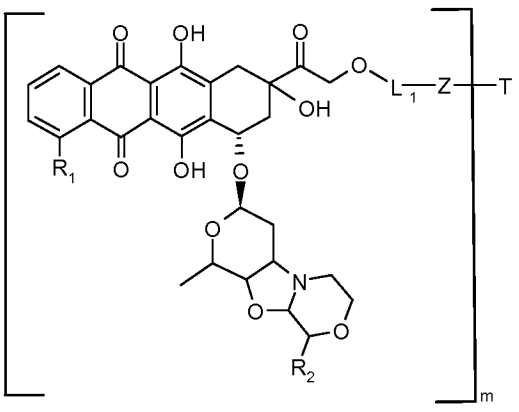
[0381] In some embodiments, an ADC comprising anthracycline. Anthracyclines are antibiotic compounds that exhibit cytotoxic activity. While not intending to be bound by any particular theory, studies have indicated that anthracyclines may operate to kill cells by a number of different mechanisms, including: 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).

[0382] 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).

[0383] 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).

[0384] A nonlimiting exemplary ADC comprising nemorubicin or nemorubicin derivatives is shown in Formula Ia:



(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;

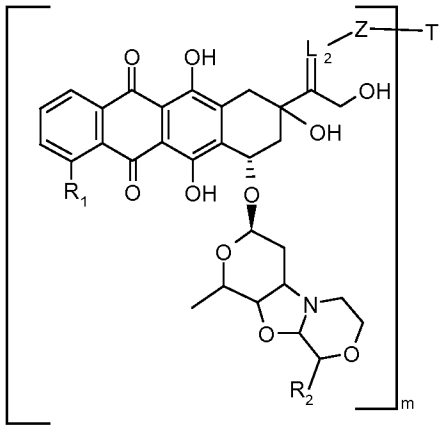
L_1 and Z together are a linker (L) as described herein;

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.

[0385] In some embodiments, R_1 and R_2 are both methoxy (-OMe).

[0386] A further nonlimiting exemplary ADC comprising nemorubicin or nemorubicin derivatives is shown in Formula Ib:



(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;

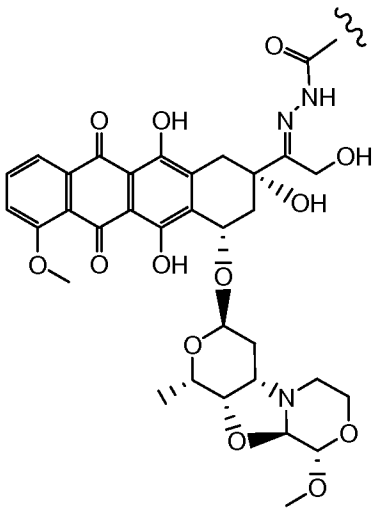
L_2 and Z together are a linker (L) as described herein;

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

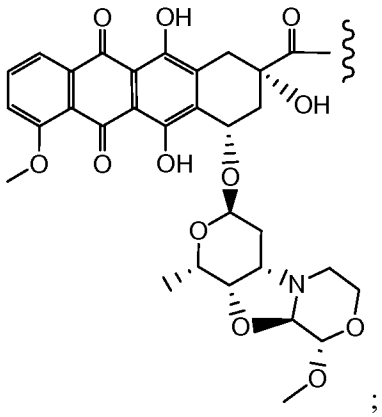
m is 1 to about 20. In some embodiments, m is 1 to 10, 1 to 7, 1 to 5, or 1 to 4.

[0387] In some embodiments, R_1 and R_2 are both methoxy (-OMe).

[0388] 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:



; or

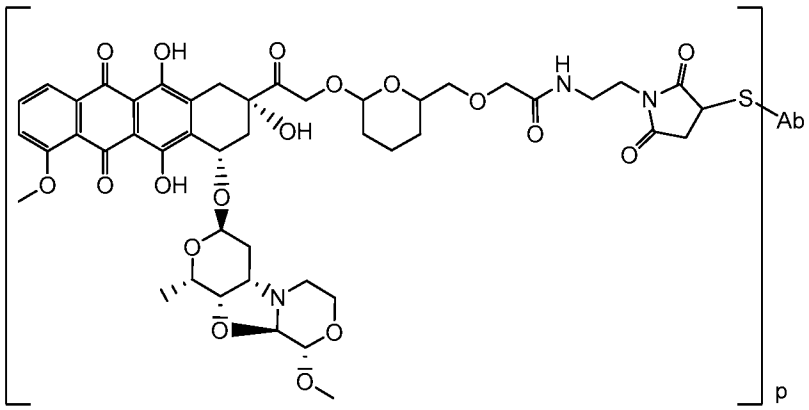


;

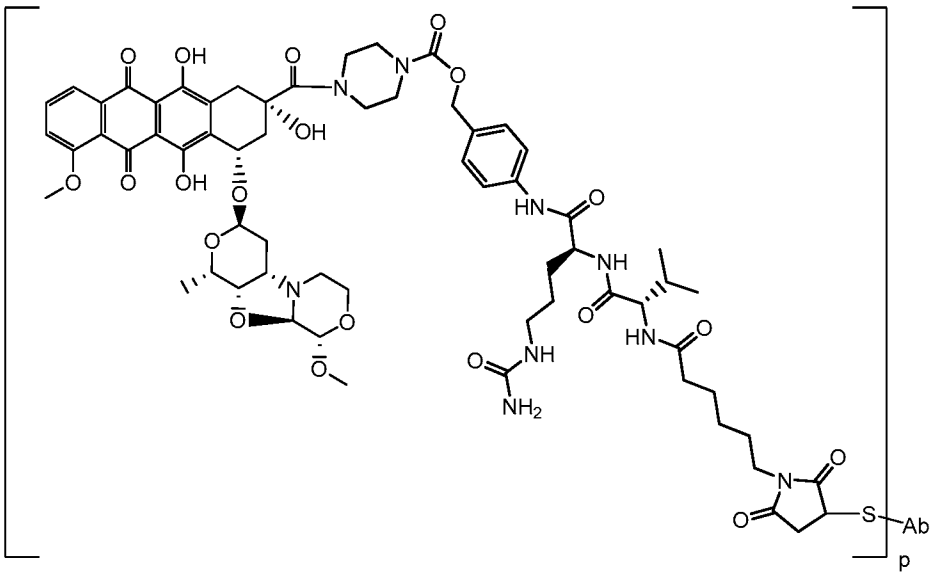
wherein the wavy line indicates the attachment to the linker (L).

[0389] 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.

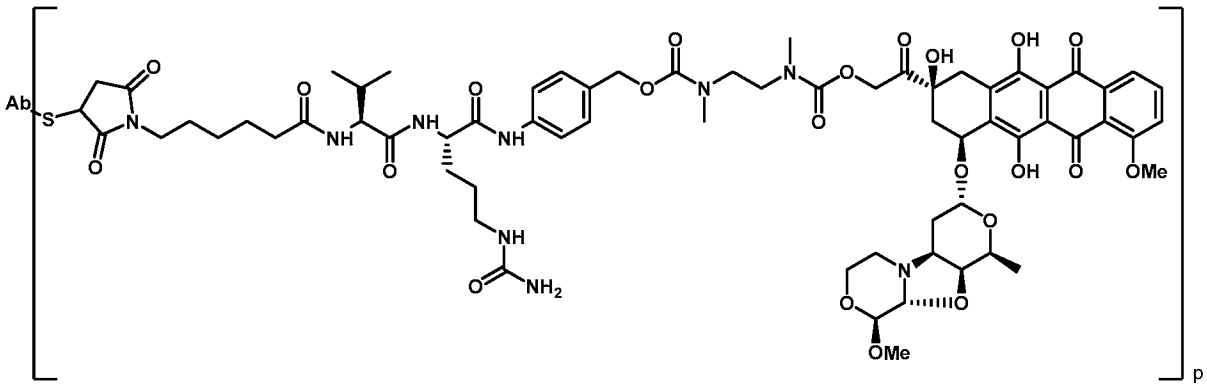
[0390] Exemplary ADCs comprising a nemorubicin and linker include, but are not limited to:



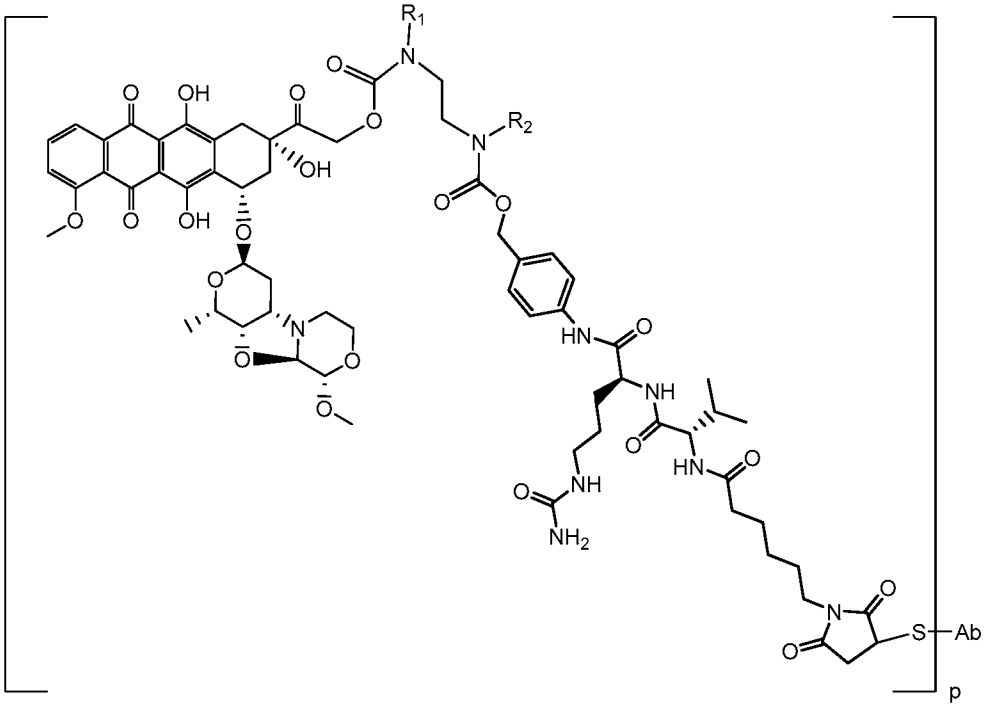
PNU-159682 maleimide acetal-Ab;



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

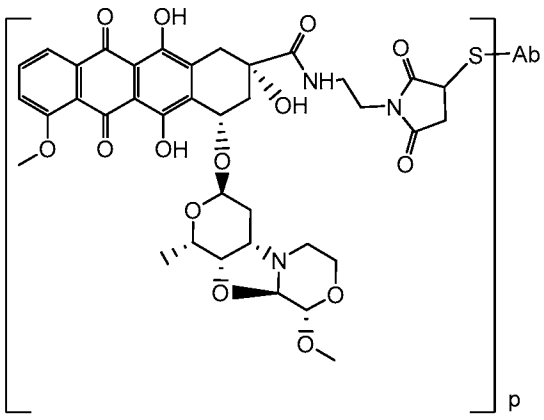


PNU-159682-val-cit-PAB-spacer-Ab;



PNU-159682-val-cit-PAB-spacer(R^1R^2)-Ab, wherein:

R_1 and R_2 are independently selected from H and C_1-C_6 alkyl; and



PNU-159682-maleimide-Ab.

[0391] 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^1R^2)-Ab are protease cleavable.

(6) Other Drug Moieties

[0392] 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.

[0393] Drug moieties also include compounds with nucleolytic activity (*e.g.*, a ribonuclease or a DNA endonuclease).

[0394] 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^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} 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^{99} or I^{123}, 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).

[0395] 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^{99}, I^{123}, Re^{186}, Re^{188} and In^{111} 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.

[0396] 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 β-galactosidase and neuraminidase, which are useful for converting glycosylated prodrugs into free drugs; β-lactamase, which is useful for converting drugs derivatized with β-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

[0397] 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.

[0398] 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).

[0399] 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 tricarbonylethylphosphine (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.

[0400] 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.

[0401] 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

[0402] 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.

[0403] 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 tricarbonylethylphosphine (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).

[0404] Antibody-drug conjugates of the invention 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.

[0405] 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.

[0406] 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.

[0407] 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).

E. Methods and Compositions for Diagnostics and Detection

[0408] In certain embodiments, any of the anti-LgR5 antibodies provided herein is useful 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 colon, colorectal, small intestine, endometrial, pancreatic, or ovarian tissue).

[0409] 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 colon, colorectal, small intestine, endometrial, pancreatic, or ovarian tissue).

[0410] 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}Ga, ^{18}F, ^{64}Cu, ^{86}Y, ^{76}Br, ^{89}Zr, and ^{124}I. In a particular embodiment, the positron emitter is ^{89}Zr.

[0411] 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 colon, colorectal, small intestine, endometrial, pancreatic or ovarian 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.

[0412] Exemplary disorders that may be diagnosed or detected according to any of the above embodiments include LgR5-positive cancers, such as LgR5-positive colorectal cancer (including adenocarcinoma), LgR5-positive small intestine cancer (including adenocarcinoma, sarcoma (*e.g.,* leiomyosarcoma), carcinoid tumors, gastrointestnal stromal tumor, and lymphoma)

LgR5-positive ovarian cancer (including ovarian serous adenocarcinoma), LgR5-positive pancreatic cancer (including pancreatic ductal adenocarcinoma), and LgR5-positive endometrial cancer. 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.

[0413] 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}P, ^{14}C, ^{125}I, ^{3}H, and ^{131}I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, *e.g.*, firefly luciferase and bacterial luciferase (U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, *e.g.*, glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like. In another embodiment, a label is a positron emitter. Positron emitters include but are not limited to ^{68}Ga, ^{18}F, ^{64}Cu, ^{86}Y, ^{76}Br, ^{89}Zr, and ^{124}I. In a particular embodiment, a positron emitter is ^{89}Zr.

F. Pharmaceutical Formulations

[0414] Pharmaceutical formulations of an anti-LgR5 antibody or immunoconjugate as described herein are prepared by mixing such antibody or 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 dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include insterstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

[0415] Exemplary lyophilized antibody or immunoconjugate formulations are described in US Patent No. 6,267,958. Aqueous antibody or immunoconjugate formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

[0416] 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. For example, in some instances, it may be desirable to further provide Avastin® (bevacizumab), *e.g.*, for the treatment of LgR5-positive cancer such as LgR5-positive colon cancer or LgR5-positive colorectal cancer.

[0417] Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

[0418] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody or immunoconjugate, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules.

[0419] 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.

G. Therapeutic Methods and Compositions

[0420] Any of the anti-LgR5 antibodies or immunoconjugates provided herein may be used in methods, *e.g.*, therapeutic methods.

[0421] In one aspect, an anti-LgR5 antibody or immunoconjugate provided herein is used in a method of inhibiting proliferation of an LgR5-positive cell, the method comprising exposing the cell to the anti-LgR5 antibody or immunoconjugate under conditions permissive for binding of the anti-LgR5 antibody or immunoconjugate to LgR5 on the surface of the cell, thereby inhibiting the proliferation of the cell. In certain embodiments, the method is an *in vitro* or an *in vivo* method. In further embodiments, the cell is a colon, colorectal, small intestine, ovarian, pancreatic, or endometrial cell.

[0422] In some embodiments, an anti-LgR5 antibody or immunoconjugate provided herein is used in a method of treating cancer that comprises a mutation in a Kras gene and/or a mutation in an adenomatous polyposis coli (APC) gene in at least a portion of the cells of the cancer. In various embodiments, the cancer is selected from colon, colorectal, small intestine, ovarian, pancreatic, and endometrial cancer. In some embodiments, an anti-LgR5 antibody or immunoconjugate provided herein is used in a method of treating a colon or colorectal cancer that comprises a mutation in a Kras gene and/or a mutation in an APC gene in at least a portion of the cells of the cancer. Nonlimiting exemplary Kras mutations found in cancers (including colon and colorectal cancers) include mutations at Kras codon 12 (*e.g.*, G12D, G12V, G12R, G12C, G12S, and G12A), codon 13 (*e.g.*, G13D and G13C), codon 61 (*e.g.*, G61H, G61L, G61E, and G61K), and codon 146. *See, e.g.*, Yokota, *Anticancer Agents Med. Chem.*, 12: 163-171 (2012); Wicki et al., *Swiss Med. Wkly*, 140: w13112 (2010). Nonlimiting exemplary APC mutations found in cancers include mutations in the mutation cluster region (MCR), such as stop codons and frameshift mutations that result in a truncated APC gene product. *See, e.g.,* Chandra et al., *PLoS One*, 7: e34479 (2012); and Kohler et al., *Hum. Mol. Genet.*, 17: 1978-1987 (2008).

[0423] In some embodiments, a method of treating cancer comprises administering an anti-LgR5 antibody or immunoconjugate to a subject, wherein the subject has a cancer comprising a Kras mutation and/or an APC mutation in at least a portion of the cancer cells. In some embodiments, the cancer is selected from colon, colorectal, small intestine, ovarian, pancreatic, and endometrial cancer. In some embodiments, the cancer is colon and/or colorectal cancer. In some embodiments, the subject has previously been determined to have a cancer

comprising a Kras mutation and/or an APC mutation in at least a portion of the cancer cells. In some embodiments, the cancer is LgR5-positive.

[0424] 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.

[0425] 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).

[0426] In another aspect, an anti-LgR5 antibody or immunoconjugate for use as a medicament is provided. In further aspects, an anti-LgR5 antibody or immunoconjugate for use in a method of treatment is provided. In certain embodiments, an anti-LgR5 antibody or immunoconjugate for use in treating LgR5-positive cancer is provided. In certain embodiments, the invention provides an anti-LgR5 antibody or immunoconjugate for use in a method of treating

an individual having an LgR5-positive cancer, the method comprising administering to the individual an effective amount of the anti-LgR5 antibody or immunoconjugate. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, *e.g.*, as described below.

[0427] In a further aspect, the invention provides for the use of an anti-LgR5 antibody or immunoconjugate in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of LgR5-positive cancer. In a further embodiment, the medicament is for use in a method of treating LgR5-positive cancer, the method comprising administering to an individual having LgR5-positive cancer an effective amount of the medicament. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, *e.g.*, as described below.

[0428] 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 or 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.

[0429] An LgR5-positive cancer according to any of the above embodiments may be, *e.g.*, LgR5-positive colon or colorectal cancer (including adenocarcinoma), LgR5-positive small intestine cancer (including adenocarcinoma, sarcoma (*e.g.,* leiomyosarcoma), carcinoid tumors, gastrointestnal stromal tumor, and lymphoma)., LgR5-positive ovarian cancer (including ovarian serous adenocarcinoma), LgR5-positive pancreatic cancer (including pancreatic ductal adenocarcinoma), and LgR5-positive endometrial cancer. 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.

[0430] An "individual" according to any of the above embodiments may be a human.

[0431] In a further aspect, the invention provides pharmaceutical formulations comprising any of the anti-LgR5 antibodies or 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 or immunoconjugates provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation

comprises any of the anti-LgR5 antibodies or immunoconjugates provided herein and at least one additional therapeutic agent, *e.g.*, as described below.

[0432] Antibodies or immunoconjugates of the invention can be used either alone or in combination with other agents in a therapy. For instance, an antibody or immunoconjugate of the invention may be co-administered with at least one additional therapeutic agent. In certain embodiments, an additional therapeutic agent is Avastin® (bevacizumab), *e.g.*, for the treatment of LgR5-positive cancer such as LgR5-positive colon cancer or LgR5-positive colorectal cancer.

[0433] 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 or immunoconjugate of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant. Antibodies or immunoconjugates of the invention can also be used in combination with radiation therapy.

[0434] An antibody or immunoconjugate of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, *e.g.* by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

[0435] Antibodies or immunoconjugates of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody or immunoconjugate 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 or immunoconjugate 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.

[0436] For the prevention or treatment of disease, the appropriate dosage of an antibody or immunoconjugate of the invention (when used alone or in combination with one or more other

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

[0437] It is understood that any of the above formulations or therapeutic methods may be carried out using both an immunoconjugate of the invention and an anti-LgR5 antibody.

H. Articles of Manufacture

[0438] In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the 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 or immunoconjugate of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an

antibody or immunoconjugate of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution 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

[0439] 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.

A. Human LgR5 Gene Expression

[0440] Human LgR5 gene expression was analyzed using a proprietary database containing gene expression information (GeneExpress®, Gene Logic Inc., Gaithersburg, MD). Graphical analysis of the GeneExpress® database was conducted using a microarray profile viewer. Figure 1 is a graphic representation of human LgR5 gene expression in various tissues. The scale on the y-axis indicates gene expression levels based on hybridization signal intensity. Dots appear both to the left and to the right of the line extending from the name of each listed tissue. The dots appearing to the left of the line represent gene expression in normal tissue, and the dots appearing to the right of the line represent gene expression in tumor and diseased tissue. Figure 1 shows increased LgR5 gene expression in certain tumor or diseased tissues relative to their normal counterparts. In particular, LgR5 is substantially overexpressed in colorectal, endometrial, and ovarian tumors. Figure 1, inset, shows that LgR5 is overexpressed in at least the following colon tumors: adenocarcinoma, benign tumors, and metastatic colon tumors, and also in tissue with a colon tumor content of less than 50% ("low tumor" in Figure 1 inset); but is not overexpressed in normal colon, Crohn's disease, or ulcerative colitis. Human LgR5 expression is much lower in normal tissues, with low levels of expression in normal brain, muscle, ovarian, and placental tissues.

B. Prevalence of Human LgR5 in Colon Tumors

[0441] To evaluate the expression of LgR5 in colorectal cancer, 57 primary colorectal adenocarcinomas were acquired from multiple sources (Asterand, Detroit, MI; Bio-Options,

Fullerton, CA; University of Michigan, Ann Arbor, MI; Cytomyx, Rockville, MD; Cooperative Human Tissue Network, Nashville, TN; Indivumed, Hamburg, Germany; ProteoGenex, Culver City, CA). Forty-four percent of samples were from men, and the average age of the patients was 66 years (range 31 to 93 years). Tissue microarrays (TMAs) were assembled using duplicate cores as described in Bubendorf L, et al., *J Pathol*. 2001 Sep;195(1):72-9, and included five normal colorectal mucosa samples from matched cases.

[0442] LgR5 expression was determined by in situ hybridization using the oligonucleotide probes shown in Table 2. *See, e.g.*, Jubb AM, et al., *Methods Mol Biol* 2006; 326:255-64. ISH for β-actin was used to confirm mRNA integrity in colorectal cancer tissues prior to analysis.

Table 2. Primer sequences for isotopic in situ hybridization probes.

Gene	Genbank Accession	Nucleotides Complementary to Probe	Anti-sense (AS) or Sense (S)	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
Lgr5	NM_003667	508	AS	ACCAACTGCATCCTAAACTG (SEQ ID NO: 83)	ACCGAGTTTCACCTCAGCTC (SEQ ID NO: 84)
Lgr5	NM_003667	496	S	ACATTGCCCTGTTGCTCTTC (SEQ ID NO: 85)	ACTGCTCTGATATACTCAATC (SEQ ID NO: 86)

[0443] LgR5 hybridization intensity was scored by a trained pathologist according to the scheme below, taking into account the intensity (silver grains) as well as breadth of staining.

0 (negative): very weak or no hybridization in >90% of tumor cells

1+ (mild): predominant hybridization pattern is weak

2+ (moderate): predominant hybridization pattern is moderately strong in the majority (> 50%) of neoplastic cells

3+ (strong): predominant hybridization pattern is strong in the majority (>50%) of neoplastic cells

Sense probes were used to control for the specificity of hybridization.

[0444] Figure 2 shows exemplary colon tumor sections with 1+, 2+, and 3+ levels of staining. The top panels show dark field images and the bottom panels show bright field images. The deposition of silver grains in the dark field images indicates hybridization of the probe and expression of LgR5 mRNA. ~77% (41/53) of colon tumor sections analyzed were LgR5 positive, showing staining at the 1+, 2+, or 3+ levels, with 34% (18/53) showing 2+ or 3+ staining. Four of the 57 samples analyzed were noninformative for LgR5 expression.

[0445] To evaluate the significance of Lgr5 expression in colon tumors, a population-based series of patients who had undergone surgical resections for colorectal adenocarcinoma was compiled retrospectively from the pathology archives at St James' University Hospital (Leeds, UK) from 1988 to 2003. Tissue microarrays (TMAs) were constructed with one core of normal mucosa and three cores of adenocarcinoma per patient as described in Bubendorf L, et al., *J Pathol.* 2001 Sep;195(1):72-9. ISH was performed and scored as described above. The heterogeneity of expression across three cores from the same tumor was also determined, and is expressed as the proportion of tumors that showed a particular level of discordance in one of the three cores. For example, if three cores had scores of +1, +3, and +3, one of the three cores from that tumor is discordant by 2.

[0446] Figure 3A shows the prevalence of 0, 1+, 2+, and 3+ levels of LgR5 staining in the colon tumor tissue microarray, measured by in situ hybridization. 75% of the colon tumor tissues showed staining at the 1+, 2+, or 3+ levels, with 37% showing 2+ or 3+ staining. Figure 3B shows the heterogeneity of LgR5 expression. 67% of tumors showed no heterogeneity across the three cores. 32% shows a discordance of 1 in one of the three cores, and only 1% showed a discordance greater than 1.

C. Mouse Monoclonal Antibody Generation

[0447] Monoclonal antibodies against human LgR5 were generated using the following procedures. Human LgR5 extracellular domain (ECD; amino acids 22-557) with a C-terminal His-tagged Fc was expressed from a baculovirus expression system, and purified on a Ni-NTA column (Qiagen), followed by gel filtration on a Superdex 200 column in 20mM MES pH 6.0, 6M guanidine HCl as previously described (Kirchhofer *et al.*, 2003) and dialysis into PBS for storage at –80°C.

[0448] Fifteen Balb/c mice (Charles River Laboratories International, Inc., Hollister, CA, USA) were injected with either huLgR5 plasmid DNA in lactated Ringer's solution (via tail vein) or with recombinant human LgR5 ECD as described above (via rear footpads) in adjuvant containing metabolizable squalene (4% v/v), Tween 80 (0.2 % v/v), trehalose 6,6-dimycolate (0.05% w/v) and monophosphoryl lipid A (0.05% w/v; Sigma Aldrich, USA). Serum titers were evaluated by standard enzyme linked immunosorbant assay (ELISA) and FACS following 6-9 injections. Splenic B cells harvested from a total of 5 mice were fused with mouse myeloma cells (X63.Ag8.653; American Type Culture Collection, Manassas, VA, USA) by electrofusion (Hybrimune; Harvard Apparatus, Inc., Holliston, MA, USA). After 10-14 days, hybridoma supernatants were screened for antibody secretion by ELISA. All positive clones were then expanded and re-screened for binding to huLgR5 and muLgR5 by ELISA and FACS (i.e., for

binding to 293-huLGR5 and 293-muLGR5 cells). Hybridoma clones 8E11.1.1 (identified from the DNA immunized mice), and 2H6.3.5 and 3G12.2.1 (both from the protein immunized mice) showed high immunobinding after two rounds of subcloning (by limiting dilution) and were scaled up for purification in INTEGRA CELLine 1000 bioreactors (INTEGRA Biosciences AG, Zizers, Switzerland). Supernatants were then purified by affinity chromatography, sterile-filtered, and stored at 4°C in PBS. The isotypes of the mAbs were determined to be IgG1 (kappa light chain) using the Isostrip Mouse mAb Isotyping Kit (Roche Applied Biosciences, Indianapolis, IN, USA).

[0449] Figure 4 shows certain monoclonal antibodies generated, along with certain properties, some of which will be described in further detail below.

D. Cloning and Chimerization of Mouse Monoclonal Antibodies

[0450] Monoclonal antibodies 8E11, 3G12, and 2H6 were cloned and chimerized as follows.

[0451] Total RNA was extracted from hybridoma cells producing murine 8E11, murine 3G12, or murine 2H6 using standard methods. The variable light (VL) and variable heavy (VH) domains were amplified using RT-PCR with degenerate primers to the heavy and light chains. The forward primers were specific for the N-terminal amino acid sequence of the VL and VH regions. Respectively, the LC and HC reverse primers were designed to anneal to a region in the constant light (CL) and constant heavy domain 1 (CH1), which are highly conserved across species. The polynucleotide sequence of the inserts was determined using routine sequencing methods. The 8E11 VL and VH amino acid sequences are shown in Figures 5 and 6, respectively (SEQ ID NOs: 3 and 4, respectively). The 3G12 and 2H6 VL and VH amino acid sequences are shown in Figures 7 and 8, respectively. The VL and VH sequences of antibody 3G12 are shown in SEQ ID NOs: 21 and 22, respectively, and the VL and VH sequences of antibody 2H6 are shown in SEQ ID NOs: 23 and 24, respectively.

[0452] Each antibody was chimerized by cloning the mouse heavy chain variable region onto a human IgG1 heavy chain constant region and cloning the light chain variable region onto a human kappa light chain constant region.

E. Humanization of 8E11

[0453] Monoclonal antibody 8E11 was humanized as described below. Residue numbers are according to Kabat et al., *Sequences of proteins of immunological interest*, 5th Ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991).

Direct hypervariable region grafts onto the acceptor human consensus framework

[0454] Variants constructed during the humanization of 8E11 were assessed in the form of an IgG. The VL and VH domains from murine 8E11 were aligned with the human VL kappa IV (VL_{KIV}) and human VH subgroup I (VH_I) consensus sequences. Hypervariable regions from the murine 8E11 (mu8E11) antibody were engineered into VL_{KIV} and VH_I acceptor frameworks to generate 8E11.v1. Specifically, from the mu8E11 VL domain, positions 24-34 (L1), 50-56 (L2) and 89-97 (L3) were grafted into VL_{KIV}. From the mu8E11 VH domain, positions 26-35 (H1), 49-65 (H2) and 95-102 (H3) were grafted into VH_I. In addition, positions 71 and 78 in framework III of VH were retained from the mouse sequence in 8E11.v1. Those residues were found to be part of the framework residues acting as "Vernier" zone, which may adjust CDR structure and fine-tune the antigen fit. *See, e.g.*, Foote and Winter, *J. Mol. Biol.* 224: 487-499 (1992) (Figures 5 and 6). These CDR definitions include positions defined by their sequence hypervariability (Wu, T. T. & Kabat, E. A. (1970)), their structural location (Chothia, C. & Lesk, A. M. (1987)) and their involvement in antigen-antibody contacts (MacCallum *et al. J. Mol. Biol.* 262: 732-745 (1996)).

[0455] Additional 8E11 variants were generated to evaluate the contributions of other Vernier positions, such as position 68 in the light chain, and positions 67 and 69 in the heavy chain. Humanized 8E11.v2 was generated by retaining two addition mouse residues, at positions 67 and 69 of the heavy chain variable region. The light chain variable region sequence and heavy chain variable region sequence for 8E11.v2, and other variants, are shown in Figures 5 and 6, respectively.

[0456] The humanized variants of 8E11 were generated by Kunkel mutagenesis using a separate oligonucleotide for each hypervariable region. Correct clones were identified by DNA sequencing.

Assessment of variants

[0457] For screening purposes, IgG variants were initially produced in 293 cells. Vectors coding for VL and VH were transfected into 293 cells. IgG was purified from cell culture media by protein A affinity chromatography.

[0458] The affinity of each 8E11 IgG variant for human LgR5 was determined by surface plasmon resonance using a BIAcore™-3000. BIAcore™ research grade CM5 chips were 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 were coupled to the chips to achieve approximately 10,000 response units (RU) in each flow cell. Unreacted coupling groups were blocked with 1M ethanolamine. For kinetics measurements,

anti-LGR5 antibodies were captured to achieve approximately 300 RU. Two-fold serial dilutions of human LgR5 ECD (amino acids 22-557 fused to His-Fc expressed in a baculovirus system, or amino acids 22-558 fused to Fc expressed from CHO cells; 125 nM to 0.49 nM) were 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 µl/min. Association rates (k_{on}) and dissociation rates (k_{off}) were calculated using a 1:1 Langmuir binding model (BIAcore™ Evaluation Software version 3.2). The equilibrium dissociation constant (Kd) was calculated as the ratio k_{off}/k_{on}.

Results

[0459] The human acceptor framework used for humanization of 8E11 is based on the human VL kappa IV consensus (VL_{KIV}) and the acceptor VH framework VH_I. Eight humanized variants of mu8E11 were produced and tested for LgR5 affinity by BIAcore™. The light chain variable regions and heavy chain variable regions of each of the variants is shown in Figures 5 and 6, respectively. The results of the affinity measurements are shown in Figure 9.

