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~~ disclosure provides anti-LgR5 antibodies and immunoconjugates and methods of using the same.

[005a] In one aspect there is provided 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 $\leq$ 5 nM.

[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 $\leq$ 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

[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.

[012a] In one aspect, there is provided 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.

[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

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.

[020a] In one aspect, there is provided an isolated nucleic acid encoding an antibody as described herein.

[020b] In one aspect, there is provided a host cell comprising a nucleic acid as described herein.

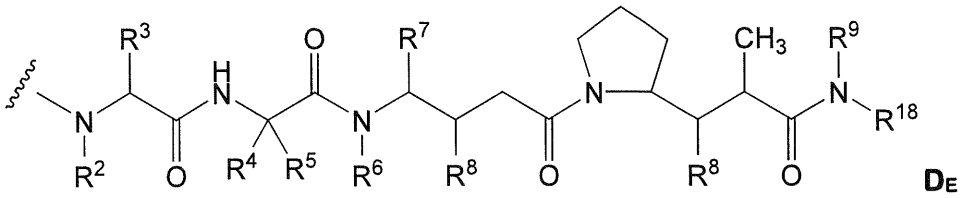
[020c] In one aspect, there is provided a method of producing an antibody comprising culturing a host cell as described herein so that the antibody is produced.

[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.

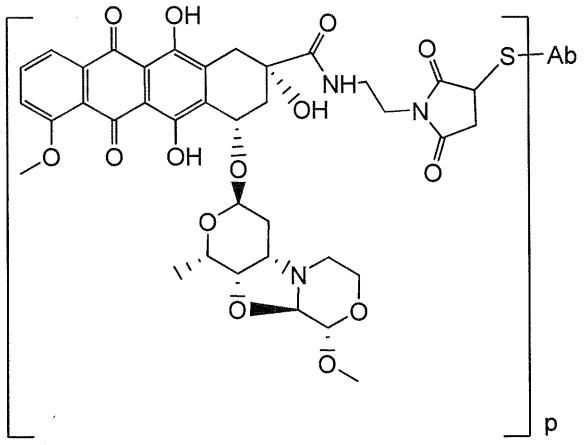
[022a] In one aspect, there is provided an immunoconjugate comprising an antibody as described herein and a cytotoxic agent.

[022b] 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:



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.

[027a] In one aspect, there is provided a pharmaceutical formulation comprising an immunoconjugate as described herein and a pharmaceutically acceptable carrier.

[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).

[028a] In one aspect, there is provided the use of an effective amount of an immunoconjugate as described herein in the manufacture of a medicament for treating an individual having an LgR5-positive cancer.

[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).

[029a] In one aspect, there is provided the use of the immunoconjugate as described herein in the manufacture of a medicament for inhibiting proliferation of an LgR5 positive cell, wherein the cell is to be exposed to the immunoconjugate under conditions permissive for binding of the immunoconjugate to LgR5 on the surface of the cell, thereby inhibiting proliferation of the cell.

[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.

[031a] In one aspect, there is provided a method of detecting human LgR5 in a biological sample comprising contacting the biological sample with an anti-LgR5 antibody as described herein 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.

[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.

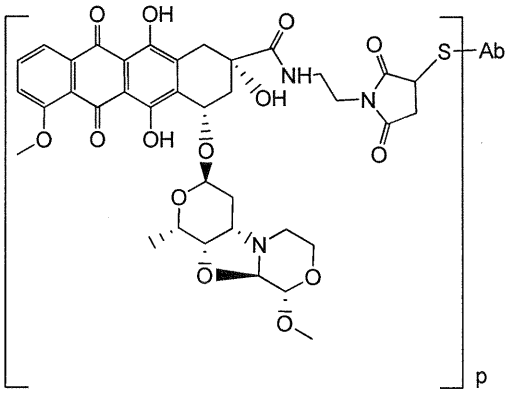
[032a] In one aspect, there is provided a method for detecting an LgR5-positive cancer comprising detecting labeled anti-LgR5 antibody in a subject having or suspected of having a LgR5-positive cancer and that has been administered the labelled anti-LgR5 antibody, wherein the labeled anti-LgR5 antibody comprises the anti-LgR5 antibody as described herein, wherein detection of the labeled anti-LgR5 antibody indicates a LgR5-positive cancer in the subject.

[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.



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~~ Use of an effective amount of the immunoconjugate of any one of claims 21 to 40 in the manufacture of a medicament for treating an individual having an LgR5-positive cancer.

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

46. The ~~method~~ use of claim 45, ~~further comprising administering~~wherein an additional therapeutic agent is to be administered to the individual.

47. The ~~method~~ use 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 use of the immunoconjugate of any one of claims 21 to 40 in the manufacture of a medicament for inhibiting proliferation of an LgR5 positive cell, wherein the cell is to be exposed to the immunoconjugate 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~~use 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 a~~the~~ subject having or suspected of having a LgR5-positive cancer and that has been administered the labelled anti-LgR5 antibody, wherein the labeled anti-LgR5 antibody comprises the anti-LgR5 antibody of any one of claims 1 to 16, 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.