

ANTI-CDH17 ANTIBODIES AND CONJUGATES THEREOF

BACKGROUND

[0001] Cadherin 17 (CDH17) is a transmembrane protein highly expressed in cancers of digestive system. In physiological conditions, expression of CDH17 in human and mice is mainly restricted to the epithelial cells in small intestine and colon, but not vital organs such as liver, stomach, heart, lung and brain. In pathophysiological conditions, the expression of CDH17 has been extensively explored in various cancers from digestive system., such as gastric cancer (GC), colorectal cancer (CRC), hepatocarcinoma (HCC), pancreatic cancer (PC), and neuroendocrine cancer. Knockdown of CDH17 suppresses tumor development and metastasis in GC, HCC, CRC and PC.

[0002] Antibody-drug conjugates (ADC) are one of the fastest growing anticancer drugs. This approach comprises a mAb conjugated to the cytotoxic payload via a chemical linker that directed toward a target antigen expressed on the cancer cell surface, reducing systemic exposure and therefore toxicity. ADCs are complex molecules that require careful attention to various components. Selection of an appropriate target, an mAb, cytotoxic payload, and the manner in which the antibody is linked to the payload are key determinants of the safety and efficacy of ADCs.

[0003] There is a need to develop more efficacious and safer clinical candidates targeting this protein and new design such as an ADC with potent cytotoxicity to the tumor cells.

SUMMARY

[0004] Anti-CDH17 antibodies and ADCs thereof are discovered herein that have potent specific binding to the human CDH17 protein. When compared to the benchmark antibodies, BMK-BI-905711 and BMK-ARB202, the instantly discovered antibodies and ADCs exhibited comparable binding affinity and specificity. The ADCs of the anti-CDH17 antibodies also exhibited potent *in vitro* cytotoxic effect on CDH17-expressing tumor cells. In particular, they had potent bystander effects on adjacent CDH17-negative cells and prominent *in vivo* anti-tumor effects.

[0005] These antibodies, therefore, can be suitably used for treating diseases such as cancer.

[0006] An antibody or antigen-binding fragment thereof that has binding specificity to a human Cadherin-17 (CDH17) protein, wherein the antibody or the fragment thereof comprises a heavy chain variable region (VH) comprising heavy chain complementarity determining regions (CDR) VH CDR1, VH CDR2, and VH CDR3, and a light chain variable region (VL) comprising VL CDR1, VL CDR2, and VL CDR3, and wherein the VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3, respectively, comprise (a) the amino acid sequences of SEQ ID NO: 11-16; (b) the amino acid sequences of SEQ ID NO: 17-22; (c) the amino acid sequences of SEQ ID NO: 23-28; (d) the amino acid sequences of SEQ ID NO: 29-34; (e) the amino acid sequences of SEQ ID NO: 35-40; or (f) the amino acid sequences of SEQ ID NO: 41-46; or (g) the amino acid sequences of SEQ ID NO: 1-6.

[0007] In some embodiments, the VH CDR1 comprises the amino acid sequence of SEQ ID NO:1, the VH CDR2 comprises the amino acid sequence of SEQ ID NO:2, the VH CDR3 comprises the amino acid sequence of SEQ ID NO:3, the VL CDR1 comprises the amino acid sequence of SEQ ID NO:4, the VL CDR2 comprises the amino acid sequence of SEQ ID NO:5, and the VL CDR3 comprises the amino acid sequence of SEQ ID NO:6.

[0008] In some embodiments, the antibody or antigen-binding fragment thereof is a chimeric antibody or a humanized antibody. In some embodiments, the VH comprises the amino acid sequence of SEQ ID NO: 7, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:7. In some embodiments, the VL comprises the amino acid sequence of SEQ ID NO: 8 or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:8.

[0009] In some embodiments, the VH CDR1 comprises the amino acid sequence of SEQ ID NO:11, the VH CDR2 comprises the amino acid sequence of SEQ ID NO:12, the VH CDR3 comprises the amino acid sequence of SEQ ID NO:13, the VL CDR1 comprises the amino acid sequence of SEQ ID NO:14, the VL CDR2 comprises the amino acid sequence of SEQ ID NO:15, and the VL CDR3 comprises the amino acid sequence of SEQ ID NO:16.