[0460] To improve the binding affinity of 8E11.v1, position 68 in the light chain and positions of 67 and 69 in the heavy chain were changed to residues found at these positions in mu8E11. Positions 71 and 78 in the heavy chain were changed to residues found at these positions in the human framework VH_I. Combinations of these altered light and heavy chains were expressed as IgG and purified as described above, and assessed for binding to human LgR5 by Biacore (Figure 9).

[0461] Variant hu8E11.v2 was generated by changing positions 67 and 69 of the hu8E11.v1 heavy chain to the residues found at those positions in mu8E11. The affinity (K_D) of hu8E11.v2 was found to be about the same as the parental ch8E11 antibody.

Summary of changes for humanized 8E11.v2

[0462] The 6 murine 8E11 CDRs (defined as positions 24-34 (L1), 50-56 (L2) and 89-97 (L3), 26-35 (H1), 49-65 (H2) and 93-102 (H3)) were grafted into the human consensus VL_{KIV} and VH_I acceptor domains. Positions 67, 69, 71, and 78 were changed back to murine residues from mu8E11. Humanized 8E11.v2 has comparable affinity for LgR5 to chimeric 8E11.

[0463] Throughout this application, mouse monoclonal antibodies 8E11, 2H6, and 3G12 are referred to in the alternative as 8E11, m8E11, or mu8E11; and 2H6, m2H6 or mu2H6; and 3G12, m3G12, or mu3G12; respectively. Chimeric monoclonal antibodies 8E11, 2H6, and 3G12 are referred to as chimeric 8E11 or ch8E11; chimeric 2H6 or ch2H6; and chimeric 3G12 or ch3G12; respectively. Humanized monoclonal antibody 8E11.v2 may also be referred to as 8E11v2, h8E11v.2, or hu8E11v.2.

F. Generation of a Human Monoclonal Antibody by Phage Display

[0464] Human LgR5 ECD (amino acids 22-555) with an N-terminal FLAG was expressed in CHO cells and purified on an anti-FLAG resin overnight, and then eluted with 0.1M acetic acid, pH 2.7. The protein was then purified by gel filtration on a Superdex 200 column in PBS and then dialyzed into PBS for storage at –80°C.

[0465] Human phage antibody libraries with synthetic diversities in the selected complementary determining regions (H1, H2, H3), mimicking the natural diversity of human IgG repertoire were used for panning. The Fab fragments were displayed bivalently on the surface of M13 bacteriophage particals (Lee et al. (2004) *J Mol Biol* **340**, 1073-93). Human LgR5 ECD (amino acids 22-555) produced as described above was used as an antigen. Nunc 96-well MaxiSorp immnoplates (Nunc) were coated overnight at 4°C with LgR5 ECD protein (10 μg/ml) and blocked for 1 hour with PBST buffer (PBS, 0.05% Tween 20) supplemented with 1% BSA. The antibody phage libraries were added and incubated overnight at room temperature. The plates were washed with PBST buffer and bound phage were eluted with 50 mM HCL/500 mM NaCl for 30 minutes and neutralized with an equal volume of 1M Tris base. Recovered phages were amplified in *E.coli* XL-1 blue cells. During subsequent selection rounds, the incubation time of the phage antibodies was decreased to 2 hours and the stringency of plate washing was gradually increased (Liang et al. (2007) *J Mol Biol* **366**, 815-829). Unique and specific phage antibodies that bind to human LgR5 ECD were identified by phage ELISA and DNA sequencing. Certain clones, including YW353, were reformatted to full length IgGs by cloning the VL and VH regions into LPG3 and LPG4 vectors, respectively. Antibodies were transiently expressed in mammalian cells and and purified on protein A columns (Carter et al. (1992) *Proc Natl Acad Sci U S A* **89**, 4285-9).

[0466] The light chain and heavy chain variable regions sequence for human antibody YW353 are shown in Figures 10 and 11, respectively (SEQ ID NOs: 26 and 25). IgG1 heavy chain and kappa light chain sequences for human antibody YW353 are shown in SEQ ID NOs: 66 and 65, respectively. Since YW353 was generated from a human antibody phage library, the terms "YW353" and "huYW353" are used interchangeably herein.

G. Species Cross-Reactivity

[0467] Monoclonal antibodies were tested to determine if they cross-react with LgR5 from species other than human. Figures 12A to C shows an alignment between human (SEQ ID NO: 67), cynomolgus monkey (SEQ ID NO: 69), rat (SEQ ID NO: 70) and mouse (SEQ ID NO: 72) LgR5. Residues that are identical among all four species are indicated by asterisks (*). Figure 4 shows the results of FACS analysis of 293 cells stably transfected with gD epitope-tagged

LgR5 (human, cynomolgus monkey, rat, or mouse LgR5); stained with 10 μg/ml YW353, ch8E11, hu8E11.v2, 2H6, or 3G12 antibody; and detected with R-Phycoerythrin conjugated goat anti-human antibody. Untransfected 293 cells do not normally express LgR5. YW353 antibody binds human and cynomolgus monkey LgR5, but not rat or mouse LgR5. Ch8E11 and hu8E11.v2 antibodies bind all four species of LgR5, although binding to rat LgR5 is not as strong as binding to human, cynomolgus monkey, or mouse LgR5. 2H6 antibody binds to human and mouse LgR5, and was not tested for binding to cynomolgus monkey or rat LgR5. 3G12 antibody shows strong binding to human LgR5, less strong binding to mouse LgR5, and was not tested for binding to cynomolgus monkey or rat LgR5.

H. Antibody Affinities

[0468] The affinity of each antibody for human LgR5 was determined by surface plasmon resonance using a BIAcore™-3000, substantially as described above in Example E.

[0469] As shown in Figure 4, YW353 antibody bound to human LgR5 with an affinity of 1.6 nM. Ch8E11 and hu8E11.v2 antibodies bound to human LgR5 with affinities of 2.4 nm and 3.1 nm, respectively. 2H6 and 3G12 antibodies bound to human LgR5 with affinities of 208 nM and 72 nM, respectively.

[0470] Scatchard analysis was performed following standard procedures (Holmes et al., *Science* 256:1205-1210 (1992)) to determine the relative binding affinities of YW353, ch8E11 and hu8E11v2 antibodies.

[0471] Anti-Lgr5 antibodies were [I^{125}] labeled using the indirect Iodogen method. The [I^{125}] labeled anti-Lgr5 antibodies were purified from free ^{125}I-Na by gel filtration using a NAP-5 column (GE Healthcare); the purified iodinated anti-Lgr5 antibodies had a range of specific activities of 13.92 to 19.01 μCi/μg. Competition assay mixtures of 50 μL volume containing a fixed concentration of [I^{125}] labeled antibody and decreasing concentrations of serially diluted, unlabeled antibody were placed into 96-well plates. 293 cells stably expression human, rat, or mouse Lgr5 were cultured in growth media at 37°C in 5% CO_2. Cells were detached from the flask using Sigma Cell Dissociation Solution and were washed with binding buffer, which consisted of Dulbecco's Modified Eagle Medium (DMEM) with 2% fetal bovine serum (FBS), 50 mM HEPES (pH 7.2) and 0.1% sodium azide. The washed cells were added to the 96 well plates at a density of 250,000 cells in 0.2 mL of binding buffer. The final concentration of the [I^{125}] labeled antibody in each well was 200 pM. The final concentration of the unlabeled antibody in the competition assay ranged from 500 nM through ten 2-fold diluation steps to a 0 nM buffer-only assay. Competition assays were carried out in triplicate. Competition assays were incubated for 2 hours at room temperature. After the 2-hour incubation, the competition assays

were transferred to a Millipore Multiscreen filter plate (Billerica, MA) and washed 4 times with binding buffer to separate the free from bound [I^{125}] labeled antibody. The filters were counted on a Wallac Wizard 1470 gamma counter (PerkinElmer Life and Analytical Sciences Inc.; Wellesley, MA). The binding data was evaluated using NewLigand software (Genentech), which uses the fitting algorithm of Munson and Robard to determine the binding affinity of the antibody (Munson and Robard 1980)

[0472] As shown in Figure 4, YW353 bound to gD-tagged human LgR5 expressed on stably transfected 293 cells with an affinity of 0.2 nM. Ch8E11 bound to gD-tagged human LgR5 and gD-tagged mouse LgR5 expressed on stably transfected 293 cells with affinities of 0.4 nM and 0.2 nM, respectively. Hu8E11v2 bound to gD-tagged human LgR5, gD-tagged mouse LgR5, and gD-tagged rat LgR5 expressed on stably transfected 293 cells with affinities 0.3-0.7 nM, 0.5-0.6 nM, and 2.4-2.8 nM, respectively. These Kd values were generally lower than those determined by BIAcore®.

I. Epitope Mapping

[0473] To determine the region of LgR5 bound by each antibody, 293 cells transiently transfected with gD epitope-tagged LgR5 with various N- and/or C-terminal deletions were stained with 10 µg/ml YW353, ch8E11, hu8E11v2, 2H6, or 3G12 antibody; and binding was detected with R-Phycoerythrin conjugated goat anti-human antibody. Antibodies YW353, 8E11, 2H6, and 3G12 all bound to gD epitope-tagged full-length LgR5. Antibodies 2H6 and 3G12 bound to gD epitope-tagged $LgR5_{324-907}$ (amino acids 324-907 of SEQ ID NO: 67). Antibodies YW353 and 8E11 did not bind to gD epitope-tagged $LgR5_{324-907}$. Only antibody YW353 bound to gD epitope-tagged $LgR5_{22-123}$ (amino acids 22-123 of SEQ ID NO: 67) with a C-terminal GPI anchor. Antibodies YW353 and 8E11 both bound to to gD epitope-tagged $LgR5_{22-323}$ (amino acids 22-323 of SEQ ID NO: 67) with a C-terminal GPI anchor, but antibodies 2H6 and 3G12 did not. Finally, none of the antibodies bound to gD epitope-tagged $LgR5_{424-907}$ (amino acids 424-907 of SEQ ID NO: 67).

[0474] Figure 4 summarizes those results in the column titled "epitope region." As shown in that figure, antibody YW353 binds to an epitope in the region of amino acids 22 to 123 of SEQ ID NO: 67; antibody 8E11 and its humanized variants bind to an epitope in the region of amino acids 22 to 323 of SEQ ID NO: 67; and antibodies 2H6 and 3G12 bind to an epitope in the region of amino acids 324 to 423 of SEQ ID NO: 67.

J. Production of Anti-LgR5 Antibody Drug Conjugates

[0475] For larger scale antibody production, antibodies were produced in CHO cells. Vectors coding for VL and VH were transfected into CHO cells and IgG was purified from cell culture media by protein A affinity chromatography.

Anti-LgR5 Antibody MMAE Conjugates

[0476] Anti-LgR5 antibody-drug conjugates (ADCs) were produced by conjugating YW353 (IgG1 heavy chain and kappa light chain sequences shown in SEQ ID NOs: 66 and 65, respectively), hu8E11v2 (IgG1 heavy chain and kappa light chain sequences shown in SEQ ID NOs: 64 and 63, respectively), mu8E11, ch8E11, 2H6, ch2H6, 3G12, and ch3G12 to the drug-linker moiety MC-vc-PAB-MMAE, which is depicted herein. For convenience, the drug-linker moiety MC-vc-PAB-MMAE is sometimes referred to in these Examples and in the Figures as "vcMMAE" or "VCE." Prior to conjugation, the antibodies were partially reduced with TCEP using standard methods in accordance with the methodology described in WO 2004/010957 A2. The partially reduced antibodies were conjugated to the drug-linker moiety using standard methods in accordance with the methodology described, *e.g.*, in Doronina et al. (2003) *Nat. Biotechnol.* 21:778-784 and US 2005/0238649 A1. Briefly, the partially reduced antibodies were combined with the drug-linker moiety to allow conjugation of the drug-linker moiety to reduced cysteine residues of the antibody. The conjugation reactions were quenched, and the ADCs were purified. The drug load (average number of drug moieties per antibody) for each ADC was determined and was between 3.3 and 4.0 for the anti-LgR5 antibodies. The structure of an antibody-vcMMAE immunoconjugate is shown in Figure 35A (p = drug load).

Anti-LgR5 Antibody PNU Conjugates

[0477] Anti-LgR5 antibody-PNU drug conjugates (ADCs) were produced by conjugating YW353 A118C thioMab (IgG1 A118C heavy chain and kappa light chain sequences shown in SEQ ID NOs: 78 and 65, respectively) or hu8E11v2 thioMab (IgG1 A118C heavy chain and kappa light chain sequences shown in SEQ ID NOs: 75 and 63, respectively) to PNU drug-linker moieties. Prior to conjugation, the antibody was reduced with dithiothreitol (DTT) to remove blocking groups (e.g. cysteine) from the engineered cysteines of the thio-antibody. This process also reduces the interchain disulfide bonds of the antibody. The reduced antibody was purified to remove the released blocking groups and the interchain disulfides were reoxidized using dehydro-ascorbic acid (dhAA).

[0478] For antibody-drug conjugates comprising a val-cit linker and PNU, the intact antibody was combined with the drug-linker moiety MC-val-cit-PAB-spacer-PNU-159682 ("val-cit" may also be referred to herein as "vc") to allow conjugation of the drug-linker moiety to the engineered cysteine residues of the antibody. The conjugation reaction was quenched by adding

excess N-acetyl-cysteine to react with any free linker-drug moiety, and the ADC was purified. The drug load (average number of drug moieties per antibody) for the ADC was in the range of about 1.8 to 2. The structure of an antibody-vcPNU immunoconjugate is shown in Figure 35B (p = drug load).

[0479] For antibody drug conjugates comprising an acetal linker and PNU, the intact antibody was combined with the drug-linker moiety MC-acetal-PNU-159682 to allow conjugation of the drug-linker moiety to the engineered cysteine residues of the antibody. The conjugation reaction was quenched by adding excess N-acetyl-cysteine to react with any free linker-drug moiety, and the ADC was purified. The drug load (average number of drug moieties per antibody) for the ADC was about 1.8 to 2. The structure of an antibody-acetal-PNU immunoconjugate is shown in Figure 35C (p = drug load).

[0480] For antibody drug conjugates comprising a noncleavable linker and PNU, the intact antibody was combined with the drug-linker moiety MC-PNU-159682 to allow conjugation of the drug-linker moiety to the engineered cysteine residues of the antibody. The conjugation reaction was quenched by adding excess N-acetyl-cysteine to react with any free linker-drug moiety, and the ADC was purified. The drug load (average number of drug moieties per antibody) for the ADC was about 1.8 to 2. The structure of an antibody-PNU immunoconjugate is shown in Figure 35D (p = drug load).

Anti-LgR5 Antibody PBD Conjugate

[0481] Anti-LgR5 antibody-PBD drug conjugates (ADCs) were produced by conjugating YW353 A118C thioMab (IgG1 A118C heavy chain and kappa light chain sequences shown in SEQ ID NOs: 78 and 65, respectively) or hu8E11v2 thioMab (IgG1 A118C heavy chain and kappa light chain sequences shown in SEQ ID NOs: 75 and 63, respectively) to PBD drug-linker moieties. Prior to conjugation, the antibody was reduced with dithiothreitol (DTT) to remove blocking groups (e.g. cysteine) from the engineered cysteines of the thio-antibody. This process also reduces the interchain disulfide bonds of the antibody. The reduced antibody was purified to remove the released blocking groups and the interchain disulfides were reoxidized using dehydro-ascorbic acid (dhAA).

[0482] For antibody-drug conjugates comprising a val-cit linker and PBD, the intact antibody was combined with the drug-linker moiety MC-val-cit-PAB-PBD ("val-cit" may also be referred to herein as "vc") to allow conjugation of the drug-linker moiety to the engineered cysteine residues of the antibody. The conjugation reaction was quenched by adding excess N-acetyl-cysteine to react with any free linker-drug moiety, and the ADC was purified. The drug load (average number of drug moieties per antibody) for the ADC was in

the range of about 1.8 to 2. The structure of an antibody-vcPBD is shown in Figure 35E (p = drug load).

K. Toxicity of Anti-LgR5 Antibody Drug Conjugate in Rats

[0483] In order to evaluate potential toxicity of anti-LgR5 antibody 8E11.v2-vcMMAE, six male Sprague-Dawley rats were administered 12 mg/kg 8E11.v2-vcMMAE once per week for four weeks, and six male Sprague-Dawley rats were administered 20 mg/kg 8E11.v2-vcMMAE once per week for two weeks. Four male Sprague-Dawley control rats were administered vehicle alone once per week for two weeks, and four male Sprague-Dawley control rats were administered vehicle alone once per week for four weeks. The rats in the two-week groups were necropsied on day 12 and the rats in the four-week groups were necropsied on day 26.

[0484] Briefly, all rats administered 8E11.v2-vcMMAE showed reduced red cell mass (red blood cells, hematocrit, hemoglobin, and reticulocytes), neutrophils, and platelets compared to control rats. Rats administered 20 mg/kg 8E11.v2-vcMMAE also showed reduced white blood cell count and lymphocytes compared to control rats. In addition, all rats administered 8E11.v2-vcMMAE showed increases in liver enzymes ALT, AST, ALP, and GGT, and increased total billirubin compared to control rats.

[0485] Histopathologic analysis of tissues collected from the study showed cellular depletion of lymphoid and hemopoietic tissues in rats administered 8E11.v2-vcMMAE, as well as increased mitotic figures in rapidly dividing tissues. Rats administered 20 mg/kg 8E11.v2-vcMMAE also showed minimal liver necrosis, minimal increased mitotic figures and single cell cryptal necrosis/apoptosis, and minimal mild alveolar histiocytosis and type II cell hyperplasia.

[0486] The pathology changes observed were similar to pathology observed in rats administered other vcMMAE antibody-drug conjugates. There did not appear to be any evidence of LgR5 antigen-dependent toxicity in the GI tract.

L. Efficacy of anti-LgR5 Antibody Drug Conjugates in LoVo Colon Cancer Cell Line Xenograft

[0487] The efficacy of the anti-LgR5 ADCs was investigated using a LoVo colon cancer xenograft model. LoVo cells are a colorectal adenocarcinoma cell line with an APC mutation (ATCC #CCL 229). LgR5 is highly expressed in LoVo cells, and was confirmed by microarray, TaqMan® quantitative RT-PCR, in situ hybridization, FACS, and Western blot. Five million LoVo cells (LgR5-positive by FACS using YW353) in HBSS-matrigel were injected subcutaneously into the dorsal flank of NCR nude mice and six days post-inoculation mice were given a single intravenous injection of 5 mg/kg murine anti-gp120-vcMMAE control antibody-drug conjugate, human anti-gD 5B6-vcMMAE control antibody-drug conjugate, huYW353-

vcMMAE antibody-drug conjugate, mu8E11-vcMMAE antibody-drug conjugate, mu2H6-vcMMAE antibody-drug conjugate, or mu3G12-vcMMAE antibody-drug conjugate; or with vehicle (PBS) alone. The presence of the antibodies was confirmed by PK bleeds one day post injection.

[0488] As shown in Figure 13, substantial tumor growth inhibition was achieved with all four anti-LgR5 antibody-drug conjugates tested.

M. Efficacy of anti-LgR5 Antibody Drug Conjugates in D5124 Pancreatic Cancer Xenograft

[0489] The efficacy of the anti-LgR5 ADCs was investigated using a D5124 pancreatic cancer xenograft model, which has a β-catenin mutation. LgR5 is highly expressed in D5124 tumors, and was confirmed by microarray, TaqMan® quantitative RT-PCR, in situ hybridization, FACS, and Western blot. Twenty to 30 mm^3 D5124 tumor fragments (LgR5-positive by FACS using YW353 and 8E11) were implanted subcutaneously into the dorsal flank area of NCR nude mice and 18 days post-transplantation the mice were given a single intravenous injection of 6 mg/kg human anti-gD 5B6-vcMMAE control antibody-drug conjugate, 3 mg/kg or 6 mg/kg huYW353-vcMMAE antibody-drug conjugate, 3 mg/kg or 6 mg/kg ch8E11-vcMMAE antibody-drug conjugate, 3 mg/kg or 6 mg/kg ch2H6-vcMMAE antibody-drug conjugate, or 3 mg/kg or 6 mg/kg ch3G12-vcMMAE antibody-drug conjugate; or with vehicle (histidine buffer: 20mM histidine acetate, 240 mM sucrose, 0.02% Tween 20, pH5.5; "HB#8" in Figure 14) alone. The presence of the antibodies was confirmed by PK bleeds one and eight days post injection.

[0490] As shown in Figure 14, substantial tumor growth inhibition was achieved at both doses of huYW353-vcMMAE, ch8E11-vcMMAE, and ch3G12-vcMMAE. Substantial tumor growth inhibition was also achieved at 6 mg/kg ch2H6-vcMMAE.

[0491] The efficacy of various doses of YW353-vcMMAE was then tested in the D5124 pancreatic cancer xenograft model described above. Twenty to 30 mm^3 D5124 tumor fragments (LgR5-positive by FACS using YW353 and 8E11) were implanted subcutaneously into the dorsal flank area of NCR nude mice and 23 days post-transplantation mice were given a single intravenous injection of 0.5 mg/kg, 1 mg/kg, 3 mg/kg, 6 mg/kg, or 12 mg/kg huYW353-vcMMAE antibody-drug conjugate; or 12 mg/kg huYW353; or 7.2 mg/kg or 14.4 mg/kg human anti-gD 5B6-vcMMAE control antibody-drug conjugate; or vehicle (histidine buffer: 20mM histidine acetate, 240 mM sucrose, 0.02% Tween 20, pH5.5; "HB#8" in Figure 15) alone. The presence of the antibodies was confirmed by PK bleeds one, four, and 14 days post injection.

[0492] As shown in Figure 15, substantial tumor growth inhibition was achieved at 3 mg/kg huYW353-vcMMAE, and almost complete tumor growth inhibition was achieved at 6 mg/kg and 12 mg/kg huYW353-vcMMAE.

N. Efficacy of mu8E11 and mu8E11-vcMMAE in Murine Intestinal Tumorigenesis Model

[0493] The efficacy of mu8E11 and mu8E11-vcMMAE was investigated in a murine intestinal tumorigenesis model, $APC^{min/+}$; $LSL\text{-}Kras^{G12D}$; VillinCre ("AKV mice"). AKV mice are the result of crossing $APC^{min/+}$; VillinCre ("AV mice") with $LSL\text{-}Kras^{G12D}$ mice. While AV mice develop 0-4 adenomas in the colon and 100 adenomas in the small intestine, AKV mice develop an average of 140 adenomas in the colon and 100 adenomas in the small intestine (data not shown). LgR5 mRNA expression was measured in normal tissue and polyps of AV and AKV mice, and LgR5 was found to be significantly overexpressed in polyps from both the small intestine and colon in AKV mice (Figure 16). To visualize expression of LgR5 in the small intestine and colon of AKV mice, AKV mice were crossed with mice having a cassette containing an enhanced green fluorescent protein (EGFP) linked in frame to human diphtheria toxin receptor cDNA located in the *Lgr5* gene. *See* Tian et al., *Nature*, 478: 255-260 (2011). The area of EGFP expression in small intestine polyps and large intestine polyps were visualized in the AKV $Lgr5^{DTR/+}$ mice. The results of that experiment are shown in Figure 20. It was found that LgR5 expression did not significantly differ between small intestine polyps and colon polyps, and further, there was no correlation between tumor size and LgR5+ area. The mean LgR5+ area of the tumors was 8%, but varied widely between tumors. Preliminary results show that the LgR5+ area in colorectal tumors of humans may be significantly higher than in mice, suggesting that the therapeutic index of anti-LgR5 ADC therapy in humans may be even better than in mice.

[0494] To assess Lgr5 expression differences between intestinal crypts and tumors within these animals, intestinal tracts from AKV $Lgr5^{DTR/+}$ mice were obtained and direct visualization of GFP was performed on tissue sections. Tumors and normal crypts were thereafter quantitated for the intensity of each GFP postive pixel. To determine relative GFP intensity, the intensity score is divided by the GFP+ area. As shown in Figure 23, LgR5 expression is higher in tumors than in intestinal crypts in of AKV $Lgr5^{DTR/+}$ mice.

[0495] An overall survival study was carried out with AKV mice to determine whether anti-LgR5 antibody can increase survival. Ten AKV mice were administered 15 mg/kg mu8E11-MC-vc-PAB-MMAE; six AKV mice were administered 15 mg/kg mu8E11, and 9 mice were administered 15 mg/kg control antibody anti-gp120-MC-vc-MMAE. The antibodies and ADCs were administered weekly beginning at 6 weeks of age until the mice either died or were deemed

moribund (as determined by standard criteria related to signs of severe lethargy, weight loss and anemia), in which case the mice were sacrificed.

[0496] The results of the overall survival study are shown in Figure 17. The untreated control data represents historical survival rates for 22 AKV mice. In that experiment, AKV mice administered either mu8E11 or mu8E11-MC-vc-PAB-MMAE had significantly longer survival times than untreated AKV mice or AKV mice administered a control ADC. Based on these results, additional animals were evaluated as described above. The results of that experiment are shown in Figure 19. In the larger experiment, AKV mice administered mu8E11-MC-vc-PAB-MMAE had significantly longer survival times than untreated AKV mice or AKV mice administered a control ADC, and also had a longer survival time than mice administered mu8E11. At the time of death, the AKV mice administered control ADC and anti-LgR5-ADC had similar numbers and sizes of polyps, suggesting that anti-LgR5-ADC may slow the disease and thereby extend survival.

[0497] In order to determine whether anti-LgR5 antibody and/or anti-LgR5 ADC caused apoptosis in the gastrointestinal tumors of AKV mice, the presence of cleaved caspase 3 was measured as a function of tumor area. Formalin fixed paraffin embedded (FFPE) small intestine and colon tissue collected at time of death were subjected to immunohistochemical staining for cleaved caspase 3 (Cell Signaling Technologies; Danvers, MA, cat# 9661L). Images of the stained slides were acquired by the Olympus Nanozoomer automated slide scanning platform and manually identified tumor-specific areas were analyzed in the Matlab software package (Mathworks, Natick, MA). Postively stained area and total tumor area were quantified. Although rare, cleaved caspase 3 was visible in the crypts following treatment with anti-LgR5 ADC, but was not observed in control ADC treated animals, suggesting that LgR5-expressing cells are being specifically targeted.

[0498] The results of that experiment are shown in Figure 18. Both anti-LgR5 antibody and anti-LgR5 ADC administration caused a statistically significant increase in the percentage of tumor area in AKV mice that was positive for the presence of cleaved caspase 3, compared to control ADC-treated AKV mice.

[0499] In order to demonstrate that the apoptosis is occurring in LgR5+ cells, AKV *Lgr5*$^{DTR/+}$ mice were administered 15 mg/kg mu8E11-MC-vc-PAB-MMAE or 15 mg/kg control antibody anti-gp120-MC-vc-MMAE (day 1). On day 4, the mice were sacrificed and tumors from the gastrointestinal tract (small and large intestine) were visualized for expression of EGFP and cleaved caspase 3. The amount of CC3+GFP+ area per total cellular area was then determined. As shown in Figure 21A, anti-LgR5-ADC treated mice tended to have a greater proportion of CC3+GFP+ area than control treated mice, although not stastically significant in that experiment.

Figure 21B shows exemplary immunohistochemical staining from control ADC treated mice (left panels) and anti-LgR5-ADC treated mice (right panels). These results demonstrate a trend towards increased apoptosis in LgR5-expressing cells upon anti-LgR5-ADC treatment.

[0500] To determine whether cell proliferation of LgR5 expressing cells is affected by anti-LgR5 treatment, the Ki67+ area per cellular area was measured in the EGFP+ cell population and EGFP- cell population from the gastrointestinal tract of control-ADC treated and anti-LgR5-ADC treated AKV *Lgr5*$^{DTR/+}$ mice. Ki67 is a nuclear protein associated with cellular proliferation. Ki67 antibodies for immunohistochemical staining were obtained from Neomarker. The results of that experiment are shown in Figure 22. There was significantly less proliferating cell area, as measured by Ki67 staining, in tumors from AKV *Lgr5*$^{DTR/+}$ mice treated with anti-LgR5-ADC than control ADC, in both the GFP+ and GFP- cell populations. These results suggest that anti-LgR5-ADC reduces proliferation and/or inhibits formation of proliferative progeny.

O. Efficacy of anti-LgR5 Antibody Drug Conjugates in D5124 Pancreatic Cancer Xenograft

[0501] The efficacy of the anti-LgR5 ADCs was investigated using a D5124 pancreatic cancer xenograft model, which has a β-catenin mutation. LgR5 is highly expressed in D5124 tumors, and was confirmed by microarray, TaqMan® quantitative RT-PCR, in situ hybridization, FACS, and Western blot. Twenty to 30 mm^3 D5124 tumor fragments were implanted subcutaneously into the dorsal flank area of NCR nude mice and 22 days post-transplantation the mice were given a single intravenous injection of 2.62 mg/kg or 5.23 mg/kg huYW353-vcMMAE antibody-drug conjugate, 3 mg/kg or 6 mg/kg ch8E11-vcMMAE antibody-drug conjugate, or 3 mg/kg or 6 mg/kg hu8E11v2-vcMMAE antibody-drug conjugate; or 6 mg/kg humanized anti-gD 5B6-vcMMAE control antibody-drug conjugate; or with vehicle (histidine buffer: 20mM histidine acetate, 240 mM sucrose, 0.02% Tween 20, pH5.5) alone (n = 8 mice per group). The presence of the antibodies was confirmed by PK bleeds one and eight days post injection.

[0502] As shown in Figure 24, substantial tumor growth inhibition was achieved at both doses of huYW353-vcMMAE, both doses of hu8E11v2-vcMMAE, and 6 mg/kg ch8E11v2-vcMMAE.

[0503] The efficacy of various doses of hu8E11v2-vcMMAE antibody-drug conjugate was then tested in the D5124 pancreatic cancer xenograft model described above. Twenty to 30 mm^3 D5124 tumor fragments were implanted subcutaneously into the dorsal flank area of NCR nude mice and 26 days post-transplantation mice were given a single intravenous injection of 0.5 mg/kg, 1 mg/kg, 3 mg/kg, 6 mg/kg, or 12 mg/kg hu8E11v2-vcMMAE antibody-drug conjugate;

or 15 mg/kg hu8E11v2; or 6.37 mg/kg or 12.73 mg/kg human anti-gD 5B6-vcMMAE control antibody-drug conjugate; or 15 mg/kg humanized anti-gD control antibody; or vehicle (histidine buffer: 20mM histidine acetate, 240 mM sucrose, 0.02% Tween 20, pH5.5) alone (n = 8 mice per group).

[0504] As shown in Figure 25, substantial tumor growth inhibition was achieved at 3 mg/kg and 6 mg/kg hu8E11v2-vcMMAE, and tumor regression was achieved at 12 mg/kg hu8E11v2-vcMMAE.

P. Efficacy of anti-LgR5 Antibody Drug Conjugates in LoVoX1.1 Colon Cancer Cell Line Xenograft

[0505] The efficacy of the anti-LgR5 ADCs was investigated using a LoVo colon cancer xenograft model. LoVo cells are a colorectal adenocarcinoma cell line with an APC mutation (ATCC #CCL 229), and subline LoVoX1.1 was derived for optimal growth in mice. Briefly, mice were inoculated with LoVo cells. Once tumors were growing, a tumor with a desirable growth rate was harvested. The tumor was minced and grown in culture to establish cell line LoVoX1.1. LgR5 is expressed in LoVoX1.1 cells, and was confirmed by microarray, TaqMan® quantitative RT-PCR, in situ hybridization, FACS, and Western blot. Five million LoVoX1.1 cells in HBSS-matrigel were injected subcutaneously into the dorsal flank of C.B-17 SCIDmice and 13 days post-inoculation mice were given a single intravenous injection of 3 mg/kg or 6 mg/kg huYW353-vcMMAE antibody-drug conjugate, 3 mg/kg or 6 mg/kg hu8E11v2-vcMMAE antibody-drug conjugate; 15 mg/kg hu8E11v2 antibody; 15 mg/kg humanized anti-gD 5B6 control antibody; or 6 mg/kg humanized anti-gD 5B6-vcMMAE control antibody-drug conjugate; or with vehicle (histidine buffer: 20mM histidine acetate, 240 mM sucrose, 0.02% Tween 20, pH5.5) alone (n = 10 mice per group).

[0506] As shown in Figure 26, substantial tumor growth inhibition was achieved at 6 mg/kg hu8E11v2-vcMMAE and 6 mg/kg huYW353-vcMMAE.

[0507] The efficacy of various doses of hu8E11v2-vcMMAE antibody-drug conjugate was then tested in the LoVoX1.1 colorectal adenocarcinoma xenograft model described above. Five million LoVoX1.1 cells in HBSS-matrigel were injected subcutaneously into the dorsal flank of C.B-17 SCID mice and 10 days post-inoculation mice were given a single intravenous injection of 1 mg/kg, 3 mg/kg, 6 mg/kg, 10 mg/kg, or 15 mg/kg hu8E11v2-vcMMAE antibody-drug conjugate; or 15 mg/kg hu8E11v2; or 6 mg/kg or 15 mg/kg humanized anti-gD 5B6-vcMMAE control antibody-drug conjugate; or vehicle (histidine buffer: 20mM histidine acetate, 240 mM sucrose, 0.02% Tween 20, pH5.5) alone (n = 9 mice per group). The presence of the antibodies was confirmed by PK bleeds one, seven, and 14 days post injection.

[0508] As shown in Figure 27, substantial tumor growth inhibition was achieved at 6 mg/kg, 10 mg/kg, and 15 mg/kg hu8E11v2-vcMMAE.

Q. Efficacy of anti-LgR5 Antibody Drug Conjugates in D5124 Pancreatic Cancer Xenograft

[0509] The efficacy of the anti-LgR5 ADCs was investigated using a D5124 pancreatic cancer xenograft model, which has a β-catenin mutation. LgR5 is highly expressed in D5124 tumors, and was confirmed by microarray, TaqMan® quantitative RT-PCR, in situ hybridization, FACS, and Western blot. Twenty to 30 mm^3 D5124 tumor fragments were implanted subcutaneously into the dorsal flank area of NCR nude mice and 26 days post-transplantation the mice were given a single intravenous injection of 1 mg/kg huYW353-vcMMAE antibody-drug conjugate, 1 mg/kg huYW353-vcPNU antibody-drug conjugate, 1 mg/kg huYW353-PNU antibody-drug conjugate, 1 mg/kg huYW353-acetal-PNU antibody-drug conjugate; or 1 mg/kg humanized anti-gD 5B6-vcPNU control antibody-drug conjugate, 1 mg/kg humanized anti-gD 5B6-PNU control antibody-drug conjugate, or 1 mg/kg humanized anti-gD 5B6-acetal-PNU control antibody-drug conjugate; or with vehicle (histidine buffer: 20mM histidine acetate, 240 mM sucrose, 0.02% Tween 20, pH5.5) alone (n = 9 mice per group). The presence of the antibodies was confirmed by PK bleeds three, seven, and 14 days post injection.

[0510] As shown in Figure 28, substantial tumor growth inhibition was achieved with 1 mg/kg huYW353-vcMMAE and 1 mg/kg huYW353-acetal-PNU, and almost complete tumor growth inhibition was achieved with 1 mg/kg huYW353-vcPNU. One of the mice treated with 1 mg/kg huYW353-acetal-PNU showed a complete response (i.e., the mouse had no detectable tumor at the end of the study). In addition, two of the mice treated with 1 mg/kg huYW353-vcPNU showed a partial response (i.e., >50% reduction of the initial tumor volume at day 0).

R. Efficacy of anti-LgR5 Antibody Drug Conjugates in D5124 Pancreatic Cancer Xenograft

[0511] The efficacy of various doses of hu8E11v2 antibody-drug conjugate was tested in the D5124 pancreatic cancer xenograft model. Twenty to 30 mm^3 D5124 tumor fragments were implanted subcutaneously into the dorsal flank area of NCR nude mice and 22 days post-transplantation mice were given a single intravenous injection of 2 mg/kg hu8E11v2-vcMMAE antibody-drug conjugate; or 2 mg/kg hu8E11v2-vcPNU; or 2 mg/kg or 10 mg/kg hu8E11v2-acetal-PNU; or 2 mg/kg or 10 mg/kg hu8E11v2-PNU; 2 mg/kg humanized anti-gD 5B6-vcPNU control antibody-drug conjugate, 10 mg/kg humanized anti-gD 5B6-acetal-PNU control antibody drug conjugate, or 10 mg/kg humanized anti-gD 5B6-PNU control antibody drug conjugate; or

vehicle (histidine buffer: 20mM histidine acetate, 240 mM sucrose, 0.02% Tween 20, pH5.5) alone (n = 8 mice per group).

[0512] As shown in Figure 29, substantial tumor growth inhibition was achieved at 2 mg/kg hu8E11v2-acetal-PNU, and almost complete tumor growth inhibition was achieved at 10 mg/kg hu8E11v2-acetal-PNU, 2 mg/kg hu8E11v2-vcPNU, and 2 mg/kg and 10 mg/kg hu8E11v2-PNU.

S. Efficacy of anti-LgR5 Antibody Drug Conjugates in LoVo Colon Cancer Cell Line Xenograft

[0513] The efficacy of the anti-LgR5 ADCs was investigated using a LoVo colon cancer xenograft model. LoVo cells are a colorectal adenocarcinoma cell line with an APC mutation (ATCC #CCL 229), and subline LoVoX1.1 was derived for optimal growth in mice. Briefly, mice were inoculated with LoVo cells. Once tumors were growing, a tumor with a desirable growth rate was harvested. The tumor was minced and grown in culture to establish cell line LoVoX1.1. LgR5 is expressed in LoVoX1.1 cells, and was confirmed by microarray, TaqMan® quantitative RT-PCR, in situ hybridization, FACS, and Western blot. Five million LoVoX1.1 cells in HBSS-matrigel were injected subcutaneously into the dorsal flank of C.B-17 SCID mice and 11 days post-inoculation mice were given a single intravenous injection of 2 mg/kg hu8E11v2-vcMMAE antibody-drug conjugate, 2 mg/kg hu8E11v2-vcPNU, 2 mg/kg hu8E11v2-acetal-PNU, or 2 mg/kg hu8E11v2-PNU; or 2 mg/kg humanized anti-gD 5B6-vcPNU control antibody-drug conjugate, 2 mg/kg humanized anti-gD 5B6-acetal-PNU control antibody drug conjugate, or 2 mg/kg humanized anti-gD 5B6-PNU control antibody drug conjugate; or with vehicle (histidine buffer: 20mM histidine acetate, 240 mM sucrose, 0.02% Tween 20, pH5.5) alone (n = 10 mice per group). The presence of the antibodies was confirmed by PK bleeds one, seven, and 14 days post injection.

[0514] As shown in Figure 30, in this experiment, certain control antibodies appeared to show substantial tumor growth inhibition (*see* 2 mg/kg humanized anti-gD 5B6-vcPNU and 2 mg/kg humanized anti-gD 5B6-acetal-PNU control antibody drug conjugate).

[0515] Because of the apparent non-specific effects of the control antibody in the prior experiment, the LoVoX1.1 colorectal adenocarcinoma model was tested with a different control antibody-drug conjugate (that binds to a different antigen not expressed on the surface of LoVo cells), and with administration of an excess of anti-gD control antibody to block possible nonspecific antibody binding sites on the tumor cells. Five million LoVoX1.1 cells in HBSS-matrigel were injected subcutaneously into the dorsal flank of C.B-17 SCID mice and seven days post-inoculation mice were given a single intravenous injection of 10 mg/kg hu8E11v2-acetal-

PNU antibody-drug conjugate; or 10 mg/kg thioAb-acetal-PNU control antibody-drug conjugate; or vehicle (50 mM sodium phosphate, 240 mM sucrose, 0.02% Tween20, pH 7) alone (n = 5 mice per group). In addition, the mice were administered 30 mg/kg humanized anti-gD control antibody i.p. once per week until the end of the study, beginning on the same day as, but 4 hours prior to, administration of the test antibodies.

[0516] As shown in Figure 31, substantial tumor growth inhibition was achieved with 10 mg/kg hu8E11v2-acetal-PNU and the control antibody did not inhibit tumor growth.

T. Efficacy of anti-LgR5 Antibody Drug Conjugates in D5124 Pancreatic Cancer Xenograft

[0517] The efficacy of the YW353 anti-LgR5 ADCs was investigated using the D5124 pancreatic cancer xenograft model, which has a β-catenin mutation. LgR5 is highly expressed in D5124 tumors, and was confirmed by microarray, TaqMan® quantitative RT-PCR, in situ hybridization, FACS, and Western blot. Twenty to 30 mm^3 D5124 tumor fragments were implanted subcutaneously into the dorsal flank area of NCR nude mice and 26 days post-transplantation the mice were given a single intravenous injection of 1 mg/kg huYW353-vcMMAE antibody-drug conjugate, 1 mg/kg huYW353-vcPBD antibody-drug conjugate; or 1 mg/kg humanized anti-gD 5B6-vcPBD control antibody-drug conjugate; or with vehicle (histidine buffer: 20mM histidine acetate, 240 mM sucrose, 0.02% Tween 20, pH5.5) alone (n = 9 mice per group). The presence of the antibodies was confirmed by PK bleeds one, seven, and fourteen days post injection.

[0518] As shown in Figure 32, substantial tumor growth inhibition was achieved with 1 mg/kg huYW353-vcMMAE and 1 mg/kg huYW353-vcPBD. One of the mice treated with 1 mg/kg huYW353-vcPBD showed a complete response (i.e., the mouse had no detectable tumor at the end of the study).

[0519] In a separate experiment, efficacy of the hu8E11v2 anti-LgR5 ADCs was investigated using the D5124 pancreatic cancer xenograft model. Twenty to 30 mm^3 D5124 tumor fragments were implanted subcutaneously into the dorsal flank area of NCR nude mice and 22 days post-transplantation the mice were given a single intravenous injection of 2 mg/kg hu8E11v2-vcMMAE antibody-drug conjugate, 2 mg/kg hu8E11v2-vcPBD antibody-drug conjugate; or 2 mg/kg humanized anti-gD 5B6-vcPBD control antibody-drug conjugate; or with vehicle (histidine buffer: 20mM histidine acetate, 240 mM sucrose, 0.02% Tween 20, pH5.5) alone (n= 8 mice per group).

[0520] As shown in Figure 33, substantial tumor growth inhibition was achieved with 2 mg/kg hu8E11v2-vcMMAE, and tumor regression was achieved with 2 mg/kg hu8E11v2-vcPBD.

U. Efficacy of anti-LgR5 Antibody Drug Conjugates in LoVoX1.1 Colon Cancer Cell Line Xenograft

[0521] The efficacy of the anti-LgR5 ADCs was investigated using a LoVoX1.1 colon cancer xenograft model. LoVo cells are a colorectal adenocarcinoma cell line with an APC mutation (ATCC #CCL 229), and subline LoVoX1.1 was derived for optimal growth in mice. Briefly, mice were inoculated with LoVo cells. Once tumors were growing, a tumor with a desirable growth rate was harvested. The tumor was minced and grown in culture to establish cell line LoVoX1.1. LgR5 is expressed in LoVoX1.1 cells, and was confirmed by microarray, TaqMan® quantitative RT-PCR, in situ hybridization, FACS, and Western blot. Five million LoVoX1.1 cells in HBSS-matrigel were injected subcutaneously into the dorsal flank of C.B-17 SCID mice and 11 days post-inoculation mice were given a single intravenous injection of 2 mg/kg hu8E11v2-vcMMAE antibody-drug conjugate, 2 mg/kg hu8E11v2-vcPBD antibody-drug conjugate; or 2 mg/kg humanized anti-gD 5B6-vcPBD control antibody-drug conjugate; or with vehicle (histidine buffer: 20mM histidine acetate, 240 mM sucrose, 0.02% Tween 20, pH5.5) alone (n = 10 mice per group). The presence of the antibodies was confirmed by PK bleeds one, seven, and 14 days post injection.

[0522] As shown in Figure 34, complete tumor growth inhibition was achieved with 2 mg/kg hu8E11v2-vcPBD.

[0523] 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
1	huκ$_{IV}$	DIVMTQSPDS LAVSLGERAT INCKSSQSVL YSSNNKNYLA WYQQKPGQPP KLLIYWASTR ESGVPDRFSG SGSGTDFTLT ISSLQAEDVA VYYCQQYYST PFTFGQGTKV EIKR
2	huVH$_1$	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYIHWVRQA PGQGLEWIGW INPGSGNTNY AQKFQGRVTI TRDTSTSTAY LELSSLRSED TAVYYCARFD YWGQGTLVTV SS
3	mu8E11 light chain variable region	NIVLTQSPAS LAVSLGQRAT ISCRASESVD NYGNSFMHWY QQKPGQPPKL LIYLASNLES GVPARFSGSG SRTDFTLTID PVEADDAATY YCQQNYEDPF TFGSGTKVEI KR
4	mu8E11 heavy chain variable region	QVQLQQSGTE LMKPGASVKI SCKATGYTFS AYWIEWIKQR PGHGLEWIGE ILPGSDSTDY NEKFKVKATF SSDTSSNTVY IQLNSLTYED SAVYYCARGG HYGSLDYWGQ GTTLKVSS
5	hu8E11.v1 light chain variable region	DIVMTQSPDS LAVSLGERAT INCRASESVD NYGNSFMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQNYEDPF TFGQGTKVEI KR
6	hu8E11.v1 heavy chain variable region	EVQLVQSGAE VKKPGASVKV SCKASGYTFS AYWIEWVRQA PGQGLEWIGE ILPGSDSTDY NEKFKVRVTI TSDTSTSTVY LELSSLRSED TAVYYCARGG HYGSLDYWGQ GTLVTVSS
7	hu8E11.v2 light chain variable region	DIVMTQSPDS LAVSLGERAT INCRASESVD NYGNSFMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQNYEDPF TFGQGTKVEI KR
8	hu8E11.v2 heavy chain variable region	EVQLVQSGAE VKKPGASVKV SCKASGYTFS AYWIEWVRQA PGQGLEWIGE ILPGSDSTDY NEKFKVRATF TSDTSTSTVY LELSSLRSED TAVYYCARGG HYGSLDYWGQ GTLVTVSS
9	hu8E11.v3 light chain variable region	DIVMTQSPDS LAVSLGERAT INCRASESVD NYGNSFMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SRTDFTLTIS SLQAEDVAVY YCQQNYEDPF TFGQGTKVEI KR
10	hu8E11.v3 heavy chain variable region	EVQLVQSGAE VKKPGASVKV SCKASGYTFS AYWIEWVRQA PGQGLEWIGE ILPGSDSTDY NEKFKVRVTI TSDTSTSTVY LELSSLRSED TAVYYCARGG HYGSLDYWGQ GTLVTVSS
11	hu8E11.v4 light chain variable region	DIVMTQSPDS LAVSLGERAT INCRASESVD NYGNSFMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SRTDFTLTIS SLQAEDVAVY YCQQNYEDPF TFGQGTKVEI KR
12	hu8E11.v4 heavy chain variable region	EVQLVQSGAE VKKPGASVKV SCKASGYTFS AYWIEWVRQA PGQGLEWIGE ILPGSDSTDY NEKFKVRATF TSDTSTSTVY LELSSLRSED TAVYYCARGG HYGSLDYWGQ GTLVTVSS
13	hu8E11.v5 light chain variable region	DIVMTQSPDS LAVSLGERAT INCRASESVD NYGNSFMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQNYEDPF TFGQGTKVEI KR
14	hu8E11.v5 heavy chain variable region	EVQLVQSGAE VKKPGASVKV SCKASGYTFS AYWIEWVRQA PGQGLEWIGE ILPGSDSTDY NEKFKVRVTI TRDTSTSTAY LELSSLRSED TAVYYCARGG HYGSLDYWGQ GTLVTVSS
15	hu8E11.v6 light chain variable region	DIVMTQSPDS LAVSLGERAT INCRASESVD NYGNSFMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQNYEDPF TFGQGTKVEI KR
16	hu8E11.v6 heavy chain variable	EVQLVQSGAE VKKPGASVKV SCKASGYTFS AYWIEWVRQA PGQGLEWIGE ILPGSDSTDY NEKFKVRVTI TADTSTSTAY

	region	LELSSLRSED TAVYYCARGG HYGSLDYWGQ GTLVTVSS
17	hu8E11.v7 light chain variable region	DIVMTQSPDS LAVSLGERAT INCRASESVD NYGNSFMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SRTDFTLTIS SLQAEDVAVY YCQQNYEDPF TFGQGTKVEI KR
18	hu8E11.v7 heavy chain variable region	EVQLVQSGAE VKKPGASVKV SCKASGYTFS AYWIEWVRQA PGQGLEWIGE ILPGSDSTDY NEKFKVRVTI TRDTSTSTAY LELSSLRSED TAVYYCARGG HYGSLDYWGQ GTLVTVSS
19	hu8E11.v8 light chain variable region	DIVMTQSPDS LAVSLGERAT INCRASESVD NYGNSFMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SRTDFTLTIS SLQAEDVAVY YCQQNYEDPF TFGQGTKVEI KR
20	hu8E11.v8 heavy chain variable region	EVQLVQSGAE VKKPGASVKV SCKASGYTFS AYWIEWVRQA PGQGLEWIGE ILPGSDSTDY NEKFKVRVTI TADTSTSTAY LELSSLRSED TAVYYCARGG HYGSLDYWGQ GTLVTVSS
21	mu3G12 light chain variable region	DVVMTQTPLS LPVSLGDQAS ISCRSSQSLV HSNGNTYLQW YLQKPGQSPK LLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGI YFCSQSTHFP YTFGGGTKLE IKR
22	mu3G12 heavy chain variable region	QVQLQQPGAE MVKPGASVKL SCKASVDTFN SYWMHWVKQR PGQGLEWIGE INPSNGRTNY IEKFKNRATV TVDKSSSTAF MQLSSLTSED SAVYYCATGW YFDVWGAGTT VTVSS
23	mu2H6 light chain variable region	DIVMTQSPSS LTVTAGEKVT MSCKSSQSLL NSGNQKNYLT WFQQKPGQPP KLLIYWASTR ESGVPDRFTG SGSGTDFTLT ISNVQAEDLA VYYCQNDYSF PFTFGQGTKV EIKR
24	mu2H6 heavy chain variable region	EVQLQQSGPE LVKPGTSMKI SCKASGYSFT GYTMNWVKQS HKNGLEWIGL INCYNGGTNY NQKFKGKATL TVDKSSSTAF MELLSLTSED SAVYYCARGG STMITPRFAY WGQGTLVTVS S
25	YW353 light chain variable region	DIQMTQSPSS LSASVGDRVT ITCRASQDVS TAVAWYQQKP GKAPKLLIYS ASFLYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ SYTTPPTFGQ GTKVEIKR
26	YW353 heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFT SYSISWVRQA PGKGLEWVAE IYPPGGYTDY ADSVKGRFTI SADTSKNTAY 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 chain (LC) framework 1 (FR1)	DIVMTQSPDS LAVSLGERAT INC
34	Hu8E11 LC FR2	WYQQKPGQPP KLLIY
35	Hu8E11.v1 LC FR3 Hu8E11.v2 LC FR3 Hu8E11.v5 LC FR3 Hu8E11.v6 LC FR3	GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YC
36	Hu8E11.v3 LC	GVPDRFSGSG SRTDFTLTIS SLQAEDVAVY YC

	FR3 Hu8E11.v4 LC FR3 Hu8E11.v7 LC FR3 Hu8E11.v8 LC FR3	
37	Hu8E11 LC FR4	FGQGTKVEIK R
38	Hu8E11 heavy chain (HC) framework1 (FR1)	EVQLVQSGAE VKKPGASVKV SCKAS
39	Hu8E11 HC FR2	WVRQAPGQGL EWIG
40	Hu8E11.v1 HC FR3 Hu8E11.v3 HC FR3	RVTITSDTST STVYLELSSL RSEDTAVYYC AR
41	Hu8E11.v2 HC FR3 Hu8E11.v4 HC FR3	RATFTSDTST STVYLELSSL RSEDTAVYYC AR
42	Hu8E11.v5 HC FR3 Hu8E11.v7 HC FR3	RVTITRDTST STAYLELSSL RSEDTAVYYC AR
43	Hu8E11.v6 HC FR3 Hu8E11.v8 HC FR3	RVTITADTST STAYLELSSL RSEDTAVYYC AR
44	Hu8E11 HC FR4	WGQGTLVTVS 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	KSSQSLLNSG NQKNYLT
52	mu2H6 HVR L2	WASTRES
53	mu2H6 HVR L3	QNDYSFPFT
54	mu2H6 HVR H1	GYSFTGYTMN
55	mu2H6 HVR H2	LINCYNGGTN YNQKFKG
56	mu2H6 HVR H3	GGSTMITPRF AY
57	YW353 HVR L1	RASQDVSTAV A
58	YW353 HVR L2	SASFLYS
59	YW353 HVR L3	QQSYTTPPT
60	YW353 HVR H1	GFTFTSYSIS
61	YW353 HVR H2	EIYPPGGYTD YADSVKG
62	YW353 HVR H3	ARLFFDY
63	hu8E11.v2 light chain	DIVMTQSPDS LAVSLGERAT INCRASESVD NYGNSFMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQNYEDPF TFGQGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS

		GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC
64	hu8E11.v2 heavy chain	EVQLVQSGAE VKKPGASVKV SCKASGYTFS AYWIEWVRQA PGQGLEWIGE ILPGSDSTDY NEKFKVRATF TSDTSTSTVY LELSSLRSED TAVYYCARGG HYGSLDYWGQ GTLVTVSSAS TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKKVEPKS CDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK
65	YW353 light chain	DIQMTQSPSS LSASVGDRVT ITCRASQDVS TAVAWYQQKP GKAPKLLIYS ASFLYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ SYTTPPTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
66	YW353 heavy chain	EVQLVESGGG LVQPGGSLRL SCAASGFTFT SYSISWVRQA PGKGLEWVAE IYPPGGYTDY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCAKAR LFFDYWGQGT LVTVSSASTK GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK
67	Human LgR5 precursor; LGR5_human NP_003658; signal sequence = amino acids 1-21	MDTSRLGVLL SLPVLLQLAT GGSSPRSGVL LRGCPTHCHC EPDGRMLLRV DCSDLGLSEL PSNLSVFTSY LDLSMNNISQ LLPNPLPSLR FLEELRLAGN ALTYIPKGAF TGLYSLKVLM LQNNQLRHVP TEALQNLRSL QSLRLDANHI SYVPPSCFSG LHSLRHLWLD DNALTEIPVQ AFRSLSALQA MTLALNKIHH IPDYAFGNLS SLVVLHLHNN RIHSLGKKCF DGLHSLETLD LNYNNLDEFP TAIRTLSNLK ELGFHSNNIR SIPEKAFVGN PSLITIHFYD NPIQFVGRSA FQHLPELRTL TLNGASQITE FPDLTGTANL ESLTLTGAQI SSLPQTVCNQ LPNLQVLDLS YNLLEDLPSF SVCQKLQKID LRHNEIYEIK VDTFQQLLSL RSLNLAWNKI AIIHPNAFST LPSLIKLDLS SNLLSSFPIT GLHGLTHLKL TGNHALQSLI SSENFPELKV IEMPYAYQCC AFGVCENAYK ISNQWNKGDN SSMDDLHKKD AGMFQAQDER DLEDFLLDFE EDLKALHSVQ CSPSPGPFKP CEHLLDGWLI RIGVWTIAVL ALTCNALVTS TVFRSPLYIS PIKLLIGVIA AVNMLTGVSS AVLAGVDAFT FGSFARHGAW WENGVGCHVI GFLSIFASES SVFLLTLAAL ERGFSVKYSA KFETKAPFSS LKVIILLCAL LALTMAAVPL LGGSKYGASP LCLPLPFGEP STMGYMVALI LLNSLCFLMM TIAYTKLYCN LDKGDLENIW DCSMVKHIAL LLFTNCILNC PVAFLSFSSL INLTFISPEV IKFILLVVVP LPACLNPLLY ILFNPHFKED LVSLRKQTYV

		WTRSKHPSLM SINSDDVEKQ SCDSTQALVT FTSSSITYDL PPSSVPSPAY PVTESCHLSS VAFVPCL
68	Human LgR5 mature, without signal sequence; amino acids 22 to 907	GSSPRSGVL LRGCPTHCHC EPDGRMLLRV DCSDLGLSEL PSNLSVFTSY LDLSMNNISQ LLPNPLPSLR FLEELRLAGN ALTYIPKGAF TGLYSLKVLM LQNNQLRHVP TEALQNLRSL QSLRLDANHI SYVPPSCFSG LHSLRHLWLD DNALTEIPVQ AFRSLSALQA MTLALNKIHH IPDYAFGNLS SLVVLHLHNN RIHSLGKKCF DGLHSLETLD LNYNNLDEFP TAIRTLSNLK ELGFHSNNIR SIPEKAFVGN PSLITIHFYD NPIQFVGRSA FQHLPELRTL TLNGASQITE FPDLTGTANL ESLTLTGAQI SSLPQTVCNQ LPNLQVLDLS YNLLEDLPSF SVCQKLQKID LRHNEIYEIK VDTFQQLLSL RSLNLAWNKI AIIHPNAFST LPSLIKLDLS SNLLSSFPIT GLHGLTHLKL TGNHALQSLI SSENFPELKV IEMPYAYQCC AFGVCENAYK ISNQWNKGDN SSMDDLHKKD AGMFQAQDER DLEDFLLDFE EDLKALHSVQ CSPSPGPFKP CEHLLDGWLI RIGVWTIAVL ALTCNALVTS TVFRSPLYIS PIKLLIGVIA AVNMLTGVSS AVLAGVDAFT FGSFARHGAW WENGVGCHVI GFLSIFASES SVFLLTLAAL ERGFSVKYSA KFETKAPFSS LKVIILLCAL LALTMAAVPL LGGSKYGASP LCLPLPFGEP STMGYMVALI LLNSLCFLMM TIAYTKLYCN LDKGDLENIW DCSMVKHIAL LLFTNCILNC PVAFLSFSSL INLTFISPEV IKFILLVVVP LPACLNPLLY ILFNPHFKED LVSLRKQTYV WTRSKHPSLM SINSDDVEKQ SCDSTQALVT FTSSSITYDL PPSSVPSPAY PVTESCHLSS VAFVPCL
69	Cynomolgus monkey LgR5 partial sequence, predicted; predicted to correspond to amino acids 33 to 907 of full-length precursor	GCPTHCHCEP DGRMLLRVDC SDLGLSELPS NLSVFTSYLD LSMNNISQLL PNPLPSLRFL EELRLAGNAL TYIPKGAFTG LYSLKVLMLQ NNQLRQVPTE ALQNLRSLQS LRLDANHISY VPPSCFSGLH SLRHLWLDDN ALTEIPVQAF RSLSALQAMT LALNKIHHIP DYAFGNLSSL VVLHLHNNRI HSLGKKCFDG LHSLETLDLN YNNLDEFPTA IRTLSNLKEL GFHSNNIRSI PEKAFVGNPS LITIHFYDNP IQFVGRSAFQ HLPELRTLTL NGASQITEFP DLTGTANLES LTLTGAQISS LPQTVCNQLP NLQVLDLSYN LLEDLPSFSV CQKLQKIDLR HNEIYEIKVD TFQQLLSLRS LNLAWNKIAI IHPNAFSTLP SLIKLDLSSN LLSSFPVTGL HGLTHLKLTG NHALQSLISS ENFPELKIIE MPYAYQCCAF GVCENAYKIS NQWNKGDNSS MDDLHKKDAG MFQVQDERDL EDFLLDFEED LKALHSVQCS PSPGPFKPCE HLLDGWLIRI GVWTIAVLAL TCNALVTSTV FRSPLYISPI KLLIGVIAVV NMLTGVSSAV LAGVDAFTFG SFARHGAWWE NGVGCQVIGF LSIFASESSV FLLTLAALER GFSVKCSAKF ETKAPFSSLK VIILLCALLA LTMAAVPLLG GSEYGASPLC LPLPFGEPST TGYMVALILL NSLCFLMMTI AYTKLYCNLD KGDLENIWDC SMVKHIALLL FTNCILYCPV AFLSFSSLLN LTFISPEVIK FILLVIVPLP ACLNPLLYIL FNPHFKEDLV SLGKQTYFWT RSKHPSLMSI NSDDVEKQSC DSTQALVTFT SSSIAYDLPP SSVPSPAYPV TESCHLSSVA FVPCL
70	Rat LgR5 precursor; LGR5_rat NP_001100254; signal sequence = amino acids 1-21	MDTSRVRMLL SLLALLQLVA AGSPPRPDTM PRGCPSYCHC ELDGRMLLRV DCSDLGLSEL PSNLSVFTSY LDLSMNNISQ LPASLLHRLR FLEELRLAGN ALTHIPKGAF AGLHSLKVLM LQNNQLRQVP EEALQNLRSL QSLRLDANHI SYVPPSCFSG LHSLRHLWLD DNALTDVPVQ AFRSLSALQA MTLALNKIHH IADHAFGNLS SLVVLHLHNN RIHSLGKKCF DGLHSLETLD

		LNYNNLDEFP TAIKTLSNLK ELGFHSNNIR SIPERAFVGN PSLITIHFYD NPIQFVGISA FQHLPELRTL TLNGASQITE FPDLTGTATL ESLTLTGAKI SSLPQTVCDQ LPNLQVLDLS YNLLEDLPSL SGCQKLQKID LRHNEIYEIK GGTFQQLFNL RSLNLARNKI AIIHPNAFST LPSLIKLDLS SNLLSSFPVT GLHGLTHLKL TGNRALQSLI PSANFPELKI IEMPYAYQCC AFGGCENVYK IPNQWNKDDS SSVDDLRKKD AGLFQVQDER DLEDFLLDFE EDLKVLHSVQ CSPPPGPFKP CEHLFGSWLI RIGVWTTAVL ALSCNALVAF TVFRTPLYIS SIKLLIGVIA VVDILMGVSS AILAVVDTFT FGSFAQHGAW WEGGIGCQIV GFLSIFASES SVFLLTLAAL ERGFSVKCSS KFEMKAPLSS LKAIILLCVL LALTIATVPL LGGSEYNASP LCLPLPFGEP STTGYMVALV LLNSLCFLIM TIAYTRLYCS LEKGELENLW DCSMVKHTAL LLFTNCILYC PVAFLSFSSL LNLTFISPEV IKFILLVIVP LPACLNPLLY IVFNPHFKED MGSLGKQTRF WTRAKHPSLL SINSDDVEKR SCDSTQALVS FTHASIAYDL PSDSGSSPAY PMTESCHLSS VAFVPCL
71	Rat LgR5 mature, without signal sequence; amino acids 22 to 907	GSPPRPDTM PRGCPSYCHC ELDGRMLLRV DCSDLGLSEL PSNLSVFTSY LDLSMNNISQ LPASLLHRLR FLEELRLAGN ALTHIPKGAF AGLHSLKVLM LQNNQLRQVP EEALQNLRSL QSLRLDANHI SYVPPSCFSG LHSLRHLWLD DNALTDVPVQ AFRSLSALQA MTLALNKIHH IADHAFGNLS SLVVLHLHNN RIHSLGKKCF DGLHSLETLD LNYNNLDEFP TAIKTLSNLK ELGFHSNNIR SIPERAFVGN PSLITIHFYD NPIQFVGISA FQHLPELRTL TLNGASQITE FPDLTGTATL ESLTLTGAKI SSLPQTVCDQ LPNLQVLDLS YNLLEDLPSL SGCQKLQKID LRHNEIYEIK GGTFQQLFNL RSLNLARNKI AIIHPNAFST LPSLIKLDLS SNLLSSFPVT GLHGLTHLKL TGNRALQSLI PSANFPELKI IEMPYAYQCC AFGGCENVYK IPNQWNKDDS SSVDDLRKKD AGLFQVQDER DLEDFLLDFE EDLKVLHSVQ CSPPPGPFKP CEHLFGSWLI RIGVWTTAVL ALSCNALVAF TVFRTPLYIS SIKLLIGVIA VVDILMGVSS AILAVVDTFT FGSFAQHGAW WEGGIGCQIV GFLSIFASES SVFLLTLAAL ERGFSVKCSS KFEMKAPLSS LKAIILLCVL LALTIATVPL LGGSEYNASP LCLPLPFGEP STTGYMVALV LLNSLCFLIM TIAYTRLYCS LEKGELENLW DCSMVKHTAL LLFTNCILYC PVAFLSFSSL LNLTFISPEV IKFILLVIVP LPACLNPLLY IVFNPHFKED MGSLGKQTRF WTRAKHPSLL SINSDDVEKR SCDSTQALVS FTHASIAYDL PSDSGSSPAY PMTESCHLSS VAFVPCL
72	Mouse LgR5 precursor; LGR5_mouse NP_034325; signal sequence = amino acids 1-21	MDTSCVHMLL SLLALLQLVA AGSSPGPDAI PRGCPSHCHC ELDGRMLLRV DCSDLGLSEL PSNLSVFTSY LDLSMNNISQ LPASLLHRLC FLEELRLAGN ALTHIPKGAF TGLHSLKVLM LQNNQLRQVP EEALQNLRSL QSLRLDANHI SYVPPSCFSG LHSLRHLWLD DNALTDVPVQ AFRSLSALQA MTLALNKIHH IADYAFGNLS SLVVLHLHNN RIHSLGKKCF DGLHSLETLD LNYNNLDEFP TAIKTLSNLK ELGFHSNNIR SIPERAFVGN PSLITIHFYD NPIQFVGVSA FQHLPELRTL TLNGASHITE FPHLTGTATL ESLTLTGAKI SSLPQAVCDQ LPNLQVLDLS YNLLEDLPSL SGCQKLQKID LRHNEIYEIK GSTFQQLFNL RSLNLAWNKI AIIHPNAFST LPSLIKLDLS SNLLSSFPVT GLHGLTHLKL TGNRALQSLI PSANFPELKI IEMPSAYQCC AFGGCENVYK ISNQWNKDDG NSVDDLHKKD AGLFQVQDER