[0010] In some embodiments, the antibody or antigen-binding fragment thereof is a chimeric antibody or a humanized antibody. In some embodiments, the VH comprises the amino acid sequence of SEQ ID NO: 47, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:47. In some embodiments, the VL comprises the amino acid

sequence of SEQ ID NO: 48 or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:48.

[0011] In some embodiments, the VH CDR1 comprises the amino acid sequence of SEQ ID NO:17, the VH CDR2 comprises the amino acid sequence of SEQ ID NO:18, the VH CDR3 comprises the amino acid sequence of SEQ ID NO:19, the VL CDR1 comprises the amino acid sequence of SEQ ID NO:20, the VL CDR2 comprises the amino acid sequence of SEQ ID NO:21, and the VL CDR3 comprises the amino acid sequence of SEQ ID NO:22.

[0012] In some embodiments, the antibody or antigen-binding fragment thereof is a chimeric antibody or a humanized antibody. In some embodiments, the VH comprises the amino acid sequence of SEQ ID NO: 49, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:49. In some embodiments, the VL comprises the amino acid sequence of SEQ ID NO: 50, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:50.

[0013] In some embodiments, the VH CDR1 comprises the amino acid sequence of SEQ ID NO:23, the VH CDR2 comprises the amino acid sequence of SEQ ID NO:24, the VH CDR3 comprises the amino acid sequence of SEQ ID NO:25, the VL CDR1 comprises the amino acid sequence of SEQ ID NO:26, the VL CDR2 comprises the amino acid sequence of SEQ ID NO:27, and the VL CDR3 comprises the amino acid sequence of SEQ ID NO:28.

[0014] In some embodiments, the antibody or antigen-binding fragment thereof is a chimeric antibody or a humanized antibody. In some embodiments, the VH comprises the amino acid sequence of SEQ ID NO: 51, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:51. In some embodiments, the VL comprises the amino acid sequence of SEQ ID NO: 52, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:52.

[0015] In some embodiments, the VH CDR1 comprises the amino acid sequence of SEQ ID NO:29, the VH CDR2 comprises the amino acid sequence of SEQ ID NO:30, the VH CDR3 comprises the amino acid sequence of SEQ ID NO:31, the VL CDR1 comprises the amino acid sequence of SEQ ID NO:32, the VL CDR2 comprises the amino acid sequence of SEQ ID NO:33, and the VL CDR3 comprises the amino acid sequence of SEQ ID NO:34.

[0016] In some embodiments, the antibody or antigen-binding fragment thereof is a chimeric antibody or a humanized antibody. In some embodiments, the VH comprises the amino acid sequence of SEQ ID NO: 53, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:53. In some embodiments, the VL comprises the amino acid sequence of SEQ ID NO: 54, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:54.

[0017] In some embodiments, the VH CDR1 comprises the amino acid sequence of SEQ ID NO:35, the VH CDR2 comprises the amino acid sequence of SEQ ID NO:36, the VH CDR3 comprises the amino acid sequence of SEQ ID NO:37, the VL CDR1 comprises the amino acid sequence of SEQ ID NO:38, the VL CDR2 comprises the amino acid sequence of SEQ ID NO:39, and the VL CDR3 comprises the amino acid sequence of SEQ ID NO:40.

[0018] In some embodiments, the antibody or antigen-binding fragment thereof is a chimeric antibody or a humanized antibody. In some embodiments, the VH comprises the amino acid sequence of SEQ ID NO: 55, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:55. In some embodiments, the VL comprises the amino acid sequence of SEQ ID NO: 56, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:56.

[0019] In some embodiments, the VH CDR1 comprises the amino acid sequence of SEQ ID NO:41, the VH CDR2 comprises the amino acid sequence of SEQ ID NO:42, the VH CDR3 comprises the amino acid sequence of SEQ ID NO:43, the VL CDR1 comprises the amino acid sequence of SEQ ID NO:44, the VL CDR2 comprises the amino acid sequence of SEQ ID NO:45, and the VL CDR3 comprises the amino acid sequence of SEQ ID NO:46.