		DLEDFLLDFE EDLKALHSVQ CSPSPGPFKP CEHLFGSWLI RIGVWTTAVL ALSCNALVAL TVFRTPLYIS SIKLLIGVIA VVDILMGVSS AVLAAVDAFT FGRFAQHGAW WEDGIGCQIV GFLSIFASES SIFLLTLAAL ERGFSVKCSS KFEVKAPLFS LRAIVLLCVL LALTIATIPL LGGSKYNASP LCLPLPFGEP STTGYMVALV LLNSLCFLIM TIAYTKLYCS LEKGELENLW DCSMVKHIAL LLFANCILYC PVAFLSFSSL LNLTFISPDV IKFILLVIVP LPSCLNPLLY IVFNPHFKED MGSLGKHTRF WMRSKHASLL SINSDDVEKR SCESTQALVS FTHASIAYDL PSTSGASPAY PMTESCHLSS VAFVPCL
73	Mouse LgR5 mature, without signal sequence; amino acids 22 to 907	GSSPGPDAI PRGCPSHCHC ELDGRMLLRV DCSDLGLSEL PSNLSVFTSY LDLSMNNISQ LPASLLHRLC FLEELRLAGN ALTHIPKGAF TGLHSLKVLM LQNNQLRQVP EEALQNLRSL QSLRLDANHI SYVPPSCFSG LHSLRHLWLD DNALTDVPVQ AFRSLSALQA MTLALNKIHH IADYAFGNLS SLVVLHLHNN RIHSLGKKCF DGLHSLETLD LNYNNLDEFP TAIKTLSNLK ELGFHSNNIR SIPERAFVGN PSLITIHFYD NPIQFVGVSA FQHLPELRTL TLNGASHITE FPHLTGTATL ESLTLTGAKI SSLPQAVCDQ LPNLQVLDLS YNLLEDLPSL SGCQKLQKID LRHNEIYEIK GSTFQQLFNL RSLNLAWNKI AIIHPNAFST LPSLIKLDLS SNLLSSFPVT GLHGLTHLKL TGNRALQSLI PSANFPELKI IEMPSAYQCC AFGGCENVYK ISNQWNKDDG NSVDDLHKKD AGLFQVQDER DLEDFLLDFE EDLKALHSVQ CSPSPGPFKP CEHLFGSWLI RIGVWTTAVL ALSCNALVAL TVFRTPLYIS SIKLLIGVIA VVDILMGVSS AVLAAVDAFT FGRFAQHGAW WEDGIGCQIV GFLSIFASES SIFLLTLAAL ERGFSVKCSS KFEVKAPLFS LRAIVLLCVL LALTIATIPL LGGSKYNASP LCLPLPFGEP STTGYMVALV LLNSLCFLIM TIAYTKLYCS LEKGELENLW DCSMVKHIAL LLFANCILYC PVAFLSFSSL LNLTFISPDV IKFILLVIVP LPSCLNPLLY IVFNPHFKED MGSLGKHTRF WMRSKHASLL SINSDDVEKR SCESTQALVS FTHASIAYDL PSTSGASPAY PMTESCHLSS VAFVPCL
74	hu8E11.v2 V205C cysteine engineered light chain (Igκ)	DIVMTQSPDS LAVSLGERAT INCRASESVD NYGNSFMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQNYEDPF TFGQGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPCT KSFNRGEC
75	hu8E11.v2 A118C cysteine engineered heavy chain (IgG1)	EVQLVQSGAE VKKPGASVKV SCKASGYTFS AYWIEWVRQA PGQGLEWIGE ILPGSDSTDY NEKFKVRATF TSDTSTSTVY LELSSLRSED TAVYYCARGG HYGSLDYWGQ GTLVTVSSCS TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKKVEPKS CDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK
76	hu8E11.v2 S400C cysteine	EVQLVQSGAE VKKPGASVKV SCKASGYTFS AYWIEWVRQA PGQGLEWIGE ILPGSDSTDY NEKFKVRATF TSDTSTSTVY

	engineered heavy chain (IgG1)	LELSSLRSED TAVYYCARGG HYGSLDYWGQ GTLVTVSSAS TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKKVEPKS CDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD CDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK
77	YW353 V205C cysteine engineered light chain (Igκ)	DIQMTQSPSS LSASVGDRVT ITCRASQDVS TAVAWYQQKP GKAPKLLIYS ASFLYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ SYTTPPTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPCTKSFN RGEC
78	YW353 A118C cysteine engineered heavy chain (IgG1)	EVQLVESGGG LVQPGGSLRL SCAASGFTFT SYSISWVRQA PGKGLEWVAE IYPPGGYTDY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCAKAR LFFDYWGQGT LVTVSSCSTK GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK
79	YW353 S400C cysteine engineered heavy chain (IgG1)	EVQLVESGGG LVQPGGSLRL SCAASGFTFT SYSISWVRQA PGKGLEWVAE IYPPGGYTDY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCAKAR LFFDYWGQGT LVTVSSASTK GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDCD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK
80	V205C cysteine engineered light chain constant region (Igκ)	TVAAPSVFIF PPSDEQLKSG TASVVCLLNN FYPREAKVQW KVDNALQSGN SQESVTEQDS KDSTYSLSST LTLSKADYEK HKVYACEVTH QGLSSPCTKS FNRGEC
81	A118C cysteine engineered heavy chain constant region (IgG1)	CSTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK

82	S400C cysteine engineered heavy chain constant region (IgG1)	ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDCDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK

WHAT IS CLAIMED IS:

1. An isolated antibody that binds to LgR5, wherein the 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 ≤ 5 nM.

2. The antibody of claim 1, which is a monoclonal antibody.

3. The antibody of claim 1, which is a human, humanized, or chimeric antibody.

4. The antibody of claim 1, which is an antibody fragment that binds LgR5.

5. The antibody of claim 1, wherein LgR5 is human LgR5 of SEQ ID NO: 67.

6. The antibody of claim 1, wherein the antibody comprises:

a) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 29, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 31; or

b) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 62, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 59, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 61.

7. The antibody of claim 1, wherein the 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; or

b) 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.

8. The antibody of claim 7, wherein the antibody comprises HVR-H1 comprising the amino acid sequence of SEQ ID NO: 30, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 31, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32, and:

a) heavy chain framework FR3 sequence of SEQ ID NO: 40;

b) heavy chain framework FR3 sequence of SEQ ID NO: 41;

c) heavy chain framework FR3 sequence of SEQ ID NO: 42; or

d) heavy chain framework FR3 sequence of SEQ ID NO: 43.

9. The antibody of claim 7 or claim 8, wherein the antibody comprises:

a) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 29, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 31, 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; or

b) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 62, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 59, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 61, 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.

10. The antibody of claim 1, wherein the antibody comprises:

a) 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; or

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.

11. The antibody of claim 9 or claim 10, wherein the antibody comprises 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, and further comprises a light chain framework FR3 sequence of SEQ ID NO: 35.

12. The antibody of any one of the preceding claims, wherein the antibody comprises:

a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 6;

b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 5;

c) a VH sequence as in (a) and a VL sequence as in (b);

d) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 8;

e) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 7;

f) a VH sequence as in (d) and a VL sequence as in (e);

g) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 10;

h) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 9;

i) a VH sequence as in (g) and a VL sequence as in (h);

j) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 12;

k) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 11;

l) a VH sequence as in (j) and a VL sequence as in (k);

m) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 14;

n) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 13;

o) a VH sequence as in (m) and a VL sequence as in (n);

p) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 16;

q) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 15;

r) a VH sequence as in (p) and a VL sequence as in (q);

s) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 18;

t) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 17;

u) a VH sequence as in (s) and a VL sequence as in (t);

v) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 20;

w) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 19;

x) a VH sequence as in (v) and a VL sequence as in (w);

y) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 26;

z) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 25; or

aa) a VH sequence as in (y) and a VL sequence as in (z).

13. The antibody of claim 12, comprising a VH sequence selected from SEQ ID NOs: 6, 8, 10, 12, 14, 16, 18, 20, and 26.

14. The antibody of claim 12 or claim 13, comprising a VL sequence selected from SEQ ID NOs: 5, 7, 9, 11, 13, 15, 17, 19, and 25.

15. An antibody comprising:

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.

16. The antibody of any one of claims 1 to 15, which is an IgG1, IgG2a or IgG2b antibody.

17. Isolated nucleic acid encoding the antibody of any one of claims 1 to 16.

18. A host cell comprising the nucleic acid of claim 17.

19. A method of producing an antibody comprising culturing the host cell of claim 18 so that the antibody is produced.

20. An immunoconjugate comprising the antibody of any one of claims 1 to 16 and a cytotoxic agent.

21. The immunoconjugate of claim 20 having the formula Ab-(L-D)p, wherein:

(a) Ab is the antibody of any one of claim 1 to 15;

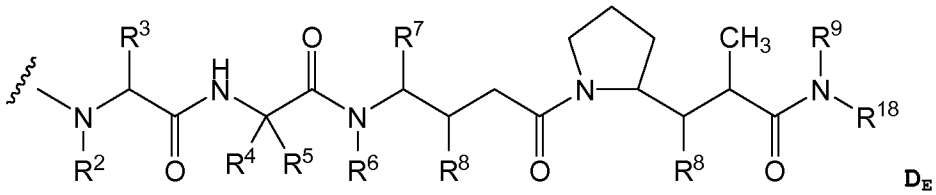
(b) L is a linker;

(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.

22. The immunoconjugate of claim 21, wherein D is an auristatin.

23. The immunoconjugate of claim 22, wherein D has formula D_E

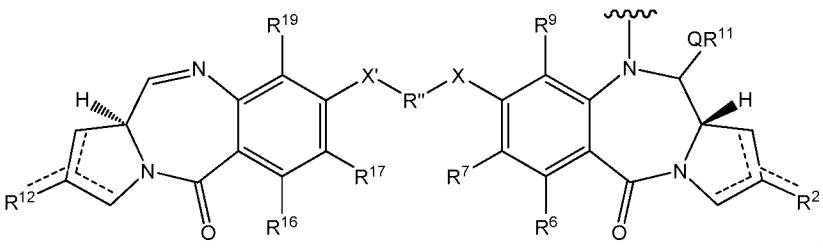


D_E

and wherein R^2 and R^6 are each methyl, R^3 and R^4 are each isopropyl, R^5 is H, R^7 is sec-butyl, each R^8 is independently selected from CH_3, O-CH_3, OH, and H; R^9 is H; and R^{18} is $–C(R^8)_2–C(R^8)_2–$aryl.

24. The immunoconjugate of claim 21, wherein the drug is MMAE.

25. The immunoconjugate of claim 21, wherein D is a pyrrolobenzodiazepine of Formula A:



A;

wherein 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, $=CH_2$, CN, R, OR, $=CH\text{-}R^D$, $=C(R^D)_2$, $O\text{-}SO_2\text{-}R$, CO_2R and COR, and optionally further selected from halo or dihalo, wherein R^D is independently selected from R, CO_2R, COR, CHO, CO_2H, and halo;

R^6 and R^9 are independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', NO_2, Me_3Sn and halo;

R^7 is independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', NO_2, Me_3Sn and halo;

Q is independently selected from O, S and NH;

R^{11} is either H, or R or, where Q is O, SO_3M, where M is a metal cation;

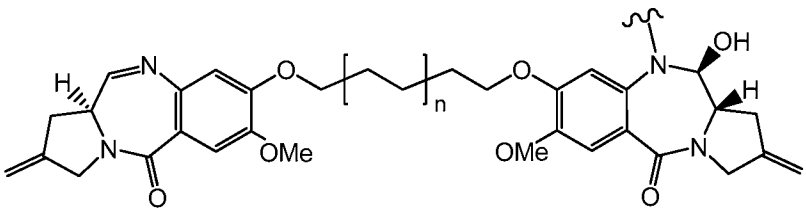
R and R' are each independently selected from optionally substituted C_{1-8} alkyl, C_{3-8} heterocyclyl and C_{5-20} aryl groups, and optionally in relation to the group NRR', R and R' together with the nitrogen atom to which they are attached form an optionally substituted 4-, 5-, 6- or 7-membered 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_{3-12} 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).

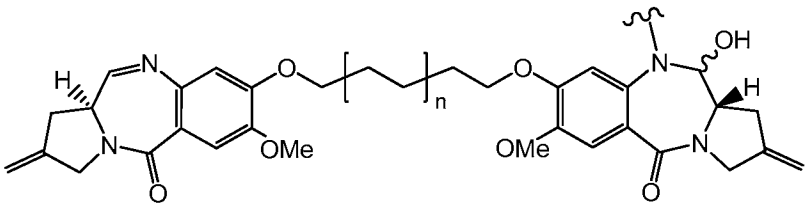
26. The immunoconjugate of claim 25, wherein D has the structure:



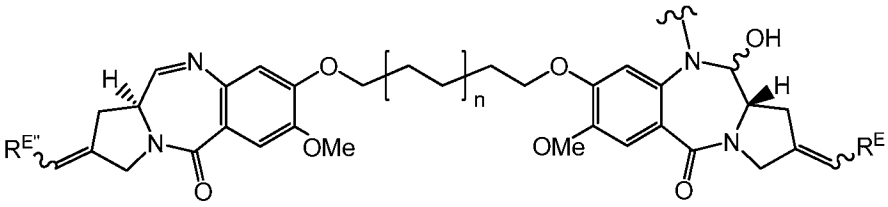
A(II);

wherein n is 0 or 1.

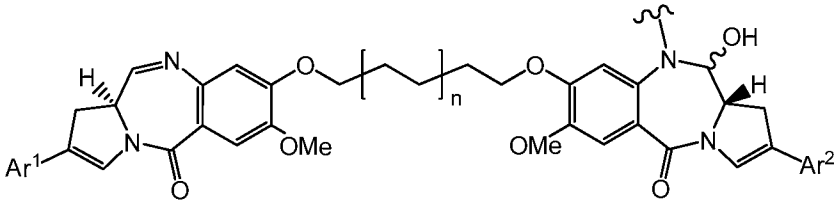
27. The immunoconjugate of claim 25, wherein D has a structure selected from:



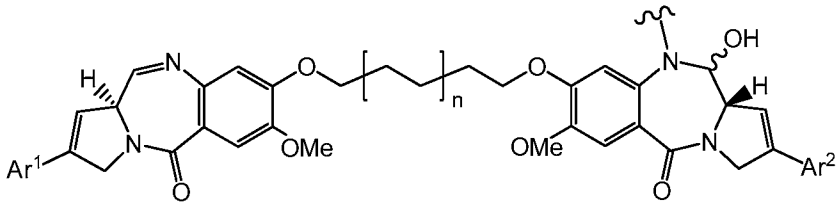
A(I);



A(III);



A(IV); and



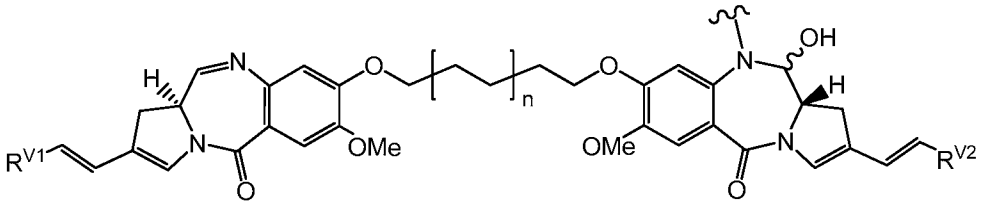
A(V);

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.

28. The immunoconjugate of claim 21, wherein D is a pyrrolobenzodiazepine of Formula B:



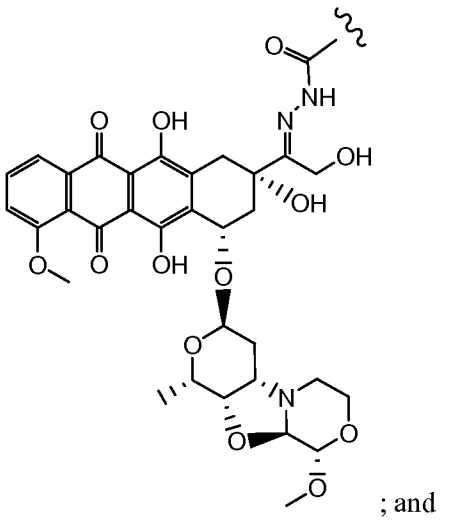
wherein the horizontal wavy line indicates the covalent attachement site to the linker;

R^{V1} and R^{V2} are independently selected from H, methyl, ethyl, phenyl, fluoro-substituted phenyl, and C_{5-6} heterocyclyl; and

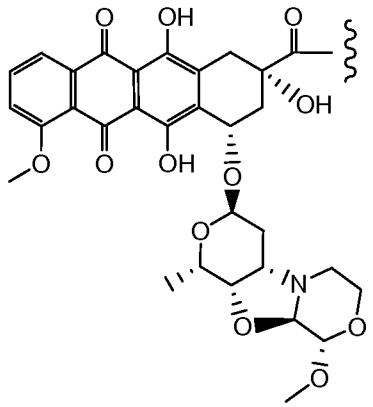
n is 0 or 1.

29. The immunoconjugate of claim 21, wherein D is a nemorubicin derivative.

30. The immunoconjugate of claim 29, wherein D has a structure selected from:



; and



.

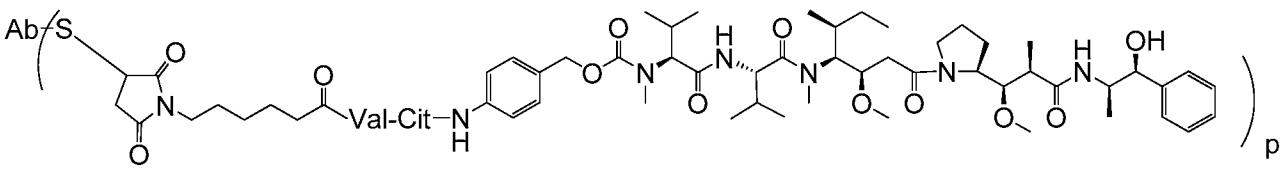
31. The immunoconjugate of any one of claims 21 to 30, wherein the linker is cleavable by a protease.

32. The immunoconjugate of claim 31, wherein the linker comprises a val-cit dipeptide or a Phe-Lys dipeptide.

33. The immunoconjugate of any one of claims 21 to 30, wherein the linker is acid-labile.

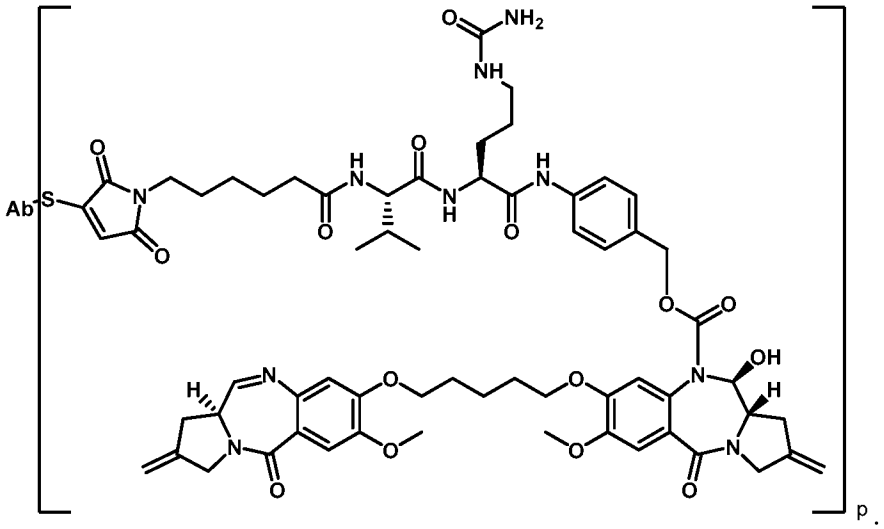
34. The immunoconjugate of claim 33, wherein the linker comprises hydrazone.

35. The immunoconjugate of claim 23 having the formula:

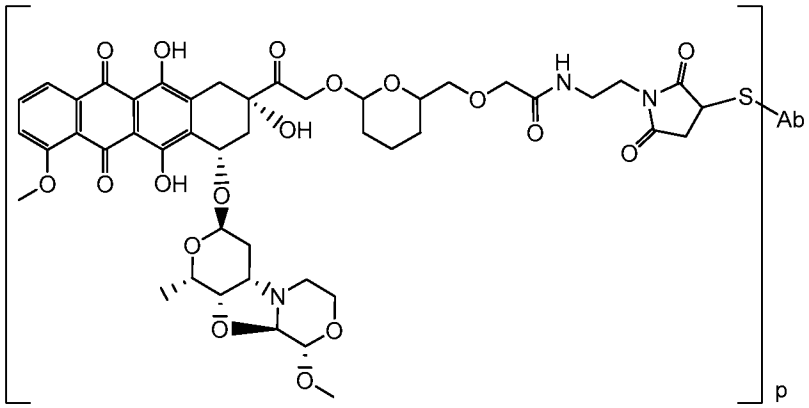


wherein S is a sulfur atom.

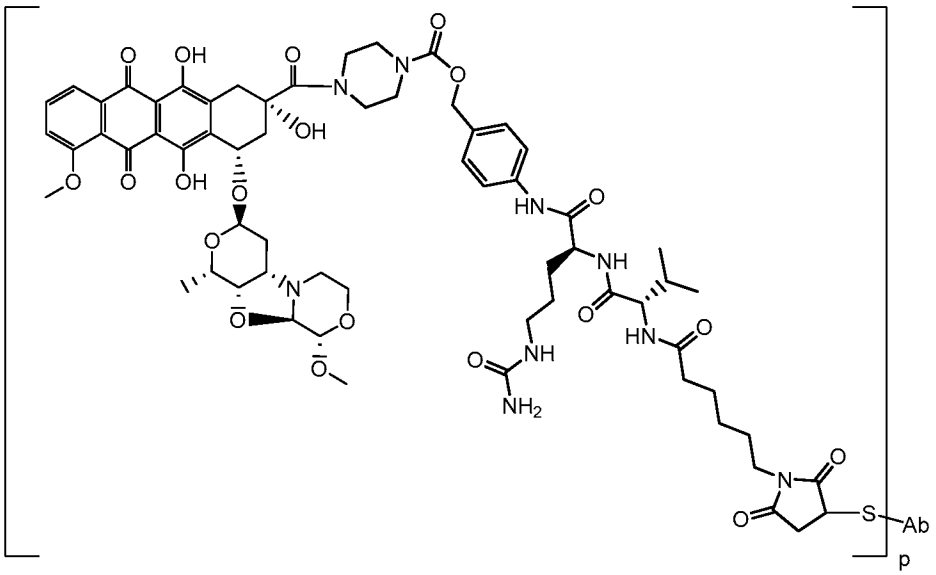
36. The immunoconjugate of claim 26 having the formula:

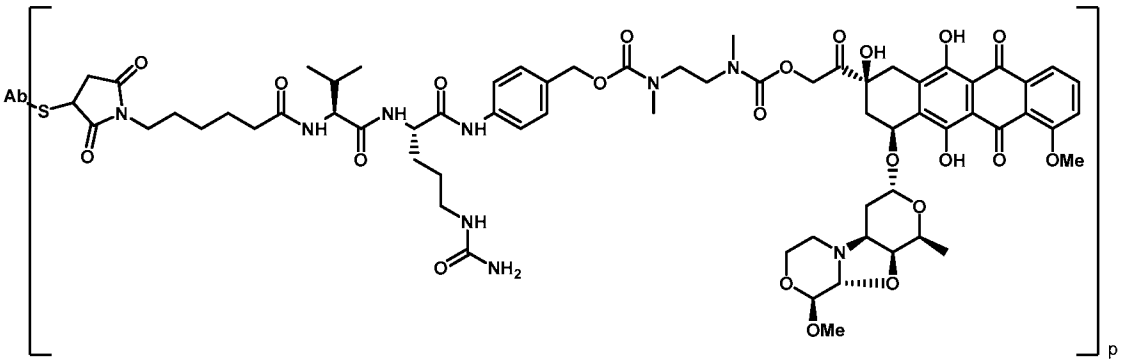


37. The immunoconjugate of claim 30 having a formula selected from:

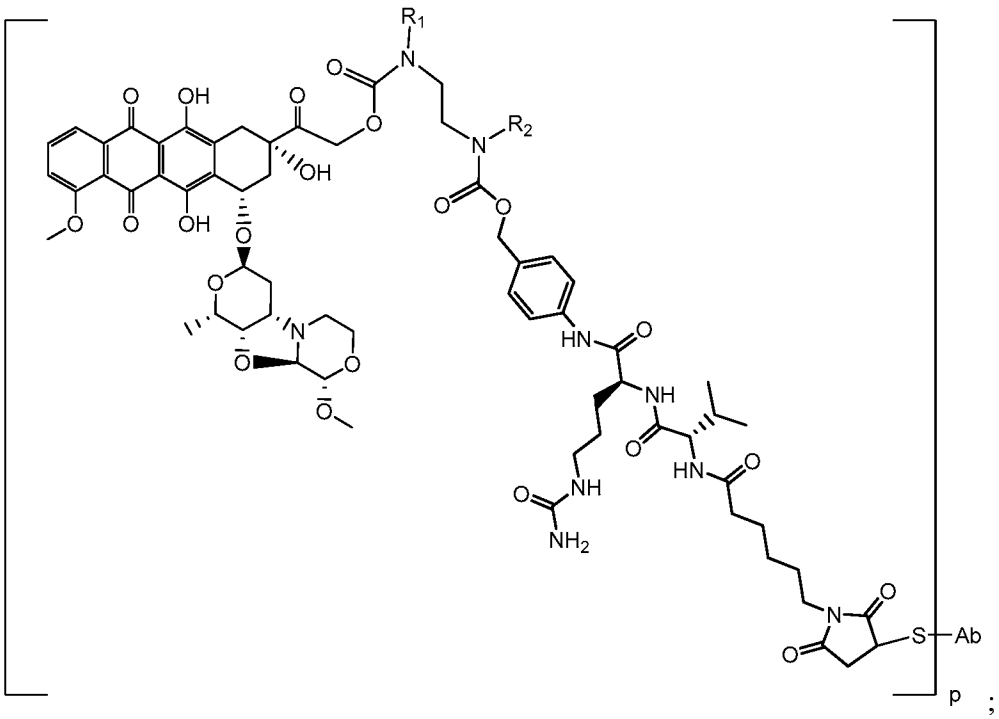


;



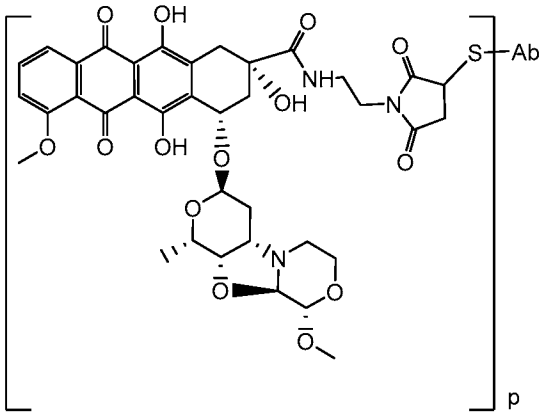


;



;

and



38. The immunoconjugate of any one of claims 21 to 37, wherein p ranges from 2-5.

39. The immunoconjugate of any one of claims 21 to 38, comprising the antibody of claim 9.

40. The immunoconjugate of any one of claims 21 to 38, comprising the antibody of claim 15.

41. A pharmaceutical formulation comprising the immunoconjugate of any one of claims 21 to 40 and a pharmaceutically acceptable carrier.

42. The pharmaceutical formulation of claim 41, further comprising an additional therapeutic agent.

43. The pharmaceutical formulation of claim 42, wherein the additional therapeutic agent is Avastin® (bevacizumab).

44. A method of treating an individual having an LgR5-positive cancer, the method comprising administering to the individual an effective amount of the immunoconjugate of any one of claims 21 to 40.

45. The method of claim 44, wherein the LgR5-positive cancer is selected from colorectal cancer, pancreatic cancer, ovarian cancer, and endometrial cancer.

46. The method of claim 45, further comprising administering an additional therapeutic agent to the individual.

47. The method of claim 46, wherein the additional therapeutic agent is Avastin® (bevacizumab).

48. A method of inhibiting proliferation of an LgR5-positive cell, the method comprising exposing the cell to the immunoconjugate of any one of claims 21 to 40 under conditions permissive for binding of the immunoconjugate to LgR5 on the surface of the cell, thereby inhibiting proliferation of the cell.

49. The method of claim 48, wherein the cell is a colorectal, pancreatic, ovarian, or endometrial cancer cell.

50. The antibody of any one of claims 1 to 16 conjugated to a label.

51. The antibody of claim 50, wherein the label is a positron emitter.

52. The antibody of claim 51, wherein the positron emitter is ^{89}Zr.

53. A method of detecting human LgR5 in a biological sample comprising contacting the biological sample with the anti-LgR5 antibody of any one of claims 1 to 16 under conditions permissive for binding of the anti-LgR5 antibody to a naturally occurring human LgR5, and detecting whether a complex is formed between the anti-LgR5 antibody and a naturally occurring human LgR5 in the biological sample.

54. The method of claim 53, wherein the anti-LgR5 antibody is an antibody as in claim 9 or claim 15.

55. The method of claim 53, wherein the biological sample is a colorectal cancer sample, pancreatic cancer sample, ovarian cancer sample, or endometrial cancer sample.

56. A method for detecting an LgR5-positive cancer comprising (i) administering a labeled anti-LgR5 antibody to a subject having or suspected of having a LgR5-positive cancer, wherein the labeled anti-LgR5 antibody comprises the anti-LgR5 antibody of any one of claims 1 to 16, and (ii) detecting the labeled anti-LgR5 antibody in the subject, wherein detection of the labeled anti-LgR5 antibody indicates a LgR5-positive cancer in the subject.

57. The method of claim 56, wherein the labeled anti-LgR5 antibody is an antibody as in claim 8 or claim 14 that is labeled.

58. The method of claim 56 or claim 57, wherein the labeled anti-LgR5 antibody comprises an anti-LgR5 antibody conjugated to a positron emitter.