[0020] In some embodiments, the antibody or antigen-binding fragment thereof is a chimeric antibody or a humanized antibody. In some embodiments, the VH comprises the amino acid sequence of SEQ ID NO: 57, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:57. In some embodiments, the VL comprises the amino acid sequence of SEQ ID NO: 58, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:58.

[0021] Also provided, in one embodiment, is an antibody or antigen-binding fragment thereof having specificity to a human CDH17 protein, wherein the antibody or antigen-

binding fragment thereof competes with the antibody or antigen-binding fragment thereof of any one of the preceding claims in binding to the CDH17 protein.

[0022] In some embodiments, the antibody or antigen-binding fragment thereof further comprises a heavy chain constant region, a light chain constant region, an Fc region, or the combination thereof. In some embodiments, the light chain constant region is a kappa or lambda chain constant region. In some embodiments, the antibody or antigen-binding fragment thereof is of an isotype of IgG, IgM, IgA, IgE or IgD.

[0023] In some embodiments, the antibody or antigen-binding fragment thereof provided herein is humanized.

[0024] In some embodiments, the antibody or antigen-binding fragment thereof provided herein is a F(ab')₂, a F(ab)₂, a Fab', a Fab, a Fv, or a scFv.

[0025] In some embodiments, the antibody or antigen-binding fragment thereof provided herein is of an isotype of IgG1, IgG2, IgG3 or IgG4.

[0026] In some embodiments, the antibody or antigen-binding fragment thereof provided herein is of an isotype of human IgG1.

[0027] In some embodiments, the antibody or antigen-binding fragment thereof provided herein is conjugated.

[0028] Also provided is a bifunctional molecule, comprising a first antigen-binding portion having specificity to a human CDH17 protein and a second portion having specificity to a second antigen, wherein the first antigen-binding portion comprises an antibody or antigen-binding fragment thereof of the present disclosure.

[0029] Another embodiment provides an antibody-drug conjugate comprising a drug moiety conjugated to an antibody or antigen-binding fragment thereof or a bifunctional molecule of the present disclosure.

[0030] In some embodiments, the drug moiety is a cytotoxic or cytostatic agent, an immunosuppressive agent, a radioisotope, or a toxin. In some embodiments, the drug moiety is a maytansinoid, an auristatin, a macrocyclic ketone analogue, topoisomerase inhibitor. In

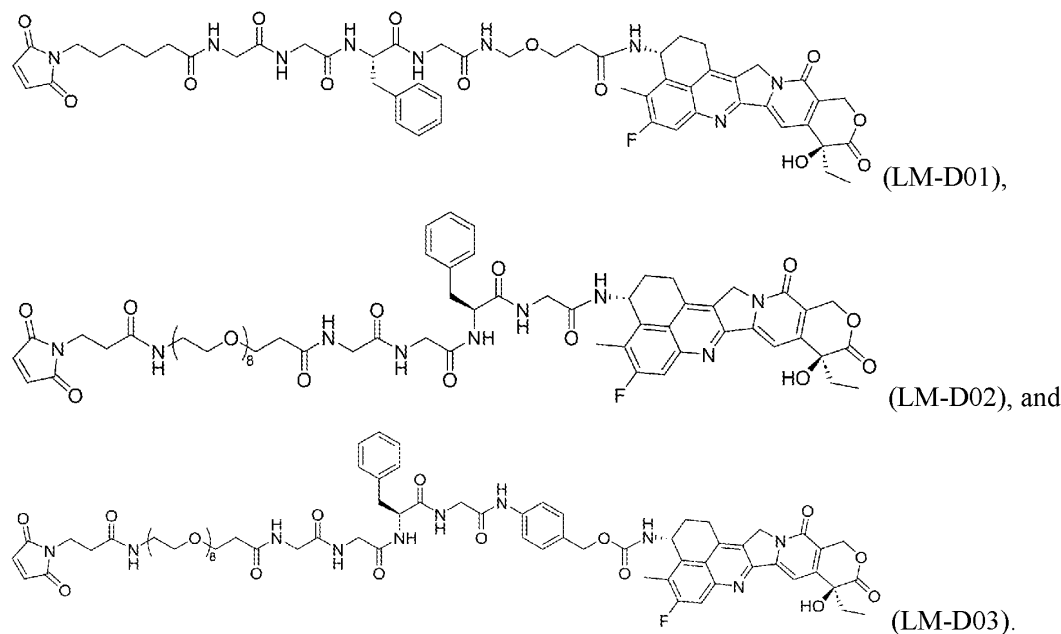
some embodiments, the drug moiety comprises monomethyl auristatin E (MMAE) or monomethyl auristatin F (MMAF).

[0031] In some embodiments, the drug moiety is a cytotoxin selected from the group consisting of auristatins, maytansinoids, macrocyclic ketone analogues, topoisomerase inhibitors, benzodiazepines, tubulysins, duocarmycin, camptothecin, calicheamicins, exatecans, irinotecans (SN38), doxorubicin, anthracycline, the pyrrolobenzodiazepenes (PBD), TLR agonist, STING agonists, pseudomonas aeruginosa exotoxin PE38, diphtheria toxin, staphylococcus aureus enterotoxin A/E-120, antibacterial antibiotic, shigatoxin, ricin, and urease.

[0032] In some embodiments, the drug moiety is a cytotoxin selected from the group consisting of monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF), maytansine, mertansine (DM1), ravtansine (DM4), tublysin A, DXd, 7-ethyl-10-hydroxycamptothecin (SN-38), DGN462, Amberstatin269, anthramycin, SG3199/SCX, IRDye®700DX, TLR7/8 agonist, diABZI STING agonist-2, or any derivative thereof.

[0033] In some embodiments, the drug moiety comprises MMAE, MMAF, or DXd.

[0034] In some embodiments, the drug moiety comprises exatecan or DXd (exatecan derivative for ADC). In some embodiments, the drug moiety comprises a compound selected from the group consisting of:



[0035] In some embodiments, the drug moiety is attached to the antibody or fragment thereof through a linker. In some embodiments, the linker is hydrolyzable under acidic conditions.

[0036] In some embodiments, the linker comprises succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), sulfo-SMCC, p-carboxycyclohexylmethylmaleimide, maleimide-caproyl (MC)-Valine-citrulline (VC)-para-aminobenzoyloxycarbonyl (PABC), CL2A, maleimide-caproyl (MC), MC-glycine-glycine-phenylalanine-glycine (GGFG), MC-PEG8-GGFG, MC-PEG8-GGFG-PAB, or maleimide propoyl (MP)-PEG8-Valine-alanine (VA)-PABC.

[0037] In some embodiments, the antibody-drug conjugate comprises ozogamicin, vedotin, mafodotin, emtansine, deruxtecan, govitecan, or tesirine.

[0038] In some embodiments, the antibody-drug conjugate has a drug moiety to antibody ratio (DAR) of 1 to 20, 2 to 16, or 4 to 8.

[0039] Also provided is a chimeric antigen receptor (CAR) having specificity to the human CDH17 protein, comprising an antigen-binding fragment thereof provided herein or a bifunctional molecule provided herein, a transmembrane domain, and an intracellular activating domain.

[0040] In some embodiments, the antigen-binding fragment is a single chain fragment (scFv).

[0041] In some embodiments, the transmembrane domain is a transmembrane domain of 4-1BB, an alpha chain of a T cell receptor, a beta chain of a T cell receptor, a zeta chain of a T cell receptor, CD3 epsilon, CD4, CD5, CD8 alpha, CD9, CD16, CD19, CD22, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD134, CD137, or CD154.

[0042] In some embodiments, the chimeric antigen receptor comprises an intracellular activation domain that is a signaling domain derived from CD3 zeta, CD3 epsilon, CD3 delta, and CD3 gamma.

[0043] In some embodiments, the chimeric antigen receptor further comprises one or more costimulatory domains selected from a signaling domain (or other suitable portion) of CD28, OX-40, 4-1BB/CD137, CD2, CD7, CD27, CD30, CD40, Programmed Death-1 (PD-1), inducible T cell costimulator (ICOS), lymphocyte function-associated antigen-1 (LFA-1, CD1-1a/CD18), CD3 gamma, CD3 delta, CD3 epsilon, CD247, CD276 (B7-H3), LIGHT,

(TNFSF14), NKG2C, Ig alpha (CD79a), DAP-10, Fc gamma receptor, MHC class 1 molecule, TNF receptor proteins, an Immunoglobulin protein, cytokine receptor, integrins, Signaling Lymphocytic Activation Molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, ICAM-1, B7-H3, CDS, ICAM-1, GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL-2R beta, IL-2R gamma, IL-7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, a ligand that specifically binds with CD83, or any combination thereof.