59. The method of claim 58, wherein the positron emitter is ^{89}Zr.

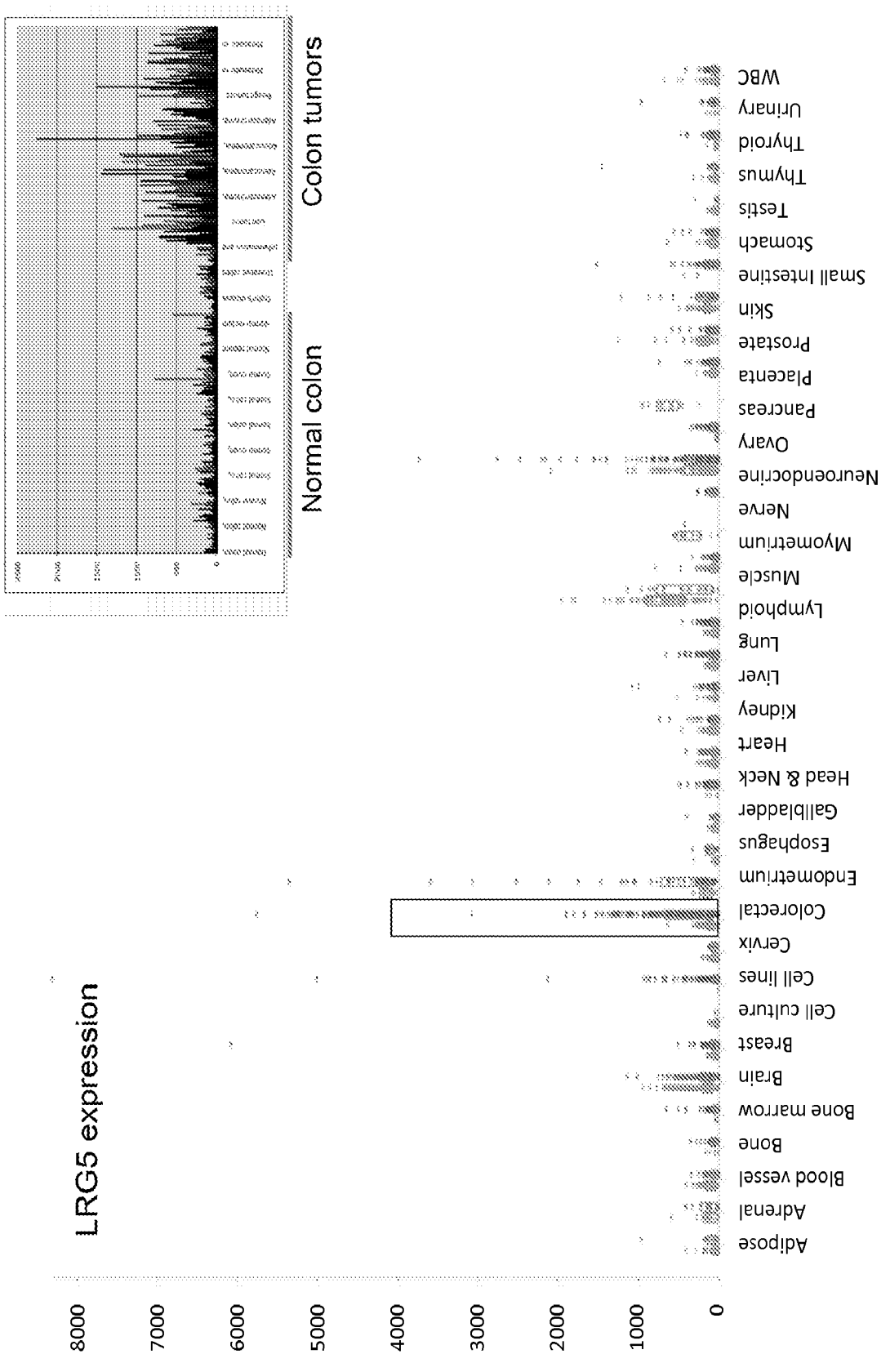

LRG5 expression
8000
7000
6000
5000
4000
3000
2000
1000
0
Adipose
Adrenal
Blood vessel
Bone
Bone marrow
Brain
Breast
Cell culture
Cell lines
Cervix
Colorectal
Endometrium
Esophagus
Gallbladder
Head & Neck
Heart
Kidney
Liver
Lung
Lymphoid
Muscle
Myometrium
Nerve
Neuroendocrine
Ovary
Pancreas
Placenta
Prostate
Skin
Small Intestine
Stomach
Testis
Thymus
Thyroid
Urinary
WBC
Normal colon
Colon tumors

FIG. 1

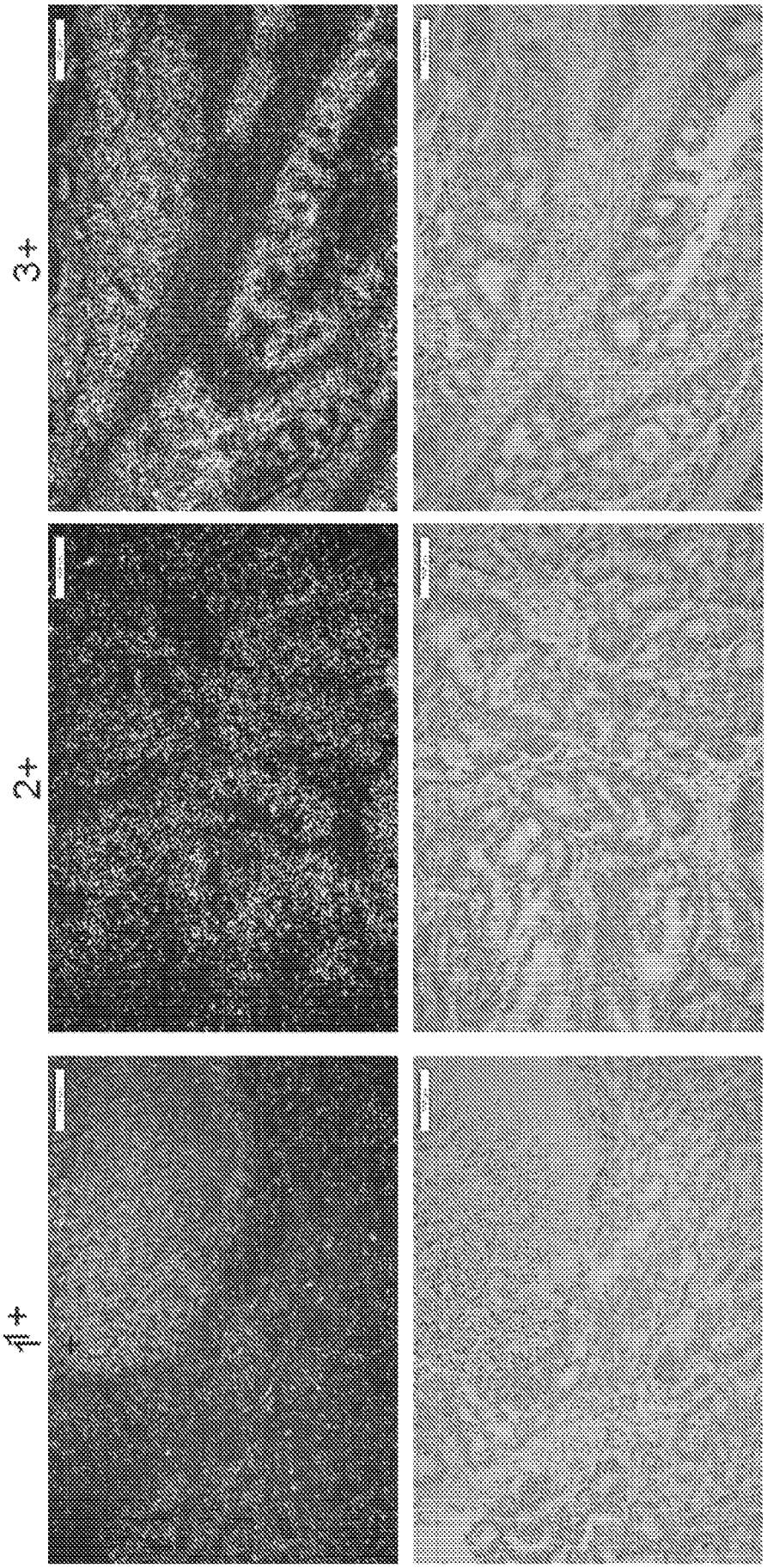


FIG. 2

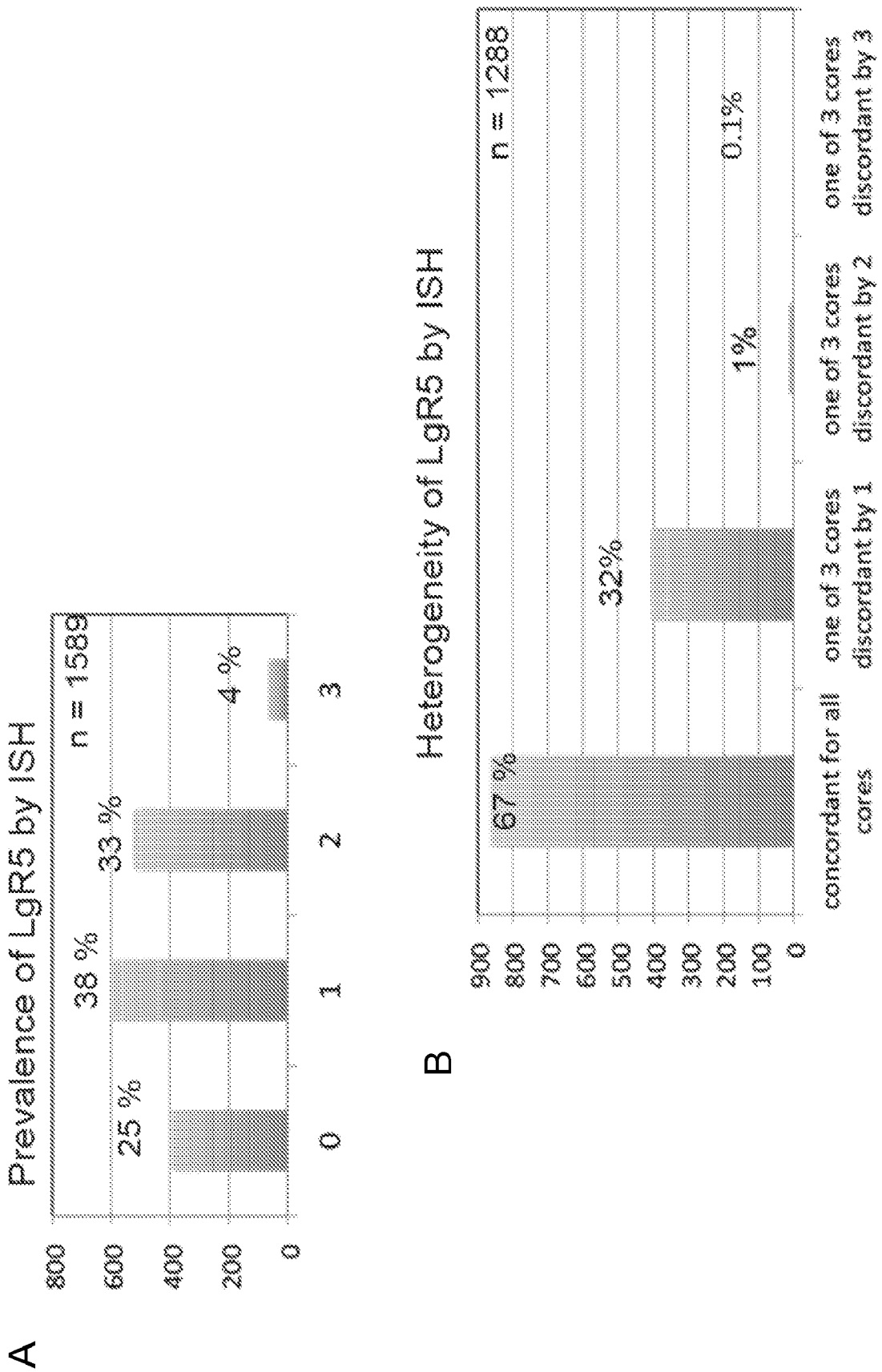

A
Prevalence of LgR5 by ISH
n = 1589
25 %
38 %
33 %
4 %
0
1
2
3
800
600
400
200
0
B
Heterogeneity of LgR5 by ISH
n = 1288
67 %
32%
1%
0.1%
900
800
700
600
500
400
300
200
100
0
concordant for all cores
one of 3 cores discordant by 1
one of 3 cores discordant by 2
one of 3 cores discordant by 3

FIG. 3

Anti-LgR5 Monoclonal Antibodies

Antibody	Epitope Region	FACS				IHC	Western Blot	Affinity (Biacore)	Affinity (Scatchard)
		Human	Cyno	Rat	Mouse				
YW353	22-123	+++	+++	neg	neg	neg	neg	1.6 nM	0.2 nM
ch8E11	22-323	+++	+++	++	+++	neg	neg	2.4 nM	0.4 nM (Hu) 0.2 nM (Mu)
hu8E11.v2	22-323	+++	+++	++	+++	neg	neg	3.1 nM	0.3-0.7 nM (Hu) 0.6-0.6 nM (Mu) 2.4-2.8 nM (Rat)
2H6	324-423	++	n.d.	n.d.	++	NS	+++	208 nM	n.d.
3G12	324-423	+++	n.d.	n.d.	+	NS	+++	72 nM	n.d.

n.d. = not determined
NS = some nonspecific binding

FIG. 4

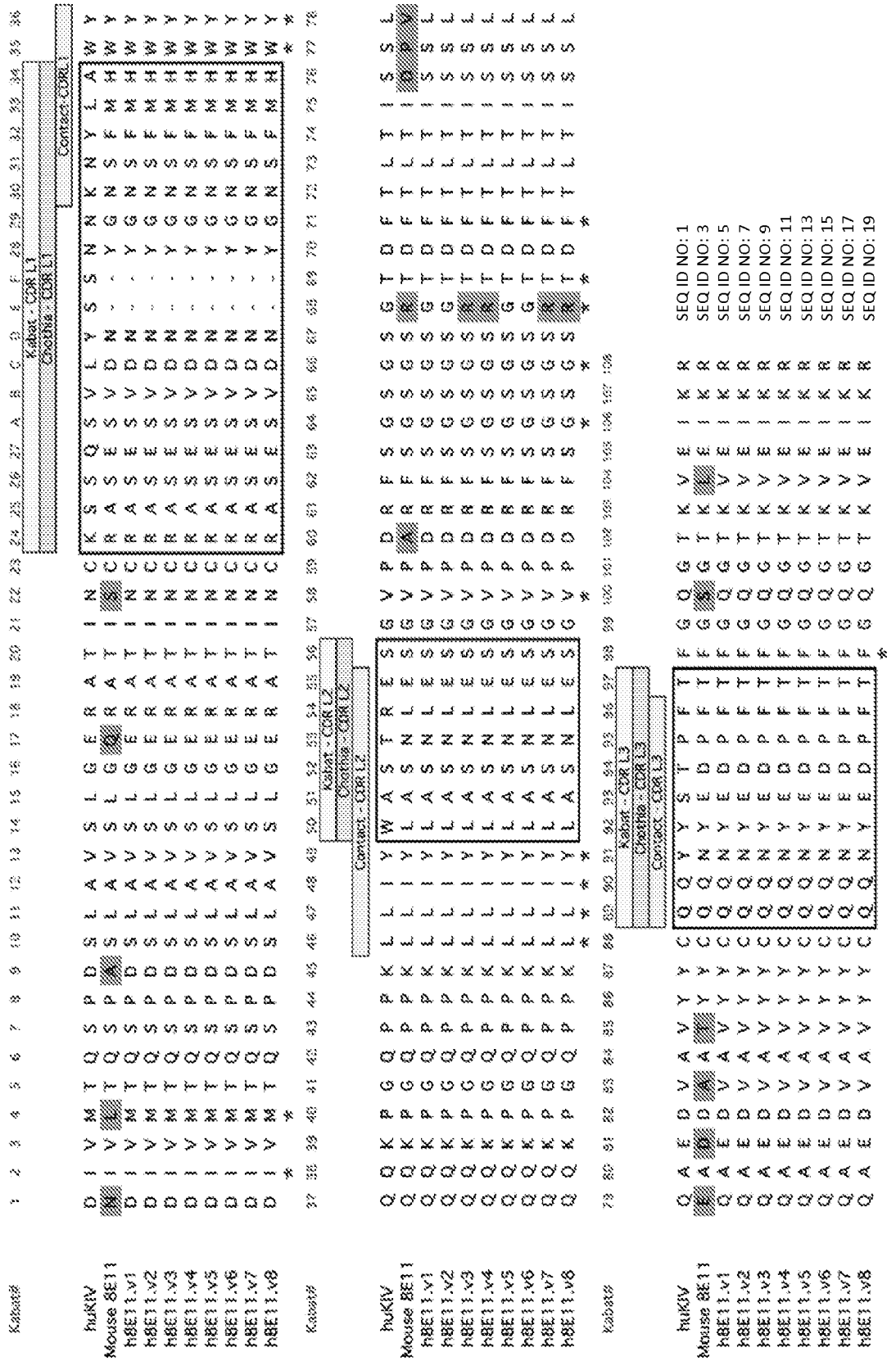

Kabat - CDR L1
Chothia - CDR L1
Contact - CDR L1
Kabat - CDR L2
Chothia - CDR L2
Contact - CDR L2
Kabat - CDR L3
Chothia - CDR L3
Contact - CDR L3
SEQ ID NO: 1
SEQ ID NO: 3
SEQ ID NO: 5
SEQ ID NO: 7
SEQ ID NO: 9
SEQ ID NO: 11
SEQ ID NO: 13
SEQ ID NO: 15
SEQ ID NO: 17
SEQ ID NO: 19

FIG. 5

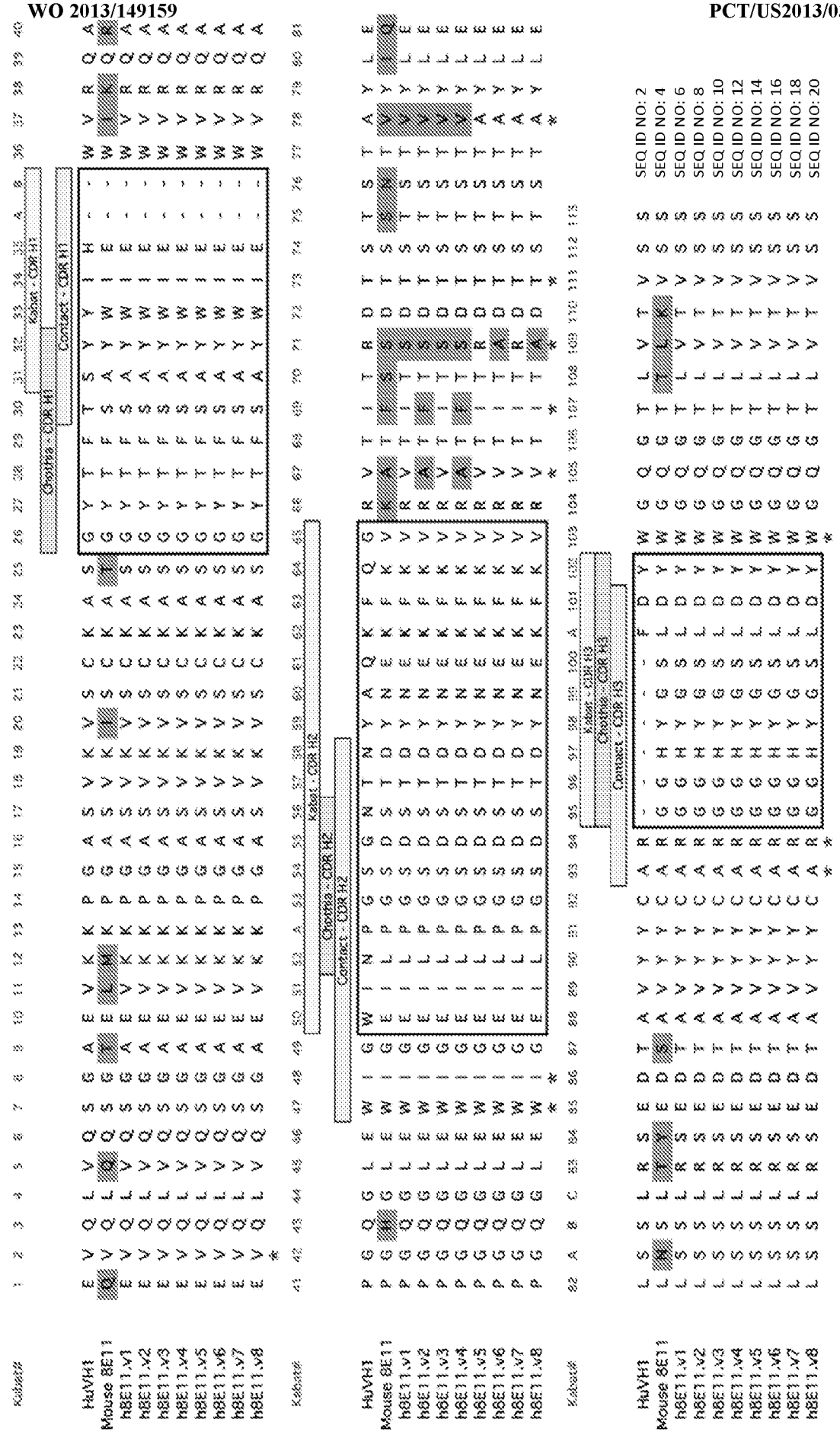

Kabat#
HuVH1
Mouse 8E11
h8E11.v1
h8E11.v2
h8E11.v3
h8E11.v4
h8E11.v5
h8E11.v6
h8E11.v7
h8E11.v8
Chothia - CDR H1
Kabat - CDR H1
Contact - CDR H1
Kabat - CDR H2
Chothia - CDR H2
Contact - CDR H2
Kabat - CDR H3
Chothia - CDR H3
Contact - CDR H3
SEQ ID NO: 2
SEQ ID NO: 4
SEQ ID NO: 6
SEQ ID NO: 8
SEQ ID NO: 10
SEQ ID NO: 12
SEQ ID NO: 14
SEQ ID NO: 16
SEQ ID NO: 18
SEQ ID NO: 20

FIG. 6

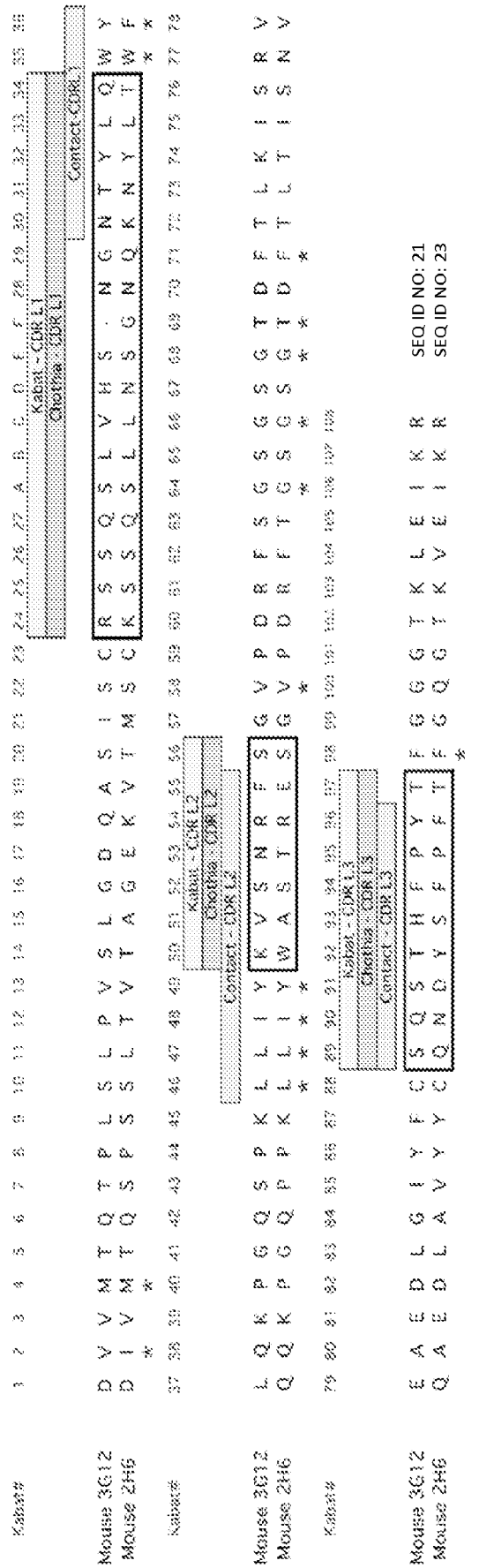

Kabat - CDR L1
Chothia - CDR L1
Contact - CDR L1
Kabat - CDR L2
Chothia - CDR L2
Contact - CDR L2
Kabat - CDR L3
Chothia - CDR L3
Contact - CDR L3
SEQ ID NO: 21
SEQ ID NO: 23

FIG. 7

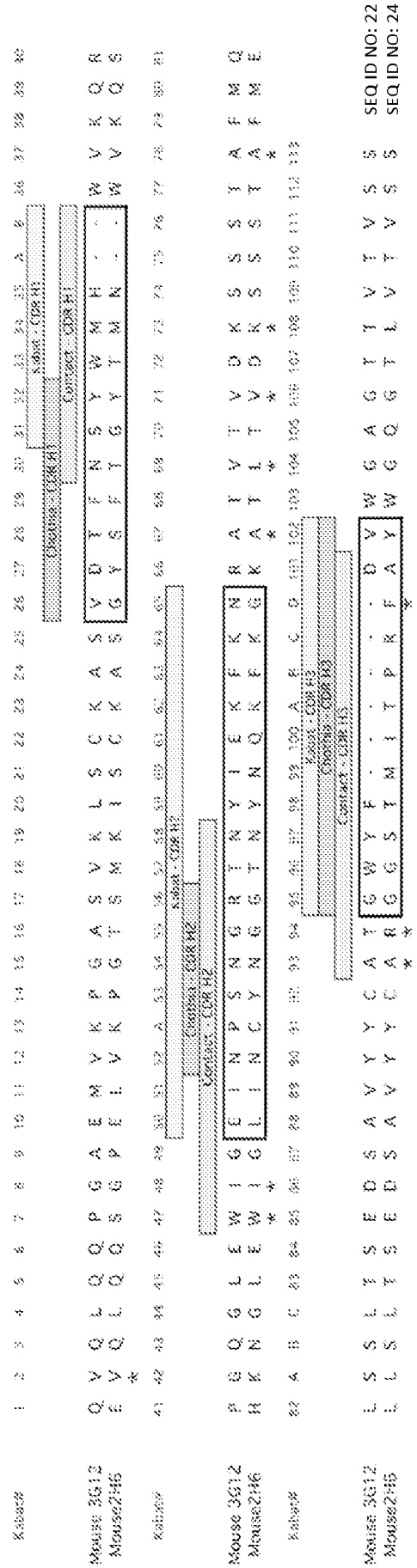

Kabat
Mouse 3G12
Mouse2H6
Kabat
Mouse 3G12
Mouse2H6
Kabat
Mouse 3G12
Mouse2H6
SEQ ID NO: 22
SEQ ID NO: 24

FIG. 8

	VL (KappaIV)	VH (Group I)	Kon($10^5 M^{-1}S^{-1}$)	Koff($10^{-4}S^{-1}$)	KD(nM)
h8E11.v1	CDR graft	CDR graft + 71S/78V	1.53	7.72	5.05
h8E11.v2	CDR graft	CDR graft + 67A/69F/71S/78V	2.79	8.53	3.10
h8E11.v3	CDR graft + 68R	CDR graft + 71S/78V	1.12	5.3	4.73
h8E11.v4	CDR graft + 68R	CDR graft + 67A/69F/71S/78V	2.54	9.22	3.63
h8E11.v5	CDR graft	CDR graft + 71R/78A	0.65	9.33	14.35
h8E11.v6	CDR graft	CDR graft + 71A/78A	1.43	20.2	14.13
h8E11.v7	CDR graft + 68R	CDR graft + 71R/78A	1.17	25.8	22.05
h8E11.v8	CDR graft + 68R	CDR graft + 71A/78A	2.42	21.7	8.97
Chimeric 8E11	Mouse 8E11 VL	Mouse 8E11 VH	2.1	6.4	3.00

FIG. 9

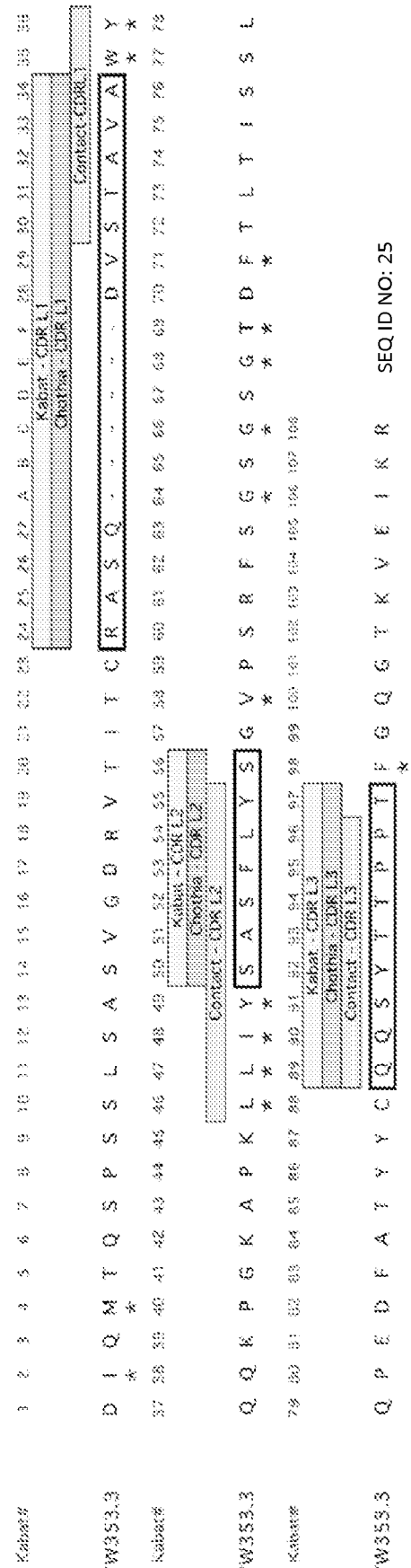

Kabat
YW353.3
Kabat - CDR L1
Chothia - CDR L1
Contact - CDR L1
Kabat - CDR L2
Chothia - CDR L2
Contact - CDR L2
Kabat - CDR L3
Chothia - CDR L3
Contact - CDR L3
SEQ ID NO: 25

FIG. 10

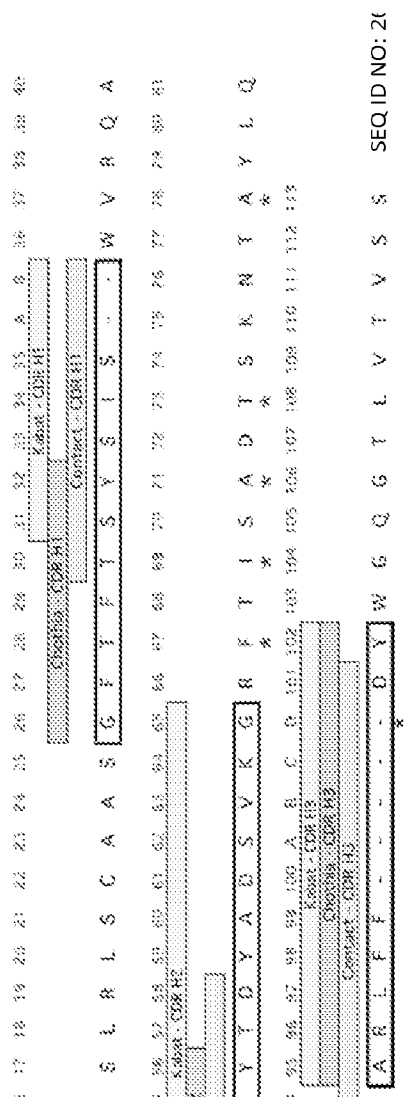

Kabat
YW353.3
Kabat - CDR H1
Chothia - CDR H1
Contact - CDR H1
Kabat - CDR H2
Chothia - CDR H2
Contact - CDR H2
Kabat - CDR H3
Chothia - CDR H3
Contact - CDR H3
SEQ ID NO: 26

FIG. 11


Signal sequence
LGR5_human MDTSRLGVLLSLPVLLQLATGGSSPRSGVLLRGCPTHCHCEPDGRMLLRVDCSDLGLSEL 60
LGR5_cyno_predicted XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXGCPTHCHCEPDGRMLLRVDCSDLGLSEL 60
LGR5_mouse MDTSCVHMLLSLLALLQLVAAGSSPGPDAIPRGCPSHCHCELDGRMLLRVDCSDLGLSEL 60
LGR5_rat MDTSRVRMLLSLLALLQLVAAGSPPRPDTMPRGCPSYCHCELDGRMLLRVDCSDLGLSEL 60
LGR5_human PSNLSVFTSYLDLSMNNISQLLPNPLPSLRFLEELRLAGNALTYIPKGAFTGLYSLKVLM 120
LGR5_cyno_predicted PSNLSVFTSYLDLSMNNISQLLPNPLPSLRFLEELRLAGNALTYIPKGAFTGLYSLKVLM 120
LGR5_mouse PSNLSVFTSYLDLSMNNISQLPASLLHRLCFLEELRLAGNALTHIPKGAFTGLHSLKVLM 120
LGR5_rat PSNLSVFTSYLDLSMNNISQLPASLLHRLRFLEELRLAGNALTHIPKGAFAGLHSLKVLM 120
LGR5_human LQNNQLRHVPTEALQNLRSLQSLRLDANHISYVPPSCFSGLHSLRHLWLDDNALTEIPVQ 180
LGR5_cyno_predicted LQNNQLRQVPTEALQNLRSLQSLRLDANHISYVPPSCFSGLHSLRHLWLDDNALTEIPVQ 180
LGR5_mouse LQNNQLRQVPEEALQNLRSLQSLRLDANHISYVPPSCFSGLHSLRHLWLDDNALTDVPVQ 180
LGR5_rat LQNNQLRQVPEEALQNLRSLQSLRLDANHISYVPPSCFSGLHSLRHLWLDDNALTDVPVQ 180
LGR5_human AFRSLSALQAMTLALNKIHHIPDYAFGNLSSLVVLHLHNNRIHSLGKKCFDGLHSLETLD 240
LGR5_cyno_predicted AFRSLSALQAMTLALNKIHHIPDYAFGNLSSLVVLHLHNNRIHSLGKKCFDGLHSLETLD 240
LGR5_mouse AFRSLSALQAMTLALNKIHHIADYAFGNLSSLVVLHLHNNRIHSLGKKCFDGLHSLETLD 240
LGR5_rat AFRSLSALQAMTLALNKIHHIADHAFGNLSSLVVLHLHNNRIHSLGKKCFDGLHSLETLD 240
LGR5_human LNYNNLDEFPTAIRTLSNLKELGFHSNNIRSIPEKAFVGNPSLITIHFYDNPIQFVGRSA 300
LGR5_cyno_predicted LNYNNLDEFPTAIRTLSNLKELGFHSNNIRSIPEKAFVGNPSLITIHFYDNPIQFVGRSA 300
LGR5_mouse LNYNNLDEFPTAIKTLSNLKELGFHSNNIRSIPERAFVGNPSLITIHFYDNPIQFVGVSA 300
LGR5_rat LNYNNLDEFPTAIKTLSNLKELGFHSNNIRSIPERAFVGNPSLITIHFYDNPIQFVGISA 300
LGR5_human FQHLPELRTLTLNGASQITEFPDLTGTANLESLTLTGAQISSLPQTVCNQLPNLQVLDLS 360
LGR5_cyno_predicted FQHLPELRTLTLNGASQITEFPDLTGTANLESLTLTGAQISSLPQTVCNQLPNLQVLDLS 360
LGR5_mouse FQHLPELRTLTLNGASHITEFPHLTGTATLESLTLTGAKISSLPQAVCDQLPNLQVLDLS 360
LGR5_rat FQHLPELRTLTLNGASQITEFPDLTGTATLESLTLTGAKISSLPQTVCDQLPNLQVLDLS 360

FIG. 12A

```
LGR5_human      YNLLEDLPSFSVCQKLQKIDLRHNEIYEIKVDTFQQLLSLRSLNLAWNKIAIIHPNAFST 420
LGR5_cyno_predicted YNLLEDLPSFSVCQKLQKIDLRHNEIYEIKVDTFQQLLSLRSLNLAWNKIAIIHPNAFST 420
LGR5_mouse      YNLLEDLPSLSGCQKLQKIDLRHNEIYEIKGSTFQQLFNLRSLNLAWNKIAIIHPNAFST 420
LGR5_rat        YNLLEDLPSLSGCQKLQKIDLRHNEIYEIKGGTFQQLFNLRSLNLARNKIAIIHPNAFST 420
                ********** :* ********************  .******:.******* ********** *****

LGR5_human      LPSLIKLDLSSNLLSSFPITGLHGLTHLKLTGNHALQSLISSENFPELKVIEMPYAYQCC 480
LGR5_cyno_predicted LPSLIKLDLSSNLLSSFPVTGLHGLTHLKLTGNHALQSLISSENFPELKIIEMPYAYQCC 480
LGR5_mouse      LPSLIKLDLSSNLLSSFPVTGLHGLTHLKLTGNRALQSLIPSANFPELKIIEMPSAYQCC 480
LGR5_rat        LPSLIKLDLSSNLLSSFPVTGLHGLTHLKLTGNRALQSLIPSANFPELKIIEMPYAYQCC 480
                ********************:**********:********.* *******:***** *****

LGR5_human      AFGVCENAYKISNQWNKGDNSSMDDLHKKDAGMFQAQDERDLEDFLLDFEEDLKALHSVQ 540
LGR5_cyno_predicted AFGVCENAYKISNQWNKGDNSSMDDLHKKDAGMFQVQDERDLEDFLLDFEEDLKALHSVQ 540
LGR5_mouse      AFGGCENVYKISNQWNKDDGNSVDDLHKKDAGLFQVQDERDLEDFLLDFEEDLKALHSVQ 540
LGR5_rat        AFGGCENVYKIPNQWNKDDSSSVDDLRKKDAGLFQVQDERDLEDFLLDFEEDLKVLHSVQ 540
                *** *** .***.******.*..*:***:******:**.********************.*****

LGR5_human      CSPSPGPFKPCEHLLDGWLIRIGVWTIAVLALTCNALVTSTVFRSPLYISPIKLLIGVIA 600
LGR5_cyno_predicted CSPSPGPFKPCEHLLDGWLIRIGVWTIAVLALTCNALVTSTVFRSPLYISPIKLLIGVIA 600
LGR5_mouse      CSPSPGPFKPCEHLFGSWLIRIGVWTTAVLALSCNALVALTVFRTPLYISSIKLLIGVIA 600
LGR5_rat        CSPPPGPFKPCEHLFGSWLIRIGVWTTAVLALSCNALVAFTVFRTPLYISSIKLLIGVIA 600
                ***.**********:..********** ******:******; ****:******.********** 

LGR5_human      AVNMLTGVSSAVLAGVDAFTFGSFARHGAWWENGVGCHVIGFLSIFASESSVFLLTLAAL 660
LGR5_cyno_predicted VVNMLTGVSSAVLAGVDAFTFGSFARHGAWWENGVGCQVIGFLSIFASESSVFLLTLAAL 660
LGR5_mouse      VVDILMGVSSAVLAAVDAFTFGRFAQHGAWWEDGIGCQIVGFLSIFASESSIFLLTLAAL 660
LGR5_rat        VVDILMGVSSAILAVVDTFTFGSFAQHGAWWEGGIGCQIVGFLSIFASESSVFLLTLAAL 660
                .*:::* ******:** **:***** **:*******.*:**:::********** :********** 

LGR5_human      ERGFSVKYSAKFETKAPFSSLKVIILLCALLALTMAAVPLLGGSKYGASPLCLPLPFGEP 720
LGR5_cyno_predicted ERGFSVKCSAKFETKAPFSSLKVIILLCALLALTMAAVPLLGGSEYGASPLCLPLPFGEP 720
LGR5_mouse      ERGFSVKCSSKFEVKAPLFSLRAIVLLCVLLALTIATIPLLGGSKYNASPLCLPLPFGEP 720
LGR5_rat        ERGFSVKCSSKFEMKAPLSSLKAIILLCVLLALTITATVPLLGGSEYNASPLCLPLPFGEP 720
                ******* *:*** ****: **:.*:***.***** :*::******:*.********** **********
```

FIG. 12B

```
LGR5_human          STMGYMVALILLLNSLCFLMMTIAYTKLYCNLDKGDLENIWDCSMVKHIALLLFTNCILNC 780
LGR5_cyno_predicted STTGYMVALILLNSLCFLMMTIAYTKLYCNLDKGDLENIWDCSMVKHIALLLFTNCILYC 780
LGR5_mouse          STTGYMVALVLLNSLCFLIMTIAYTKLYCSLEKGELENLWDCSMVKHIALLLFANCILYC 780
LGR5_rat            STTGYMVALVLLNSLCFLIMTIAYTRLYCSLEKGELENLWDCSMVKHTALLLFTNCILYC 780
                    ** ***********:**********:****.*:***:***:********* ******:***** *

LGR5_human          PVAFLSFSSLINLTFISPEVIKFILLVVVPLPACLNPLLYILFNPHFKEDLVSLRKQTYV 840
LGR5_cyno_predicted PVAFLSFSSLLNLTFISPEVIKFILLVIVPLPACLNPLLYILFNPHFKEDLVSLGKQTYF 840
LGR5_mouse          PVAFLSFSSLLNLTFISPDVIKFILLVIVPLPSCLNPLLYIVFNPHFKEDMGSLGKHTRF 840
LGR5_rat            PVAFLSFSSLLNLTFISPEVIKFILLVIVPLPACLNPLLYIVFNPHFKEDMGSLGKQTRF 840
                    **********:**********:*****:**********:********** ** *:* .

LGR5_human          WTRSKHPSLMSINSDDVEKQSCDSTQALVTFTSSSITYDLPPSSVPSPAYPVTESCHLSS 900
LGR5_cyno_predicted WTRSKHPSLMSINSDDVEKQSCDSTQALVTFTSSSIAYDLPPSSVPSPAYPVTESCHLSS 900
LGR5_mouse          WMRSKHASLLSINSDDVEKRSCESTQALVSFTHASIAYDLPSTSGASPAYPMTESCHLSS 900
LGR5_rat            WTRAKHPSLLSINSDDVEKRSCDSTQALVSFTHASIAYDLPSDSGSSPAYPMTESCHLSS 900
                    * *:**.**:**********:**:********:** :**:*****.  * .******:********

LGR5_human          VAFVPCL 907   SEQ ID NO: 67
LGR5_cyno_predicted VAFVPCL 907   SEQ ID NO: 69
LGR5_mouse          VAFVPCL 907   SEQ ID NO: 72
LGR5_rat            VAFVPCL 907   SEQ ID NO: 70
                    *******
```

FIG. 12C

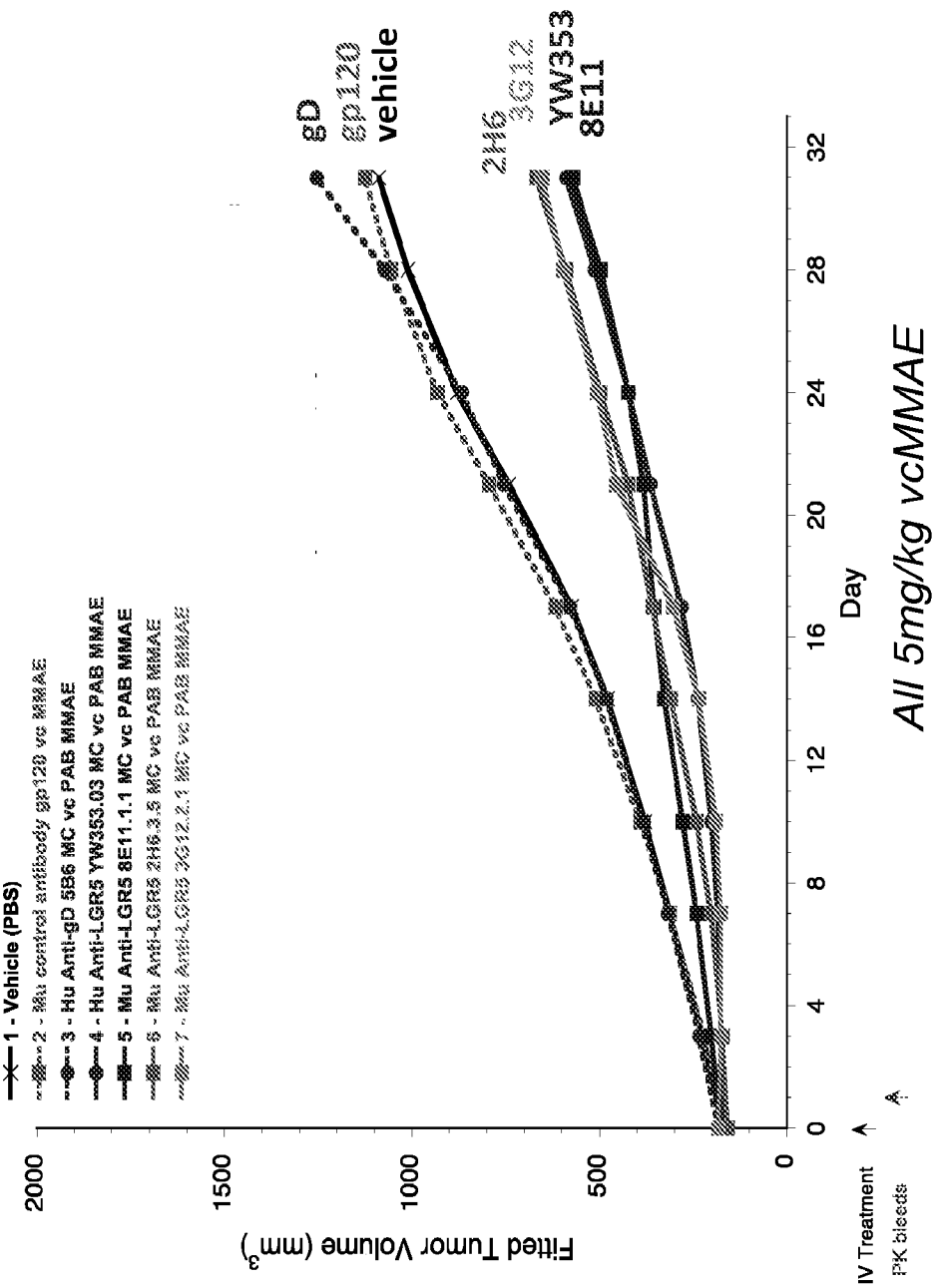

1 - Vehicle (PBS)
2 - Mu control antibody gp120 vc MMAE
3 - Hu Anti-gD 5B6 MC vc PAB MMAE
4 - Hu Anti-LGR5 YW353.03 MC vc PAB MMAE
5 - Mu Anti-LGR5 8E11.1.1 MC vc PAB MMAE
6 - Mu Anti-LGR5 2H6.3.3 MC vc PAB MMAE
7 - Mu Anti-LGR5 3G12.2.1 MC vc PAB MMAE
Fitted Tumor Volume (mm3)
2000
1500
1000
500
0
0
4
8
12
16
20
24
28
32
Day
IV Treatment
PK bleeds
gD
gp120
vehicle
2H6
3G12
YW353
8E11
All 5mg/kg vcMMAE

FIG. 13

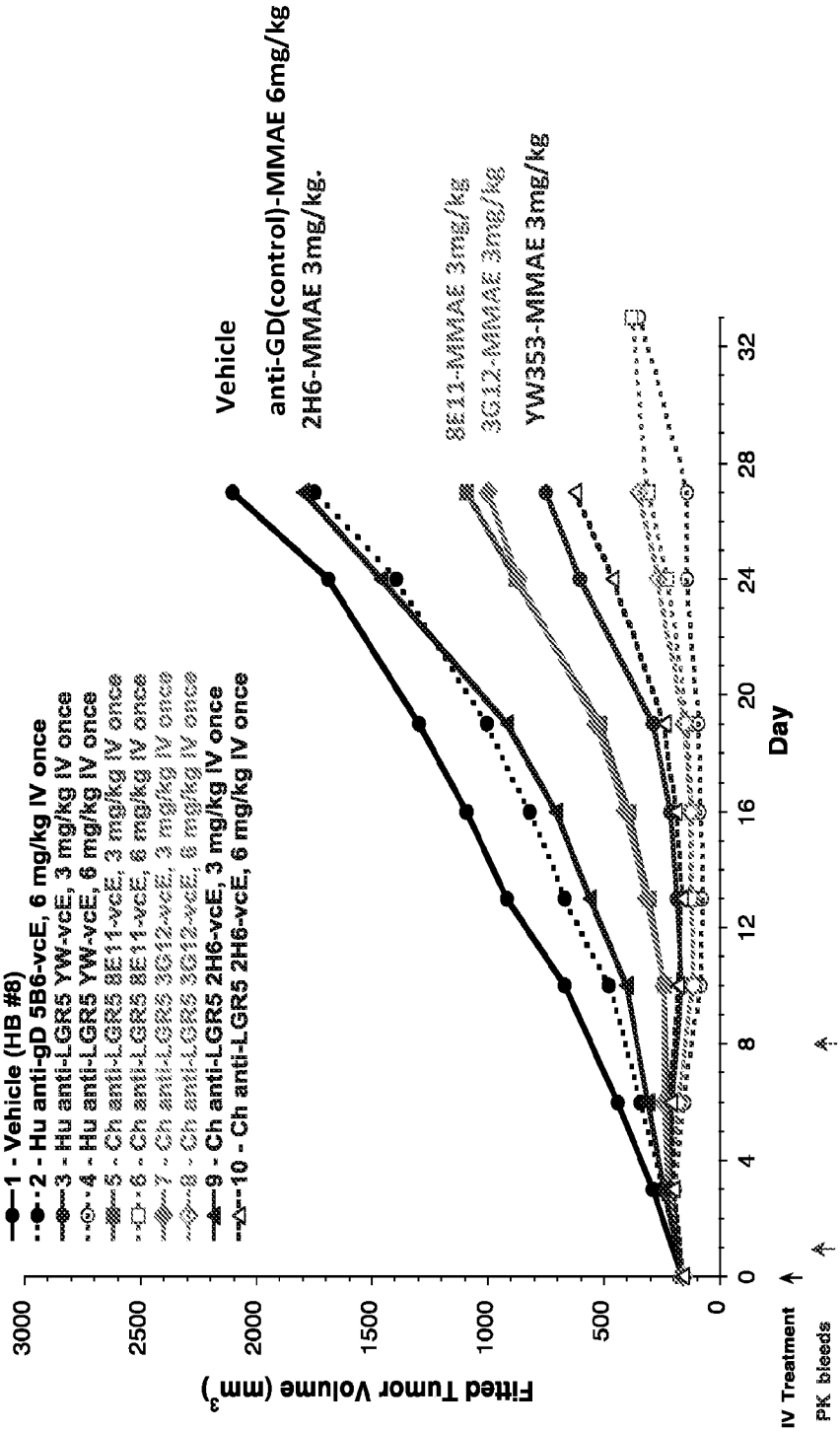

1 - Vehicle (HB #8)
2 - Hu anti-gD 5B6-vcE, 6 mg/kg IV once
3 - Hu anti-LGR5 YW-vcE, 3 mg/kg IV once
4 - Hu anti-LGR5 YW-vcE, 6 mg/kg IV once
5 - Ch anti-LGR5 8E11-vcE, 3 mg/kg IV once
6 - Ch anti-LGR5 8E11-vcE, 6 mg/kg IV once
7 - Ch anti-LGR5 3G12-vcE, 3 mg/kg IV once
8 - Ch anti-LGR5 3G12-vcE, 6 mg/kg IV once
9 - Ch anti-LGR5 2H6-vcE, 3 mg/kg IV once
10 - Ch anti-LGR5 2H6-vcE, 6 mg/kg IV once
Vehicle
anti-GD(control)-MMAE 6mg/kg
2H6-MMAE 3mg/kg.
8E11-MMAE 3mg/kg
3G12-MMAE 3mg/kg
YW353-MMAE 3mg/kg
Fitted Tumor Volume (mm3)
3000
2500
2000
1500
1000
500
0
0
4
8
12
16
20
24
28
32
Day
IV Treatment
PK bleeds

FIG. 14

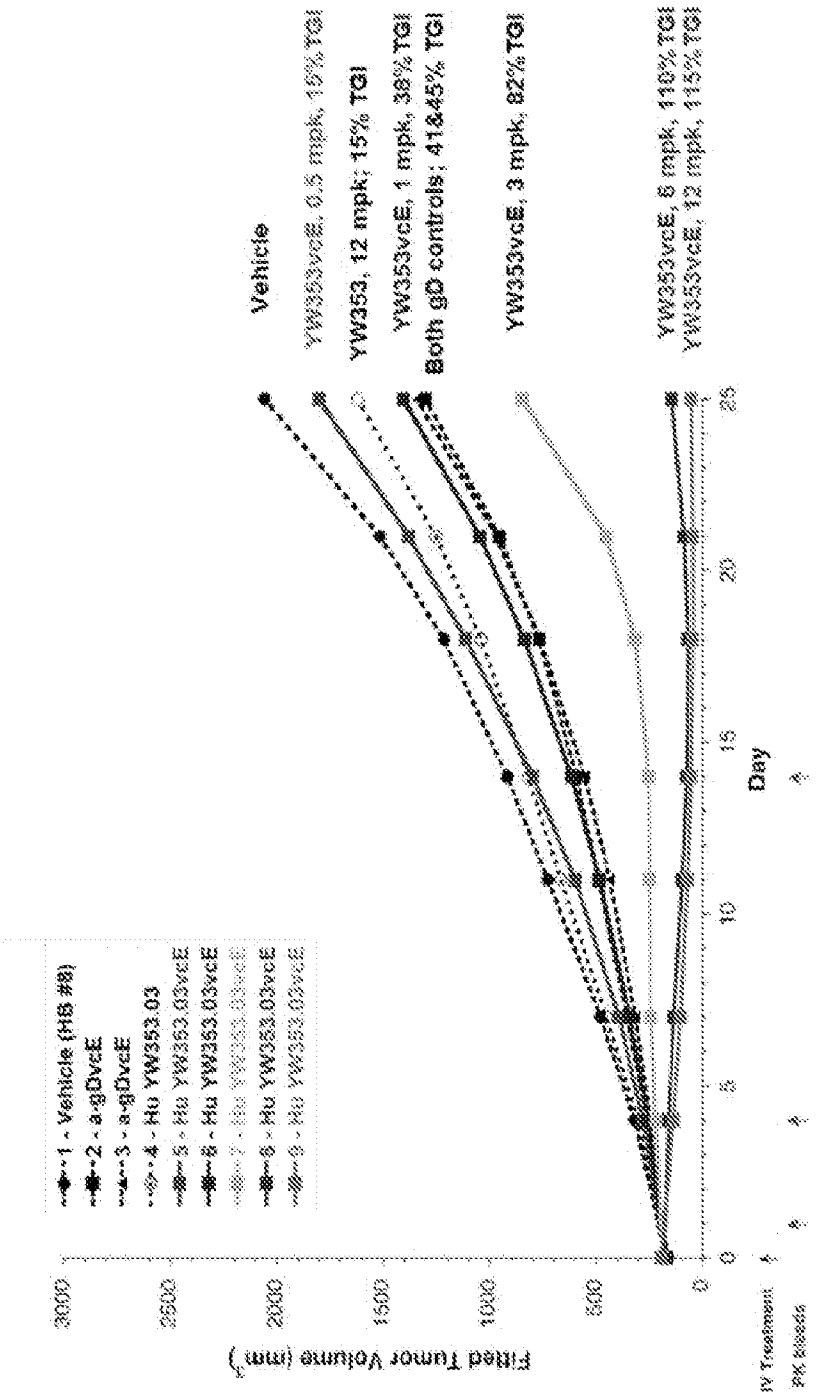

Vehicle
YW353vcE, 0.3 mpk, 15% TGI
YW353, 12 mpk; 15% TGI
YW353vcE, 1 mpk, 38% TGI
Both gD controls; 41&45% TGI
YW353vcE, 3 mpk, 82% TGI
YW353vcE, 6 mpk, 110% TGI
YW353vcE, 12 mpk, 115% TGI
1 - Vehicle (HB #8)
2 - a-gDvcE
3 - a-gDvcE
4 - Hu YW353.03
5 - Hu YW353.03vcE
6 - Hu YW353.03vcE
6 - Hu YW353.03vcE
9 - Hu YW353.03vcE
Filled Tumor Volume (mm³)
Day
IV Treatment
PK Bleeds

FIG. 15

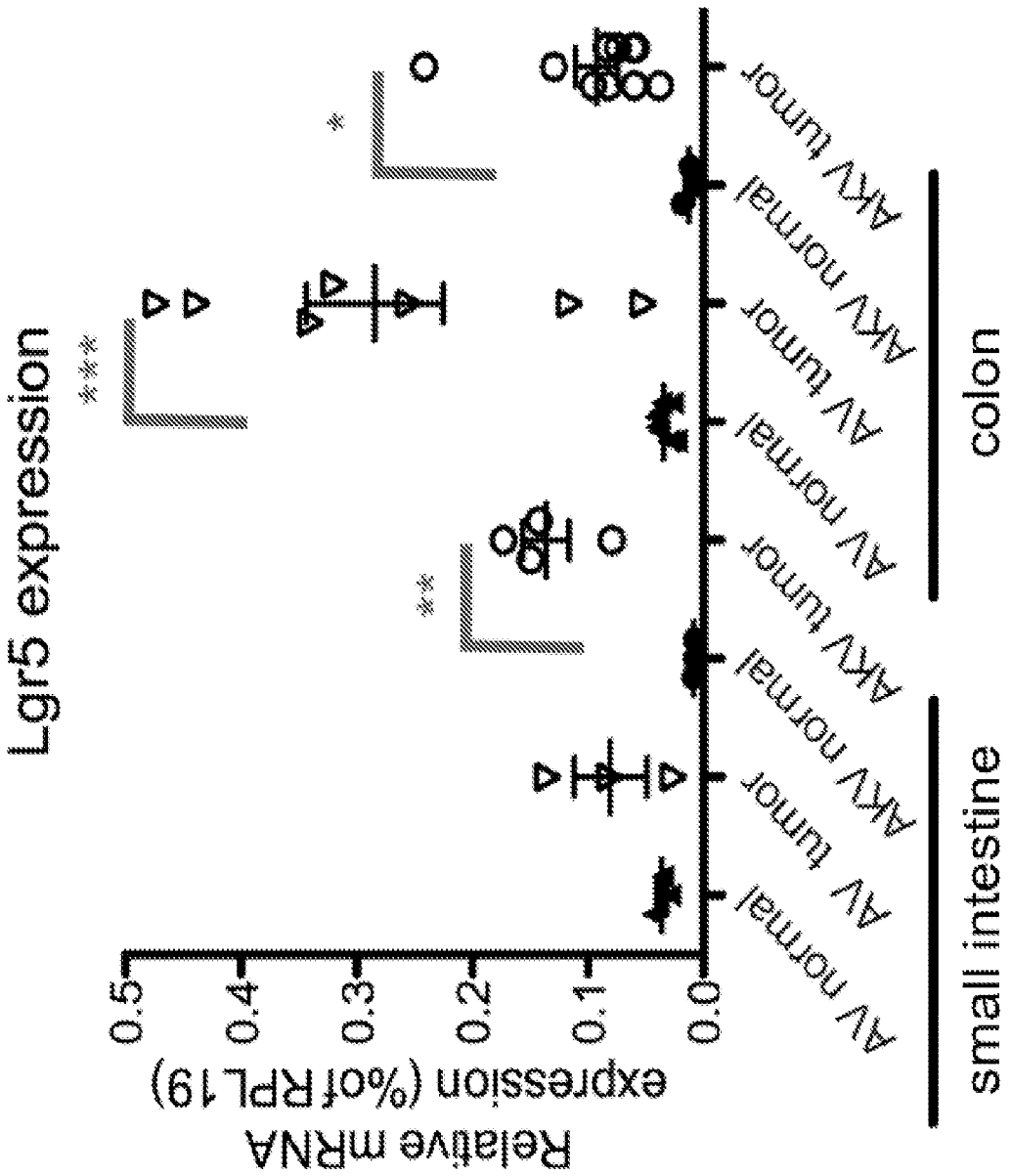

Lgr5 expression
Relative mRNA expression (%of RPL19)
0.5
0.4
0.3
0.2
0.1
0.0
***
**
*
AV normal
AV tumor
AAV normal
AAV tumor
AV normal
AV tumor
AAV normal
AAV tumor
small intestine
colon

FIG. 16

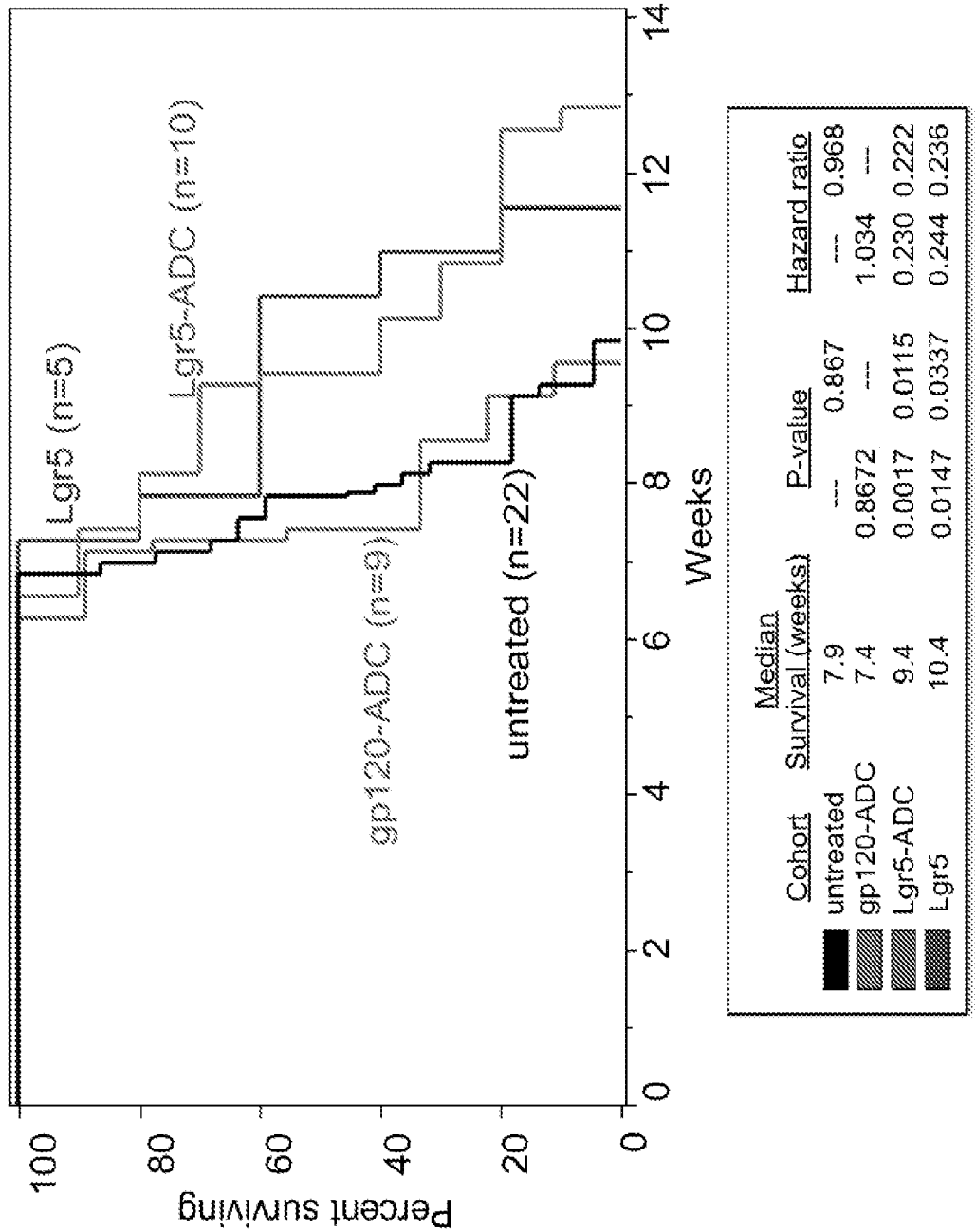

Percent surviving
100
80
60
40
20
0
0
2
4
6
8
10
12
14
Weeks
Lgr5 (n=5)
Lgr5-ADC (n=10)
gp120-ADC (n=9)
untreated (n=22)
Cohort
Median Survival (weeks)
P-value
Hazard ratio
untreated 7.9 --- 0.867 --- 0.968
gp120-ADC 7.4 0.8672 --- 1.034 ---
Lgr5-ADC 9.4 0.0017 0.0115 0.230 0.222
Lgr5 10.4 0.0147 0.0337 0.244 0.236