[0044] In some embodiments, the chimeric antigen receptor further comprises a hinge domain selected from a hinge domain of IgG1, IgG2, IgG3, IgG4, IgA, IgD, IgE, IgM, CD28, or CD8 alpha, any truncation thereof, or any combination thereof.

[0045] Also provided is a nucleic acid encoding the antibody or antigen-binding fragment thereof provided herein, the bifunctional molecule provided herein, or the CAR provided herein.

[0046] Also provided is a vector comprising the nucleic acid provided herein. Also provided is an isolated cell comprising the nucleic acid provided herein, or the vector provided herein.

[0047] Also provided is a pharmaceutical composition comprising the antibody or antigen-binding fragment thereof, the bifunctional molecule, or the antibody-drug conjugate or the CAR of the present disclosure, and a pharmaceutically acceptable carrier.

[0048] Yet another embodiment provides a method of treating cancer in a patient in need thereof, comprising administering to the patient the antibody or antigen-binding fragment thereof, the bifunctional molecule, the antibody-drug conjugate, or the CAR or use of the antigen-binding fragment thereof, the bifunctional molecule, the antibody-drug conjugate or the CAR for the preparation of a medicament for treating cancer.

[0049] In some embodiments, the cancer is selected from the group consisting of gastric cancer, colorectal cancer, hepatocarcinoma, bladder cancer, liver cancer, endometrial cancer, leukemia, lymphoma, pancreatic cancer, neuroendocrine cancer, small cell lung cancer, non-small cell lung cancer, breast cancer, urethral cancer, head and neck cancer, gastrointestinal cancer, esophageal cancer, ovarian cancer, renal cancer, melanoma, prostate cancer and thyroid cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0050] **FIG. 1A-1B** show the specific binding of 8G5G9.p1.z12 to human CDH17 overexpressing cell lines.

[0051] **FIG. 2** shows the non-specific binding test of 8G5G9.p1.z12 to blank HEK-293 cell line.

[0052] **FIG. 3A-3B** shows the specific binding of 8G5G9.p1.z12 to endogenous human CDH17 on tumor cells.

[0053] **FIG. 4A-4D** show the cross-reactivity of 8G5G9.p1.z12 to monkey and rodent CDH17 overexpressing cell lines.

[0054] **FIG. 5** shows the epitope binning assay of 8G5G9.p1.z12 against BMK-BI-905711 (anti-CDH17) on HEK-293/H_CDH17 cell line.

[0055] **FIG. 6A-6B** show the internalization potency of 8G5G9.p1.z12 on human CDH17 overexpressing cells.

[0056] **FIG. 7A-B** show the specific binding of anti-CDH17 ADCs to human CDH17 overexpressing cell lines.

[0057] **FIG. 8A-B** show the specific binding of anti-CDH17 ADCs to endogenous human CDH17 on tumor cells.

[0058] **FIG. 9A-B** show the cytotoxic effect of anti-CDH17 ADCs towards human CDH17 expressing tumor cells.

[0059] **FIG. 10A-F** show the bystander effect of anti-CDH17 ADCs towards human CDH17 negative cell lines.

[0060] FIG. 11A-B show the antitumor activity of anti-CDH17 ADCs in CRC CDX (Cell line-derived xenograft) model.

[0061] FIG. 12A-D show the cell-based binding of the anti-CDH17 antibodies 296G7E10.p4.Z9 (panel A), 312E10A9.p2.Z7 (panel B), 35E7D6.p1.z9, 135B2E11.p1.z1, 153A3D7.p3.z5, and 155B9A5.p3.V2.z2 (panel C-D) in human CDH17 overexpressing cell lines.

[0062] FIG. 13A-B show the cell-based binding of the anti-CDH17 antibodies 296G7E10.p4.Z9 (panel A) and 312E10A9.p2.Z7 (panel B) in rhesus CDH17 overexpressing HEK293 cell lines.