FIG. 17

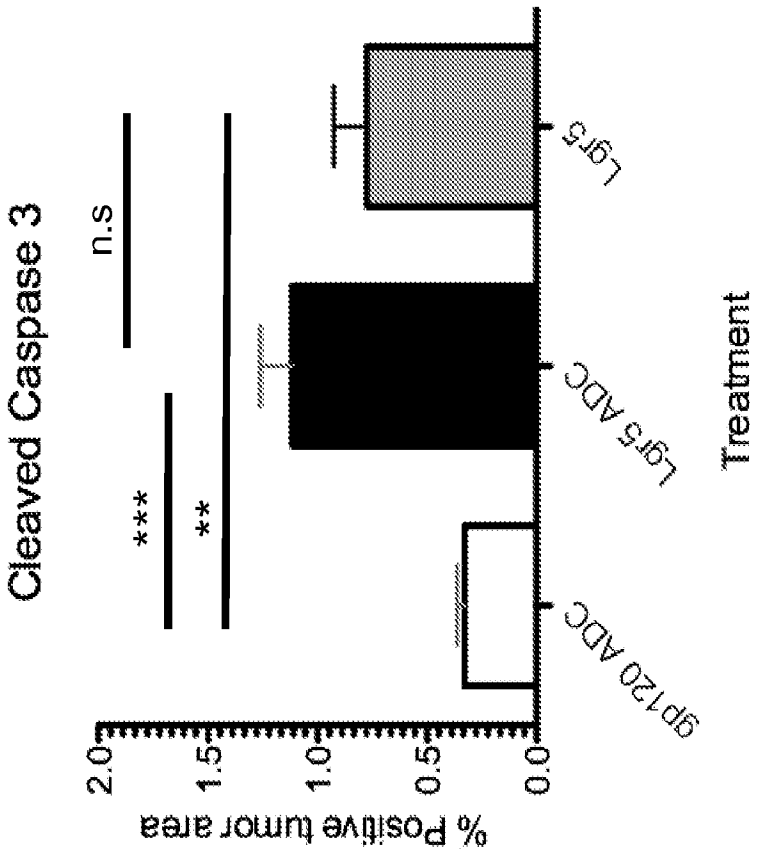
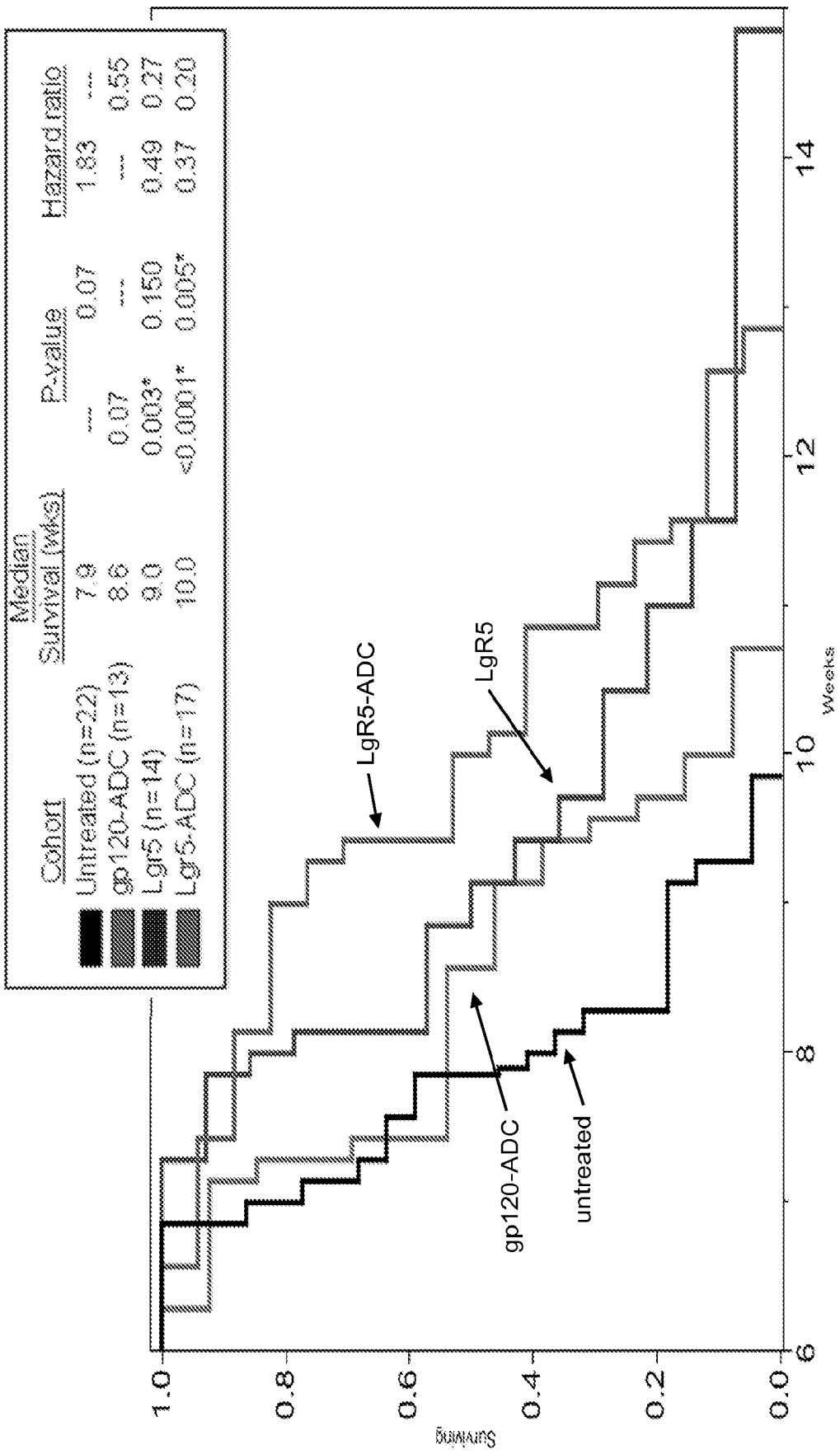

Cleaved Caspase 3
n.s
***
**
% Positive tumor area
2.0
1.5
1.0
0.5
0.0
gp120 ADC
Lgr5 ADC
Lgr5
Treatment

FIG. 18


Cohort
Untreated (n=22)
gp120-ADC (n=13)
Lgr5 (n=14)
Lgr5-ADC (n=17)
Median Survival (wks)
7.9
8.6
9.0
10.0
P-value

0.07
0.003*
<0.0001*
0.07

0.150
0.005*
Hazard ratio
1.83

0.49
0.37

0.55
0.27
0.20
LgR5-ADC
LgR5
gp120-ADC
untreated
Surviving
Weeks
1.0
0.8
0.6
0.4
0.2
0.0
6
8
10
12
14

FIG. 19

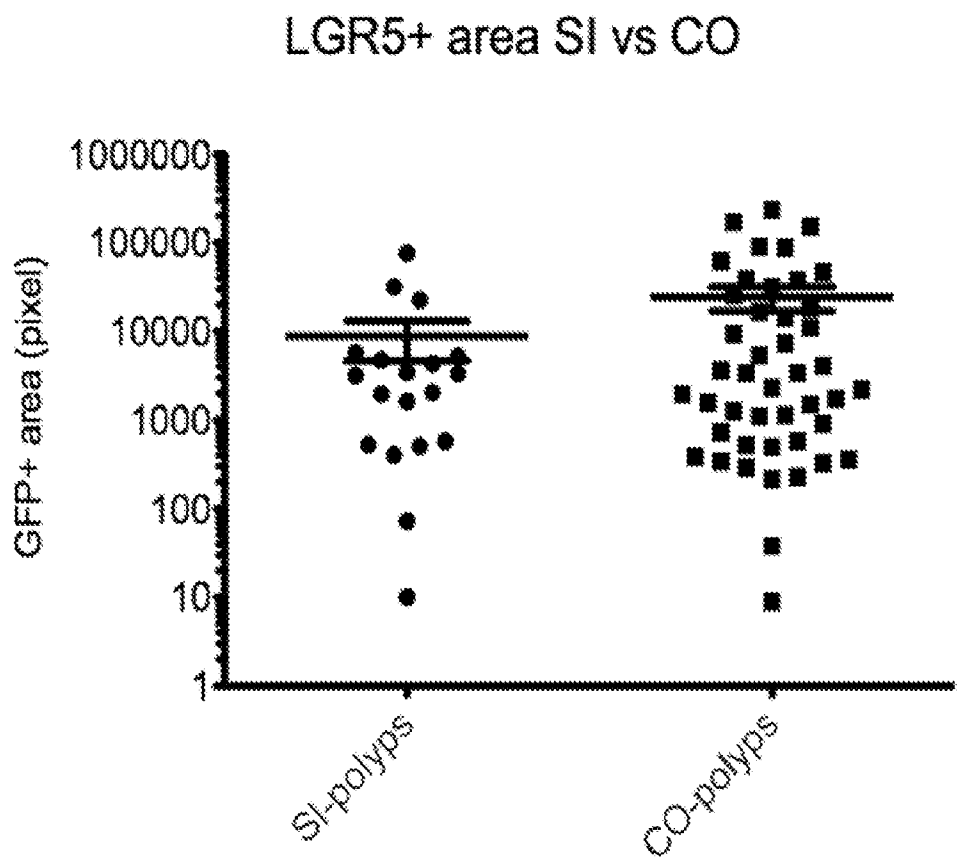

LGR5+ area SI vs CO
GFP+ area (pixel)
1000000
100000
10000
1000
100
10
1
SI-polyps
CO-polyps

FIG. 20

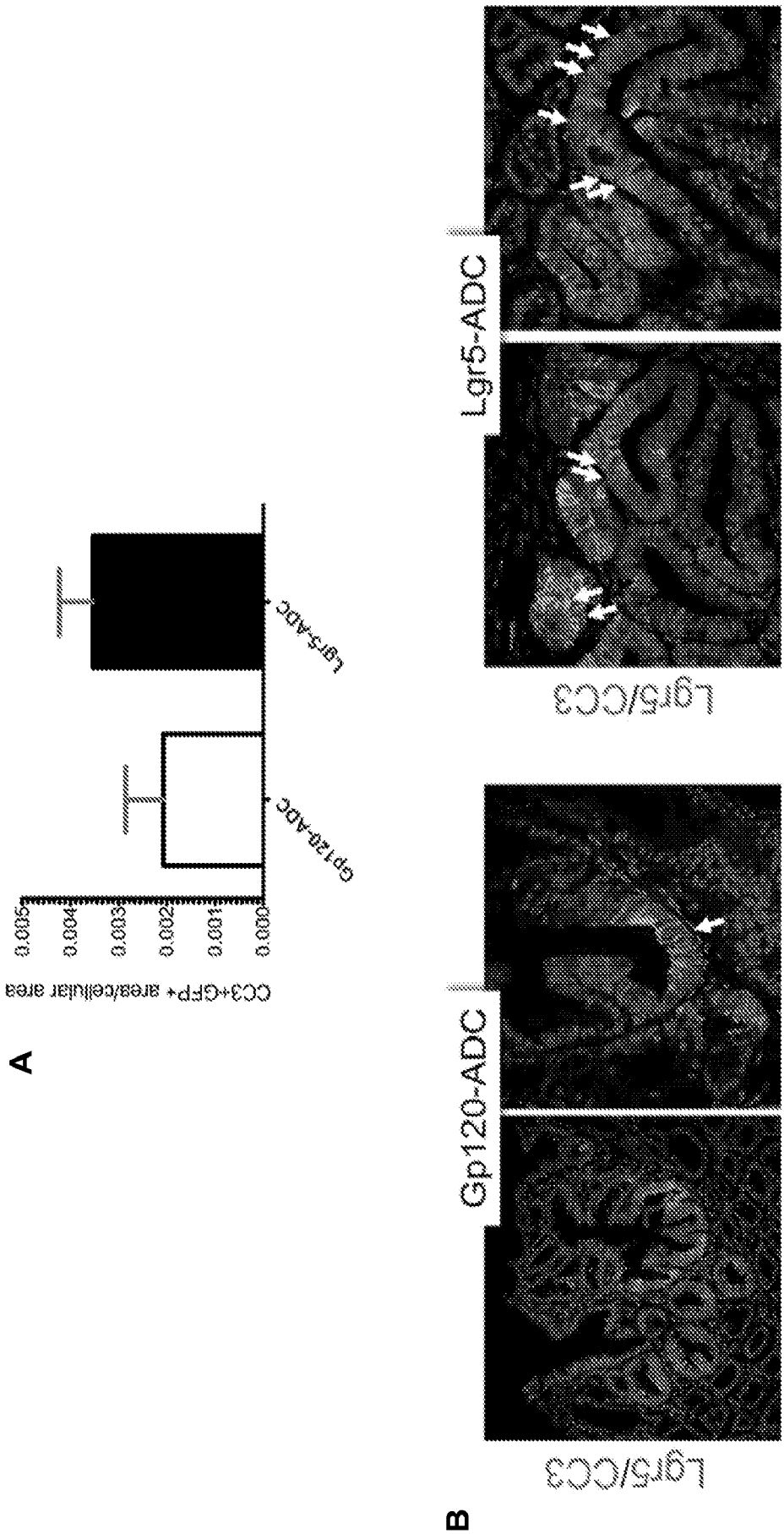

A
CC3+GFP+ area/cellular area
0.005
0.004
0.003
0.002
0.001
0.000
Gp120-ADC
Lgr5-ADC
B
Gp120-ADC
Lgr5/CC3
Lgr5-ADC
Lgr5/CC3

FIG. 21

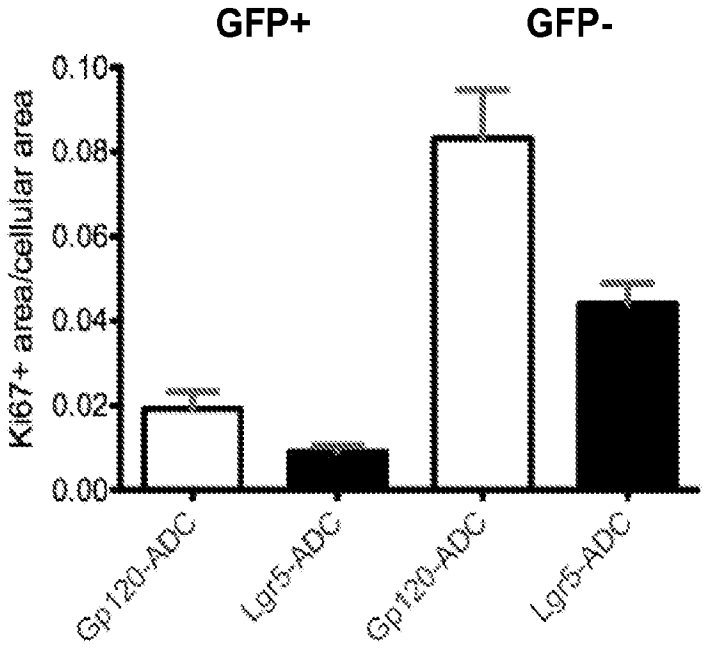

GFP+
GFP-
Ki67+ area/cellular area
0.10
0.08
0.06
0.04
0.02
0.00
Gp120-ADC
Lgr5-ADC
Gp120-ADC
Lgr5-ADC

FIG. 22

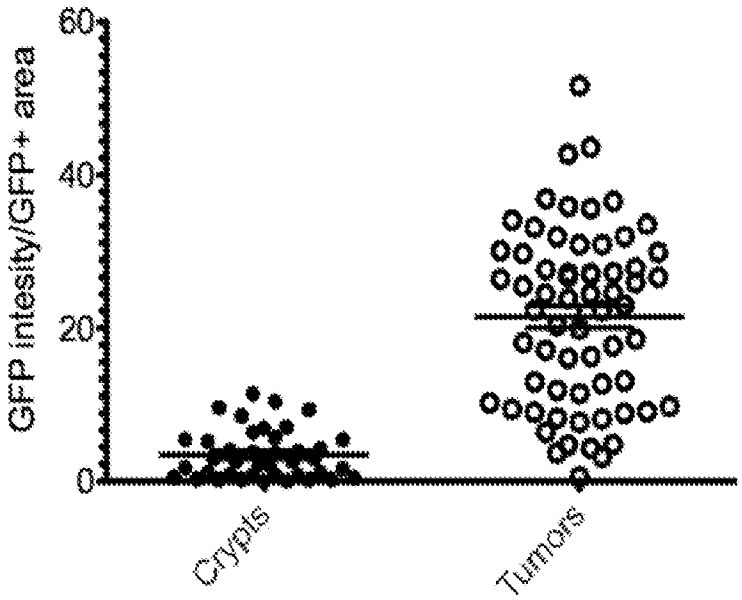

GFP intesity/GFP+ area
60
40
20
0
Crypts
Tumors

FIG. 23

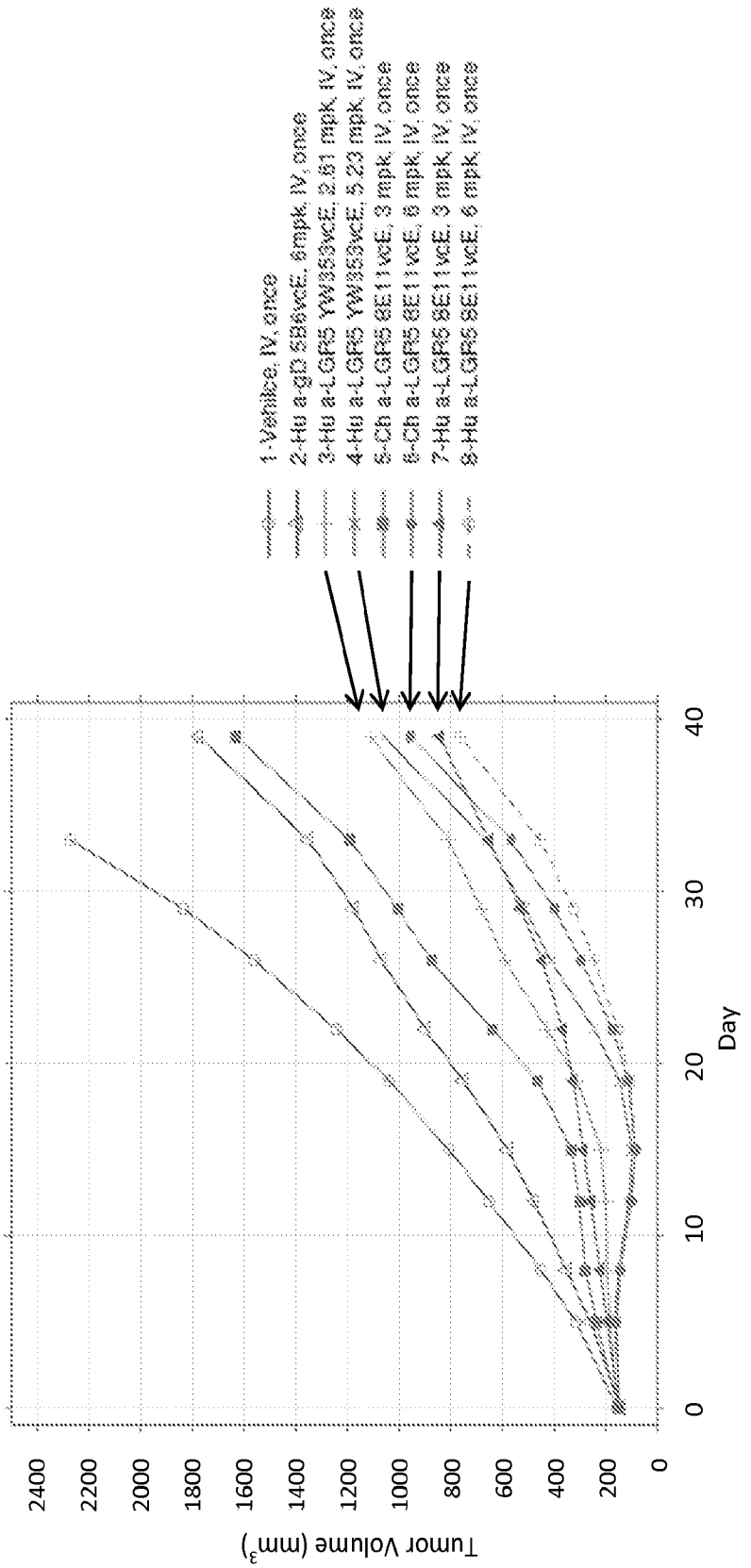

1-Vehicle, IV, once
2-Hu a-gD 5B6vcE, 6mpk, IV, once
3-Hu a-LGR5 YW353vcE, 2.61 mpk, IV, once
4-Hu a-LGR5 YW353vcE, 5.23 mpk, IV, once
5-Ch a-LGR5 8E11vcE, 3 mpk, IV, once
6-Ch a-LGR5 8E11vcE, 6 mpk, IV, once
7-Hu a-LGR5 8E11vcE, 3 mpk, IV, once
8-Hu a-LGR5 8E11vcE, 6 mpk, IV, once
Tumor Volume (mm3)
0
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
0
10
20
30
40
Day

FIG. 24

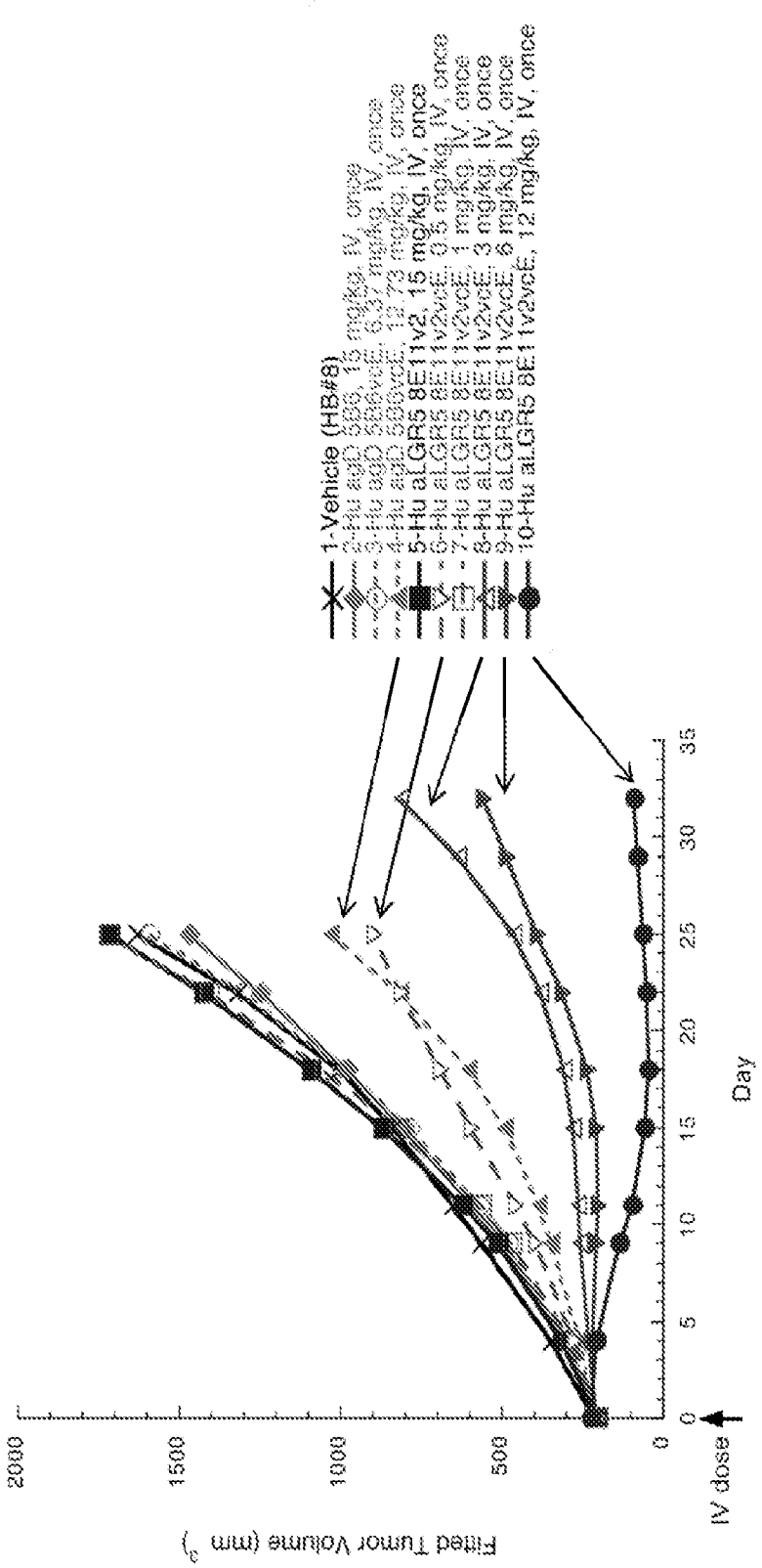

1-Vehicle (HB#8)
2-Hu aIgG 888, 15 mg/kg, IV, once
3-Hu aIgG 888vcE, 6.37 mg/kg, IV, once
4-Hu aIgG 888vcE, 12.73 mg/kg, IV, once
5-Hu aLGR5 8E11v2, 15 mg/kg, IV, once
6-Hu aLGR5 8E11v2vcE, 0.5 mg/kg, IV, once
7-Hu aLGR5 8E11v2vcE, 1 mg/kg, IV, once
8-Hu aLGR5 8E11v2vcE, 3 mg/kg, IV, once
9-Hu aLGR5 8E11v2vcE, 6 mg/kg, IV, once
10-Hu aLGR5 8E11v2vcE, 12 mg/kg, IV, once
Fitted Tumor Volume (mm 3)
2000
1500
1000
500
0
0
5
10
15
20
25
30
35
Day
IV dose

FIG. 25

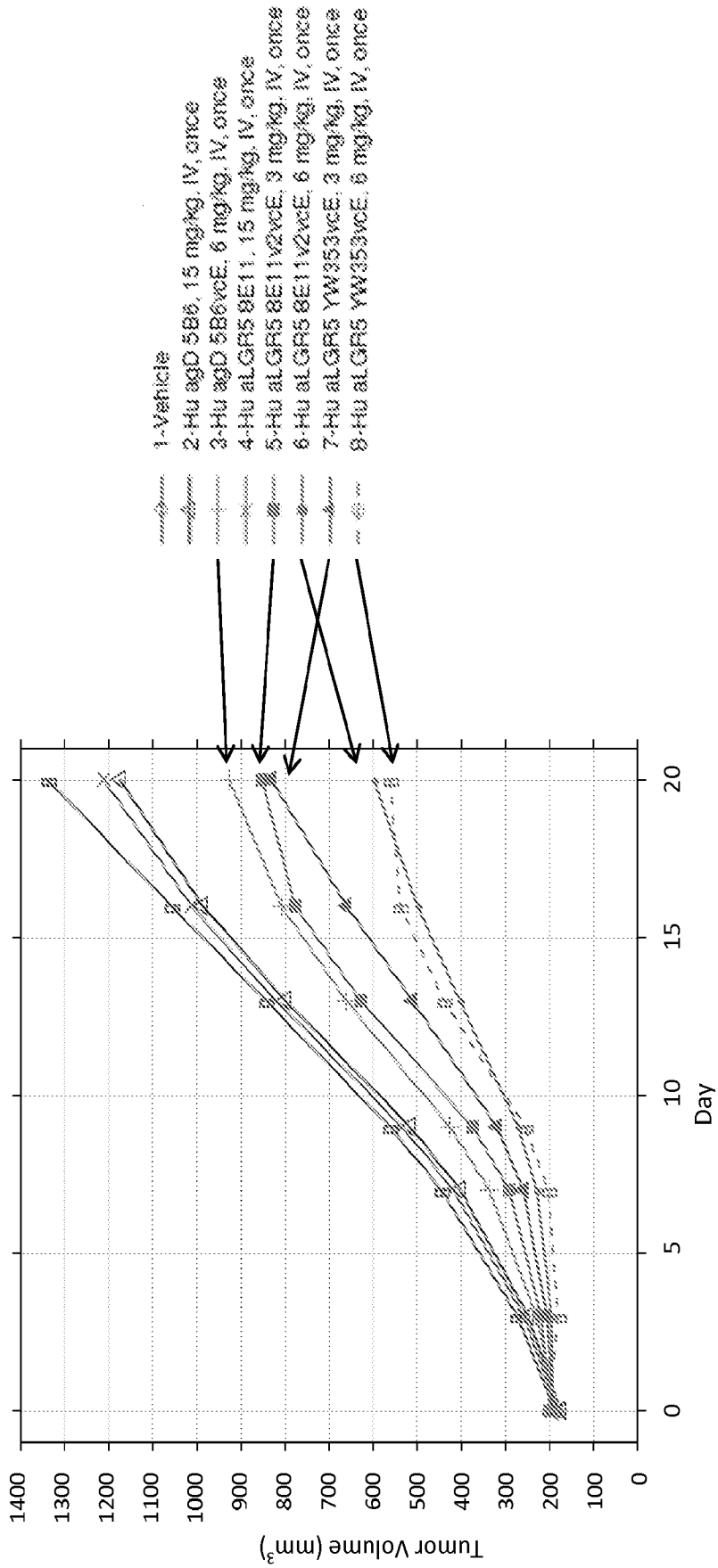

1-Vehicle
2-Hu agD 5B6, 15 mg/kg, IV, once
3-Hu agD 5B6xcE, 6 mg/kg, IV, once
4-Hu aLGR5 8E11, 15 mg/kg, IV, once
5-Hu aLGR5 8E11v2xcE, 3 mg/kg, IV, once
6-Hu aLGR5 8E11v2xcE, 6 mg/kg, IV, once
7-Hu aLGR5 YW353xcE, 3 mg/kg, IV, once
8-Hu aLGR5 YW353xcE, 6 mg/kg, IV, once
Tumor Volume (mm3)
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
Day
0
5
10
15
20

FIG. 26

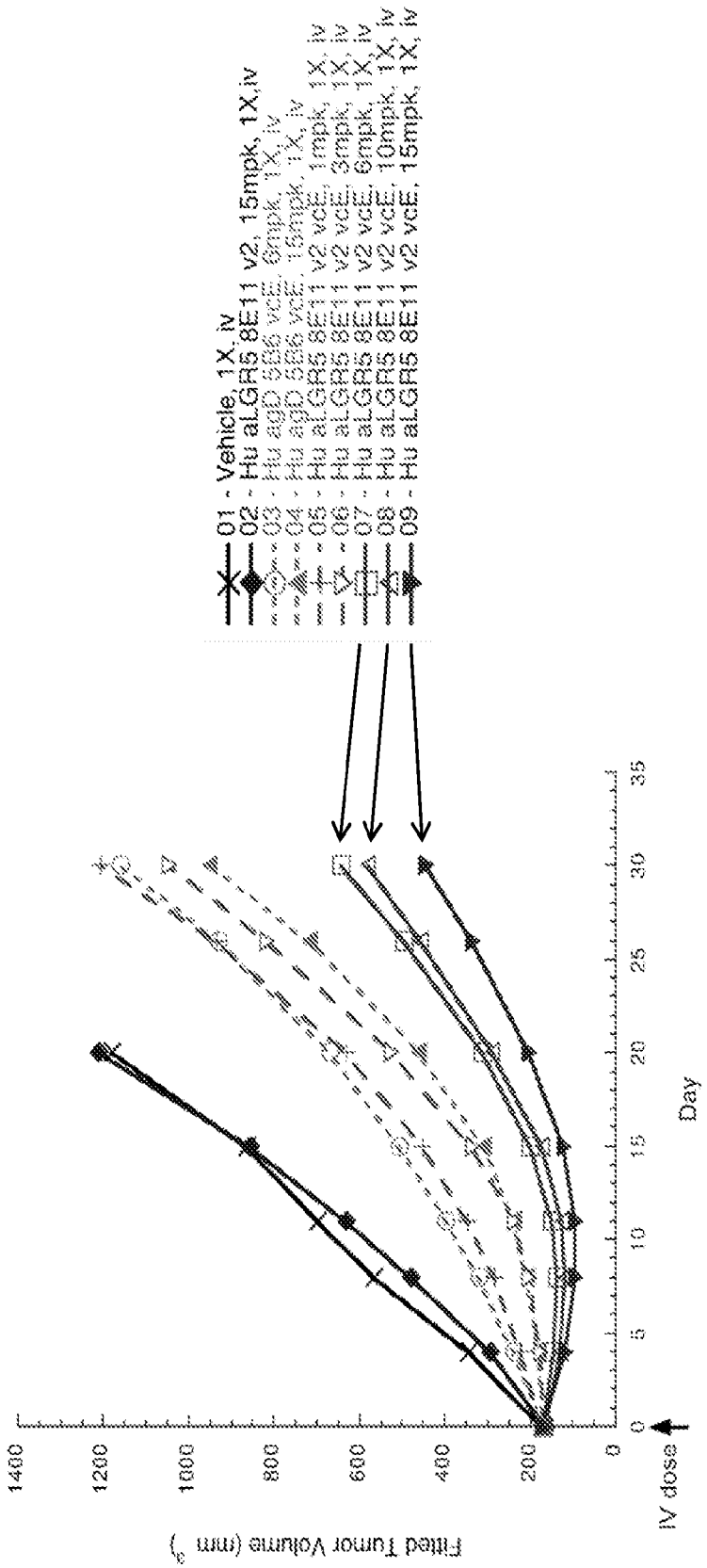

01 - Vehicle, 1X, iv
02 - Hu aLGR5 8E11 v2, 15mpk, 1X,iv
03 - Hu aglD 586 vcE, 6mpk, 1X, iv
04 - Hu aglD 586 vcE, 15mpk, 1X, iv
05 - Hu aLGR5 8E11 v2 vcE, 1mpk, 1X, iv
06 - Hu aLGR5 8E11 v2 vcE, 3mpk, 1X, iv
07 - Hu aLGR5 8E11 v2 vcE, 6mpk, 1X, iv
08 - Hu aLGR5 8E11 v2 vcE, 10mpk, 1X, iv
09 - Hu aLGR5 8E11 v2 vcE, 15mpk, 1X, iv
Tumor Volume (mm3)
Day
IV dose
0
200
400
600
800
1000
1200
1400
0
5
10
15
20
25
30
35

FIG. 27

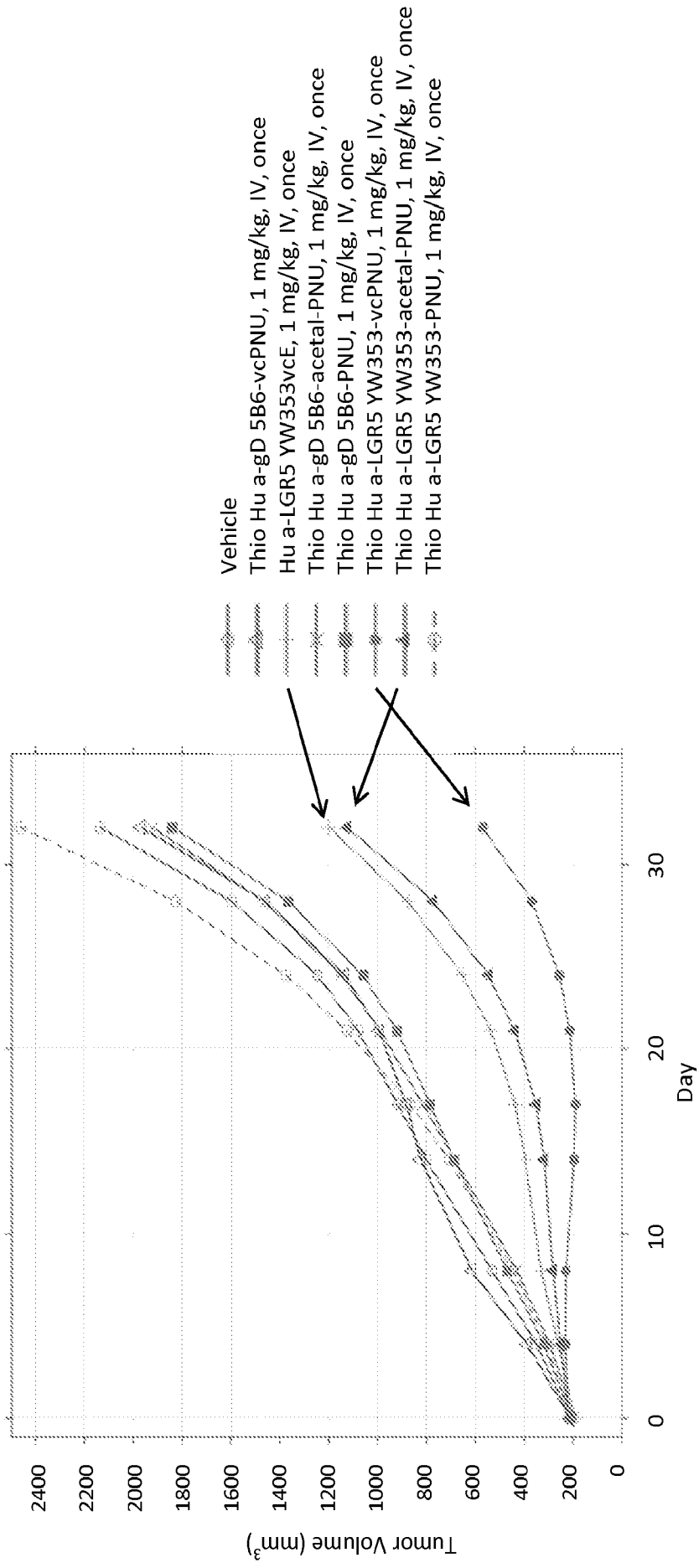

Vehicle
Thio Hu a-gD 5B6-vcPNU, 1 mg/kg, IV, once
Hu a-LGR5 YW353vcE, 1 mg/kg, IV, once
Thio Hu a-gD 5B6-acetal-PNU, 1 mg/kg, IV, once
Thio Hu a-gD 5B6-PNU, 1 mg/kg, IV, once
Thio Hu a-LGR5 YW353-vcPNU, 1 mg/kg, IV, once
Thio Hu a-LGR5 YW353-acetal-PNU, 1 mg/kg, IV, once
Thio Hu a-LGR5 YW353-PNU, 1 mg/kg, IV, once
Tumor Volume (mm3)
0
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
Day
0
10
20
30

FIG. 28

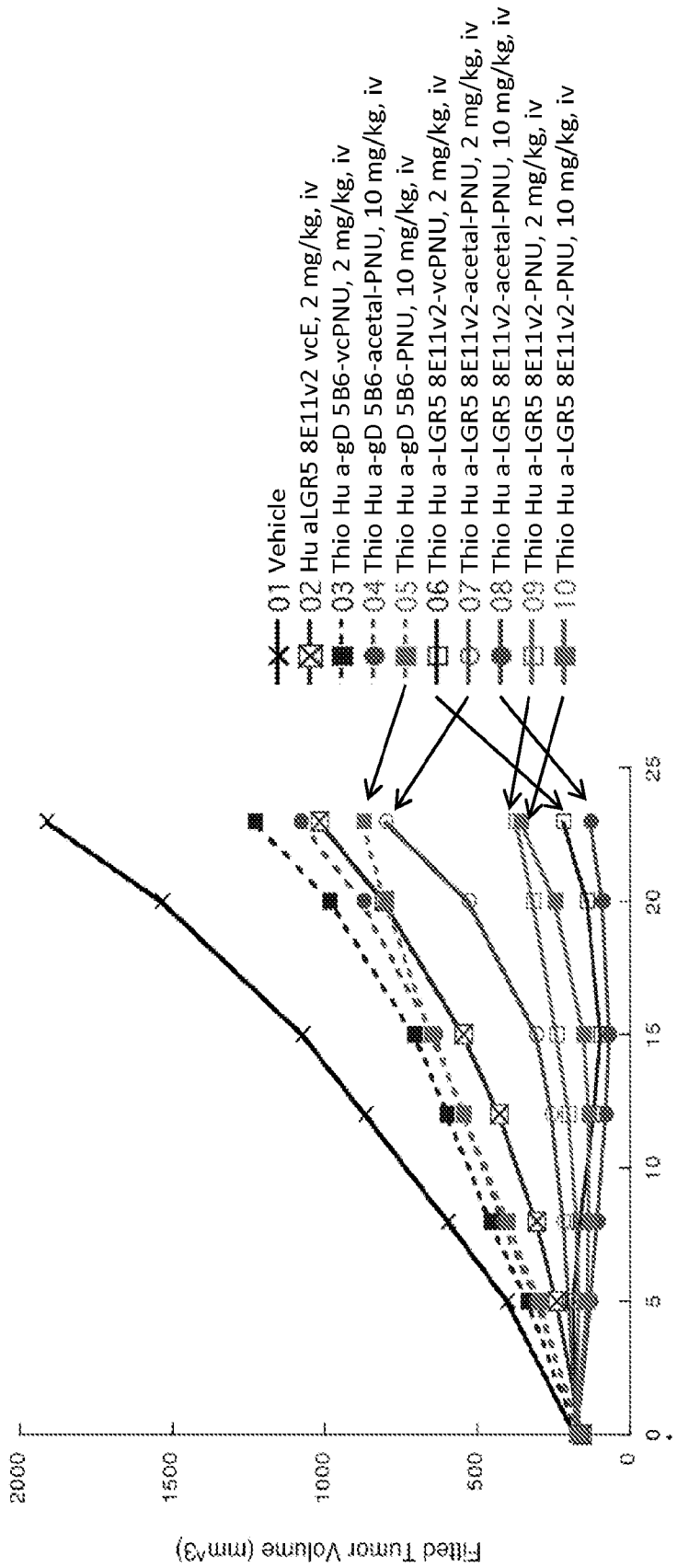

01 Vehicle
02 Hu aLGR5 8E11v2 vcE, 2 mg/kg, iv
03 Thio Hu a-gD 5B6-vcPNU, 2 mg/kg, iv
04 Thio Hu a-gD 5B6-acetal-PNU, 10 mg/kg, iv
05 Thio Hu a-gD 5B6-PNU, 10 mg/kg, iv
06 Thio Hu a-LGR5 8E11v2-vcPNU, 2 mg/kg, iv
07 Thio Hu a-LGR5 8E11v2-acetal-PNU, 2 mg/kg, iv
08 Thio Hu a-LGR5 8E11v2-acetal-PNU, 10 mg/kg, iv
09 Thio Hu a-LGR5 8E11v2-PNU, 2 mg/kg, iv
10 Thio Hu a-LGR5 8E11v2-PNU, 10 mg/kg, iv
Filled Tumor Volume (mm^3)
2000
1500
1000
500
0
0
5
10
15
20
25

FIG. 29

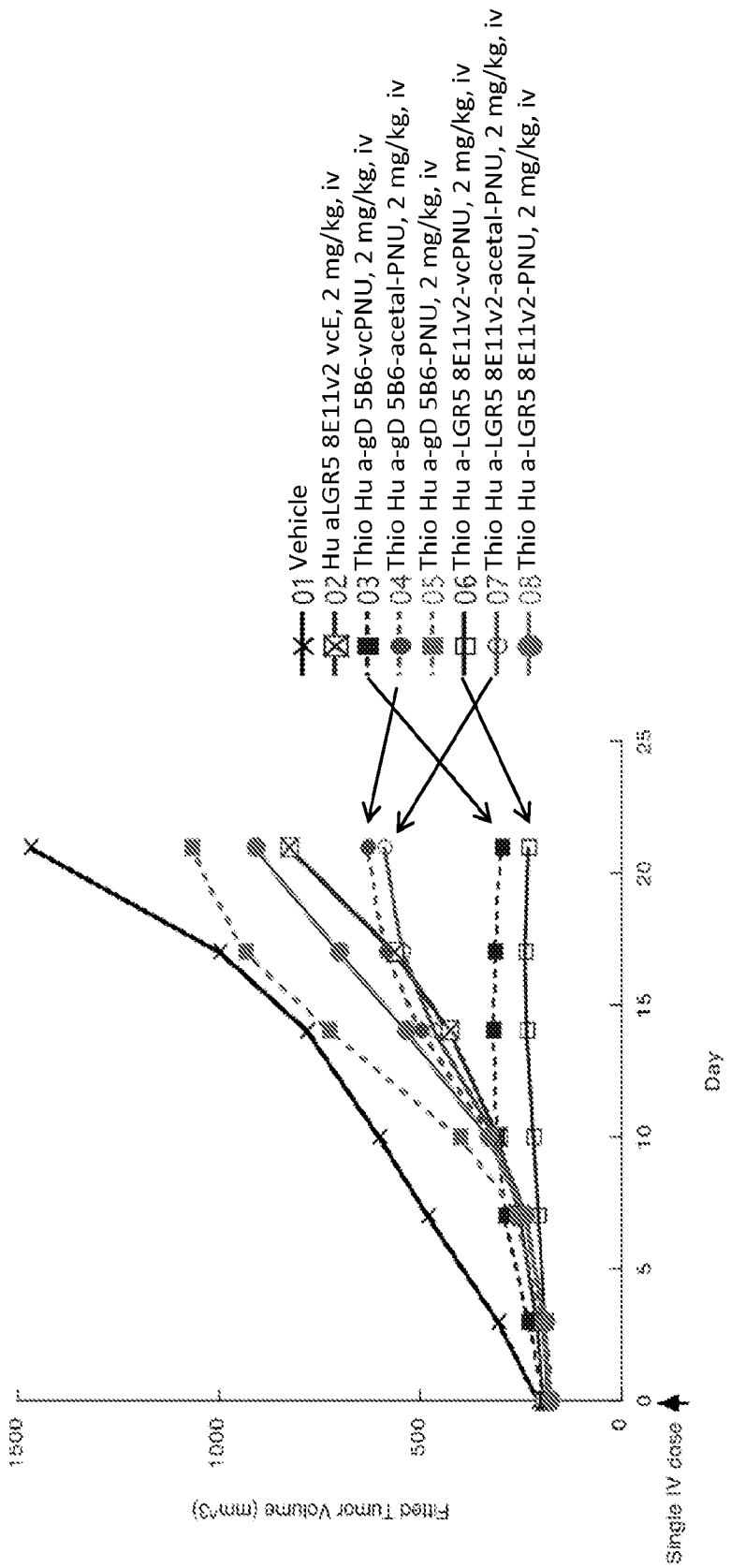

01 Vehicle
02 Hu aLGR5 8E11v2 vcE, 2 mg/kg, iv
03 Thio Hu a-gD 5B6-vcPNU, 2 mg/kg, iv
04 Thio Hu a-gD 5B6-acetal-PNU, 2 mg/kg, iv
05 Thio Hu a-gD 5B6-PNU, 2 mg/kg, iv
06 Thio Hu a-LGR5 8E11v2-vcPNU, 2 mg/kg, iv
07 Thio Hu a-LGR5 8E11v2-acetal-PNU, 2 mg/kg, iv
08 Thio Hu a-LGR5 8E11v2-PNU, 2 mg/kg, iv
Filled Tumor Volume (mm^3)
1500
1000
500
0
0
5
10
15
20
25
Day
Single IV dose

FIG. 30

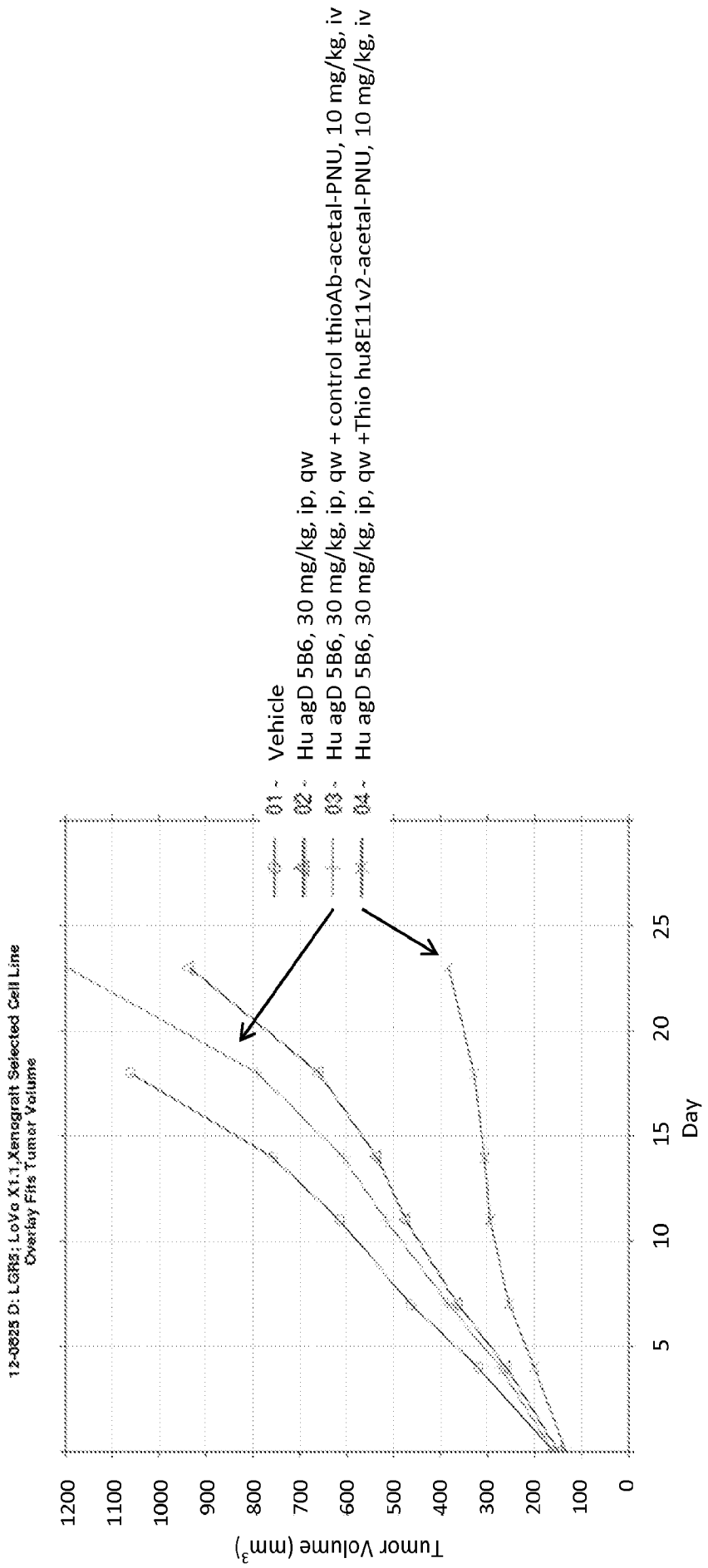

12-0825 D: LGR5; LoVo X1.1 Xenograft Selected Cell Line
Overlay Fits Tumor Volume
01 - Vehicle
02 - Hu agD 5B6, 30 mg/kg, ip, qw
03 - Hu agD 5B6, 30 mg/kg, ip, qw + control thioAb-acetal-PNU, 10 mg/kg, iv
04 - Hu agD 5B6, 30 mg/kg, ip, qw +Thio hu8E11v2-acetal-PNU, 10 mg/kg, iv
Tumor Volume (mm3)
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
5
10
15
20
25
Day

FIG. 31

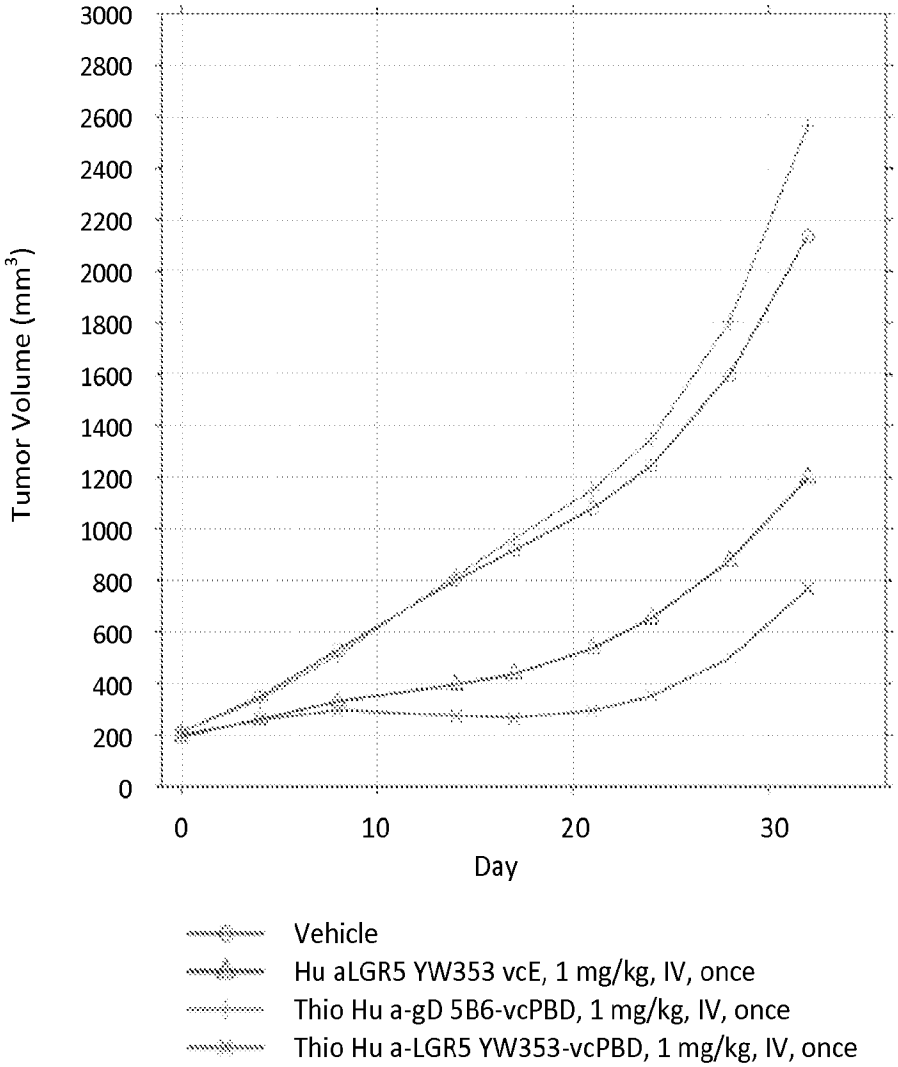

3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Tumor Volume (mm3)
0
10
20
30
Day
Vehicle
Hu aLGR5 YW353 vcE, 1 mg/kg, IV, once
Thio Hu a-gD 5B6-vcPBD, 1 mg/kg, IV, once
Thio Hu a-LGR5 YW353-vcPBD, 1 mg/kg, IV, once

FIG. 32

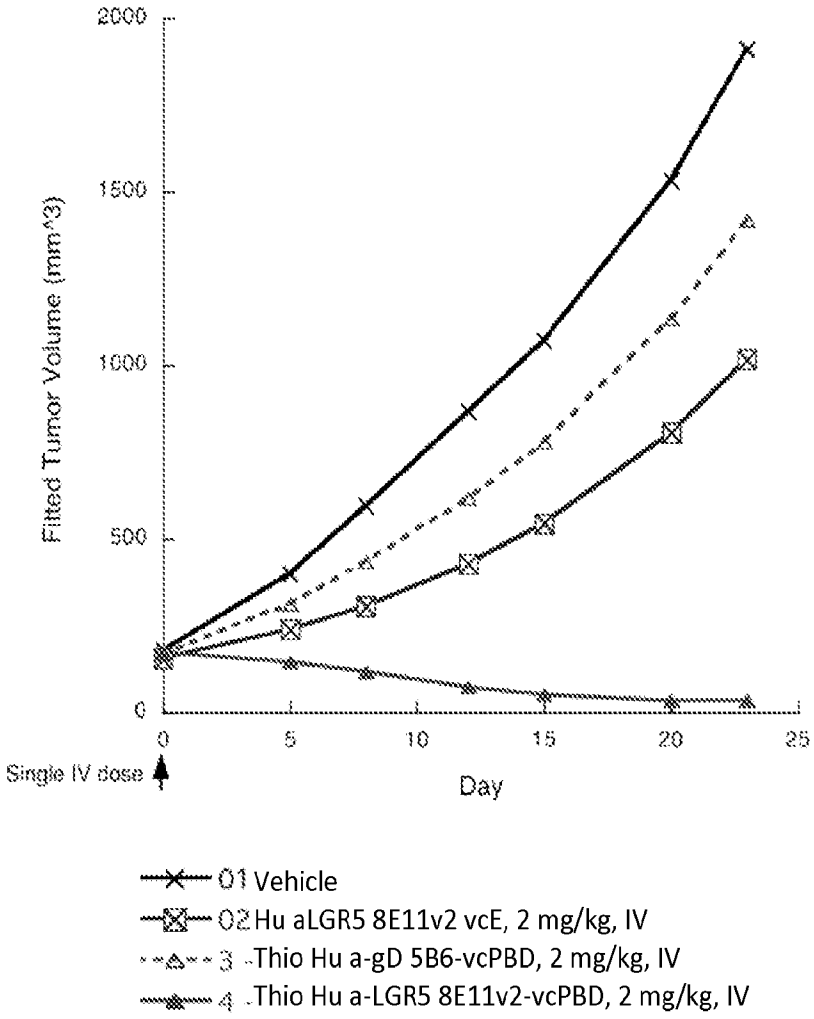

2000
1500
1000
500
0
Fitted Tumor Volume (mm^3)
0
5
10
15
20
25
Day
Single IV dose
01 Vehicle
02 Hu aLGR5 8E11v2 vcE, 2 mg/kg, IV
3 Thio Hu a-gD 5B6-vcPBD, 2 mg/kg, IV
4 Thio Hu a-LGR5 8E11v2-vcPBD, 2 mg/kg, IV

FIG. 33

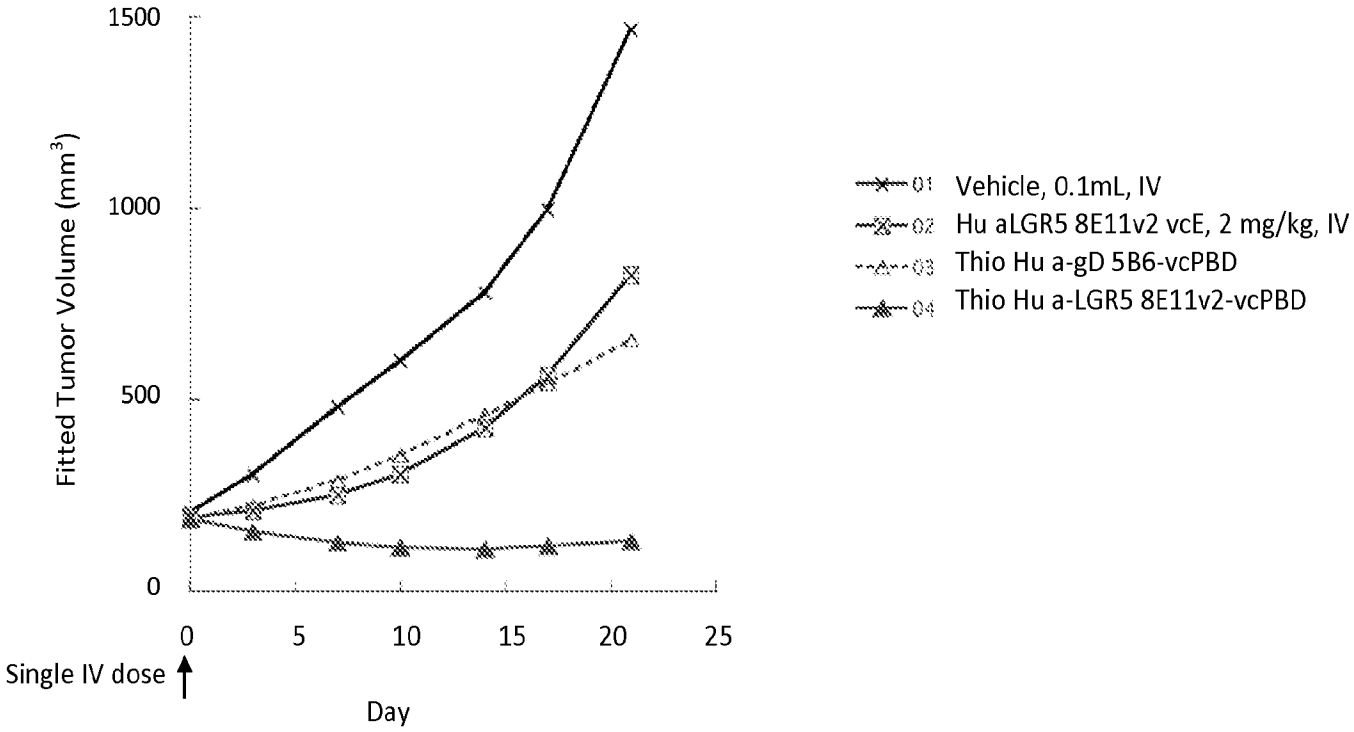
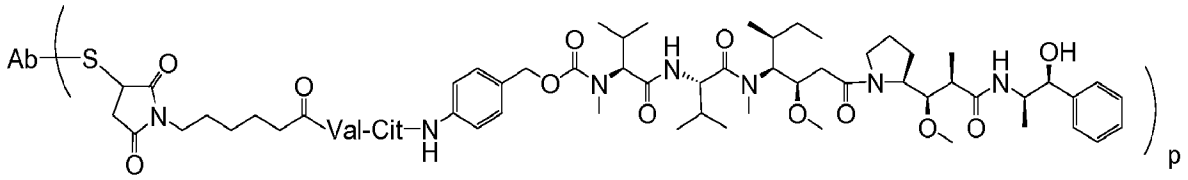
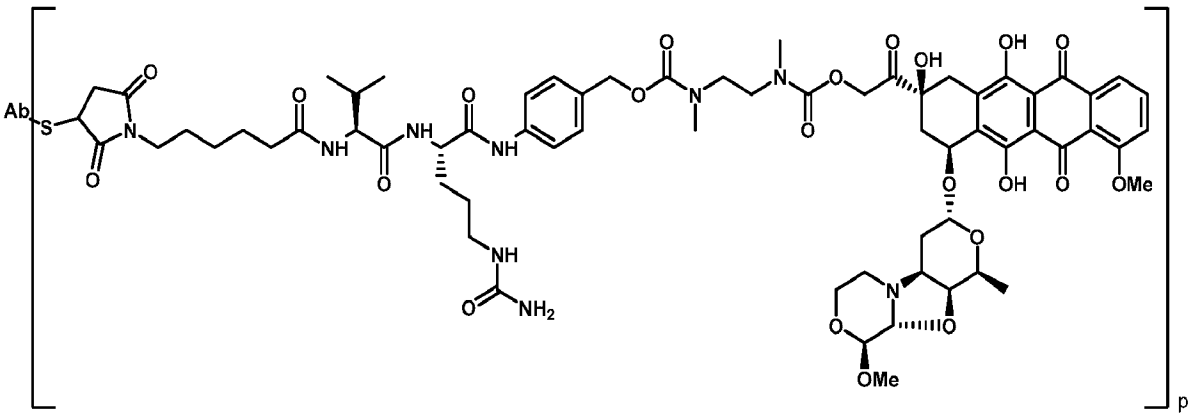

1500
1000
500
0
Fitted Tumor Volume (mm3)
0
5
10
15
20
25
Day
Single IV dose
01 Vehicle, 0.1mL, IV
02 Hu aLGR5 8E11v2 vcE, 2 mg/kg, IV
03 Thio Hu a-gD 5B6-vcPBD
04 Thio Hu a-LGR5 8E11v2-vcPBD

FIG. 34

A


Ab
S
O
N
O
O
Val-Cit
N
H
O
N
H
N
O
N
O
O
N
O
O
H
N
OH
p

B


Ab
S
O
N
O
O
NH
NH
NH
O
O
O
N
N
O
O
OH
OH
O
OH
O
OMe
NH
O
NH2
N
O
O
O
OMe
p

C

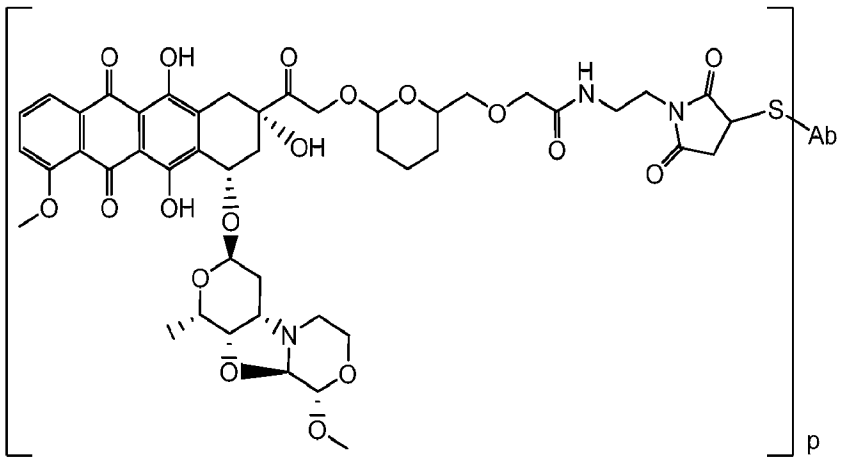
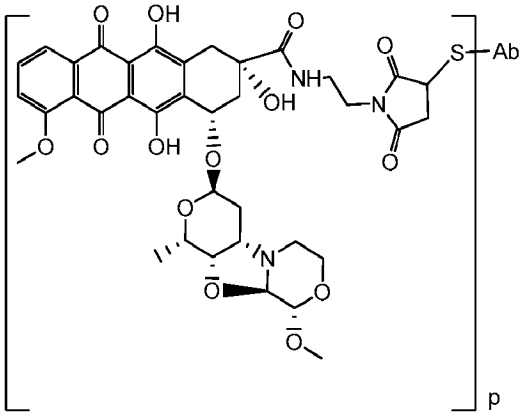

O
OH
O
O
O
O
H
N
O
N
S
Ab
O
O
OH
OH
O
O
O
N
O
O
O
p

FIG. 35

D



E

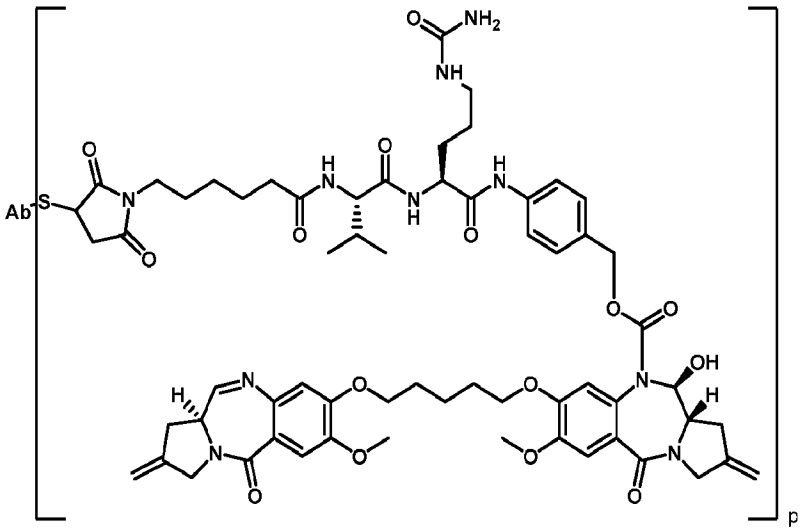


FIG. 35 (cont.)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/034629

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61K47/48 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)
C07K A61K

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 August 2013	12/08/2013

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

2

Form PCT/ISA/210 (second sheet) (April 2005)

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Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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International application No
PCT/US2013/034629

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Information on patent family members

International application No
PCT/US2013/034629

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