[0063] FIG. 14A-D show the cell-based binding of the anti-CDH17 antibodies 312E10A9.p2.Z7 (panel A, SNU16), 296G7E10.p4.Z9 (panel B, LoVo), 35E7D6.p1.z9, 135B2E11.p1.z1, 153A3D7.p3.z5, and 155B9A5.p3.V2.z2 (panel C, SNU16) and 35E7D6.p1.z9, 135B2E11.p1.z1, 153A3D7.p3.z5, and 155B9A5.p3.V2.z2 (panel D, LoVo) in CDH17 endogenously expressing cancer cell lines, respectively.

[0064] FIG. 15A-B show internalization of the anti-CDH17 antibodies on CDH17 endogenously expressing cancer cell line SNU16 (panel A) and LoVo (panel B), respectively.

[0065] FIG. 16A-B show internalization of the anti-CDH17 antibodies on CDH17 overexpressing cancer cell line HEK293 (panel A) and DLD-1 (panel B), respectively.

[0066] FIG. 17A-C show cytotoxic effect of CDH17-targeted ADCs towards human CDH17 expressing cells HEK293 (panel A), DLD-1 (panel B) and LoVo (panel C), respectively.

[0067] FIG. 18A-C show potent cytotoxic effect of CDH17-targeted ADCs towards human CDH17 expressing cells SNU-16 (panel A), HCT116 (panel B) and DLD-1/H (panel C), respectively.

[0068] FIG. 19A-B show antitumor activity of CDH17-targeted ADCs in CRC CDX (Cell line-derived xenograft) model.

[0069] FIG. 20A-B show antitumor activity of CDH17-targeted ADCs in SNU-16 (GC) xenograft model.

DETAILED DESCRIPTION

Definitions

[0070] It is to be noted that the term “a” or “an” entity refers to one or more of that entity; for example, “an antibody,” is understood to represent one or more antibodies. As such, the terms “a” (or “an”), “one or more,” and “at least one” can be used interchangeably herein.

[0071] As used herein, the term “polypeptide” is intended to encompass a singular “polypeptide” as well as plural “polypeptides,” and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term “polypeptide” refers to any chain or chains of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, “protein,” “amino acid chain,” or any other term used to refer to a chain or chains of two or more amino acids, are included within the definition of “polypeptide,” and the term “polypeptide” may be used instead of, or interchangeably with any of these terms. The term “polypeptide” is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non- naturally occurring amino acids. A polypeptide may be derived from a natural biological source or produced by recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It may be generated in any manner, including by chemical synthesis.

[0072] “Homology” or “identity” or “similarity” refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences. An “unrelated” or “non-homologous” sequence shares less than 40% identity, though preferably less than 25% identity, with one of the sequences of the present disclosure.

[0073] A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) has a certain percentage (for example, 60 %, 65 %, 70 %, 75 %, 80 %, 85 %, 90 %, 95 %,

98 % or 99 %) of “sequence identity” to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences.

[0074] The term “an equivalent nucleic acid or polynucleotide” refers to a nucleic acid having a nucleotide sequence having a certain degree of homology, or sequence identity, with the nucleotide sequence of the nucleic acid or complement thereof. A homolog of a double stranded nucleic acid is intended to include nucleic acids having a nucleotide sequence which has a certain degree of homology with or with the complement thereof. In one aspect, homologs of nucleic acids are capable of hybridizing to the nucleic acid or complement thereof. Likewise, “an equivalent polypeptide” refers to a polypeptide having a certain degree of homology, or sequence identity, with the amino acid sequence of a reference polypeptide. In some aspects, the sequence identity is at least about 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%. In some aspects, the equivalent polypeptide or polynucleotide has one, two, three, four or five addition, deletion, substitution and their combinations thereof as compared to the reference polypeptide or polynucleotide. In some aspects, the equivalent sequence retains the activity (*e.g.*, epitope-binding) or structure (*e.g.*, salt-bridge) of the reference sequence.

[0075] As used herein, an “antibody” or “antigen-binding polypeptide” refers to a polypeptide or a polypeptide complex that specifically recognizes and binds to an antigen. An antibody can be a whole antibody and any antigen binding fragment or a single chain thereof. Thus the term “antibody” includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule having biological activity of binding to the antigen. Examples of such include, but are not limited to a complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework (FR) region, or any portion thereof, or at least one portion of a binding protein.

[0076] The terms “antibody fragment” or “antigen-binding fragment”, as used herein, is a portion of an antibody such as F(ab')₂, F(ab)₂, Fab', Fab, Fv, scFv and the like. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the intact antibody. The term “antibody fragment” includes aptamers, spiegelmers, and diabodies. The term “antibody fragment” also includes any synthetic or genetically engineered protein that acts like an antibody by binding to a specific antigen to form a complex.

[0077] A “single-chain variable fragment” or “scFv” refers to a fusion protein of the variable regions of the heavy (V_H) and light chains (V_L) of immunoglobulins. In some aspects, the regions are connected with a short linker peptide of ten to about 25 amino acids. The linker can be rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the V_H with the C-terminus of the V_L , or vice versa. This protein retains the specificity of the original immunoglobulin, despite removal of the constant regions and the introduction of the linker. ScFv molecules are known in the art and are described, *e.g.*, in US patent 5,892,019.

[0078] The term antibody encompasses various broad classes of polypeptides that can be distinguished biochemically. Those skilled in the art will appreciate that heavy chains are classified as gamma, mu, alpha, delta, or epsilon (γ , μ , α , δ , ϵ) with some subclasses among them (*e.g.*, $\gamma 1$ - $\gamma 4$). It is the nature of this chain that determines the “class” of the antibody as IgG, IgM, IgA, IgG, or IgE, respectively. The immunoglobulin subclasses (isotypes) *e.g.*, IgG₁, IgG₂, IgG₃, IgG₄, IgG₅, etc. are well characterized and are known to confer functional specialization. Modified versions of each of these classes and isotypes are readily discernable to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of the instant disclosure. All immunoglobulin classes are clearly within the scope of the present disclosure, the following discussion will generally be directed to the IgG class of immunoglobulin molecules. With regard to IgG, a standard immunoglobulin molecule comprises two identical light chain polypeptides of molecular weight approximately 23,000 Daltons, and two identical heavy chain polypeptides of molecular weight 53,000-70,000. The four chains are typically joined by disulfide bonds in a “Y” configuration wherein the light chains bracket the heavy chains starting at the mouth of the “Y” and continuing through the variable region.

[0079] Antibodies, antigen-binding polypeptides, variants, or derivatives thereof of the disclosure include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized, primatized, or chimeric antibodies, single chain antibodies, epitope-binding fragments, *e.g.*, Fab, Fab' and F(ab')₂, Fd, Fvs, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), fragments comprising either a VK or VH domain, fragments produced by a Fab expression library, and anti- idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to LIGHT antibodies disclosed herein). Immunoglobulin or antibody molecules of the disclosure can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA,

and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

[0080] Light chains are classified as either kappa or lambda (K, λ). Each heavy chain class may be bound with either a kappa or lambda light chain. In general, the light and heavy chains are covalently bonded to each other, and the “tail” portions of the two heavy chains are bonded to each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are generated either by hybridomas, B cells or genetically engineered host cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain.

[0081] Both the light and heavy chains are divided into regions of structural and functional homology. The terms “constant” and “variable” are used functionally. In this regard, it will be appreciated that the variable domains of both the light (VK) and heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CK) and the heavy chain (CH1, CH2 or CH3) confer important biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the like. By convention the numbering of the constant region domains increases as they become more distal from the antigen-binding site or amino- terminus of the antibody. The N-terminal portion is a variable region and at the C-terminal portion is a constant region; the CH3 and CK domains actually comprise the carboxy-terminus of the heavy and light chain, respectively.

[0082] As indicated above, the variable region allows the antibody to selectively recognize and specifically bind epitopes on antigens. That is, the VK domain and VH domain, or subset of the complementarity determining regions (CDRs), of an antibody combine to form the variable region that defines a three-dimensional antigen-binding site. This quaternary antibody structure forms the antigen-binding site present at the end of each arm of the Y. More specifically, the antigen-binding site is defined by three CDRs on each of the VH and VK chains (*i.e.* CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3). In some instances, *e.g.*, certain immunoglobulin molecules derived from camelid species or engineered based on camelid immunoglobulins, a complete immunoglobulin molecule may consist of heavy chains only, with no light chains. *See, e.g., Hamers-Casterman et al., Nature* 363:446-448 (1993).

[0083] In naturally occurring antibodies, the six “complementarity determining regions” or “CDRs” present in each antigen-binding domain are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen-binding domain as the antibody assumes its three dimensional configuration in an aqueous environment. The remainder of the amino acids in the antigen-binding domains, referred to as “framework” regions, show less inter-molecular variability. The framework regions largely adopt a β -sheet conformation and the CDRs form loops which connect, and in some cases form part of, the β -sheet structure. Thus, framework regions act to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen-binding domain formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive antigen. This complementary surface promotes the non-covalent binding of the antibody to its cognate epitope. The amino acids comprising the CDRs and the framework regions, respectively, can be readily identified for any given heavy or light chain variable region by one of ordinary skill in the art, since they have been precisely defined (*see* “Sequences of Proteins of Immunological Interest,” Kabat, E., *et al.*, U.S. Department of Health and Human Services, (1983); and Chothia and Lesk, *J. Mol. Biol.*, 196:901-917 (1987)).

[0084] In the case where there are two or more definitions of a term which is used and/or accepted within the art, the definition of the term as used herein is intended to include all such meanings unless explicitly stated to the contrary. A specific example is the use of the term “complementarity determining region” (“CDR”) to describe the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. This particular region has been described by Kabat *et al.*, U.S. Dept. of Health and Human Services, “Sequences of Proteins of Immunological Interest” (1983) and by Chothia *et al.*, *J. Mol. Biol.* 196:901-917 (1987), which are incorporated herein by reference in their entireties. The CDR definitions according to Kabat and Chothia include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The appropriate amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth in the table below as a comparison. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can

routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

	Kabat	Chothia
CDR-H1	31-35	26-32
CDR-H2	50-65	52-58
CDR-H3	95-102	95-102
CDR-L1	24-34	26-32
CDR-L2	50-56	50-52
CDR-L3	89-97	91-96

[0085] Kabat *et al.* also defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of “Kabat numbering” to any variable domain sequence, without reliance on any experimental data beyond the sequence itself. As used herein, “Kabat numbering” refers to the numbering system set forth by Kabat *et al.*, U.S. Dept. of Health and Human Services, “Sequence of Proteins of Immunological Interest” (1983).

[0086] In addition to table above, the Kabat number system describes the CDR regions as follows: CDR-H1 begins at approximately amino acid 31 (*i.e.*, approximately 9 residues after the first cysteine residue), includes approximately 5-7 amino acids, and ends at the next tryptophan residue. CDR-H2 begins at the fifteenth residue after the end of CDR-H1, includes approximately 16-19 amino acids, and ends at the next arginine or lysine residue. CDR-H3 begins at approximately the thirty third amino acid residue after the end of CDR-H2; includes 3-25 amino acids; and ends at the sequence W-G-X-G, where X is any amino acid. CDR-L1 begins at approximately residue 24 (*i.e.*, following a cysteine residue); includes approximately 10-17 residues; and ends at the next tryptophan residue. CDR-L2 begins at approximately the sixteenth residue after the end of CDR-L1 and includes approximately 7 residues. CDR-L3 begins at approximately the thirty third residue after the end of CDR-L2 (*i.e.*, following a cysteine residue); includes approximately 7-11 residues and ends at the sequence F or W-G-X-G, where X is any amino acid.

[0087] Antibodies disclosed herein may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine, donkey, rabbit, goat, guinea pig, camel, llama, horse, or chicken antibodies. In another embodiment, the variable region may be chondrichthoid in origin (*e.g.*, from sharks).

[0088] As used herein, the term “heavy chain constant region” includes amino acid sequences derived from an immunoglobulin heavy chain. A polypeptide comprising a heavy

chain constant region comprises at least one of: a CH1 domain, a hinge (*e.g.*, upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, or a variant or fragment thereof. For example, an antigen-binding polypeptide for use in the disclosure may comprise a polypeptide chain comprising a CH1 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH2 domain; a polypeptide chain comprising a CH1 domain and a CH3 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH3 domain, or a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, a CH2 domain, and a CH3 domain. In another embodiment, a polypeptide of the disclosure comprises a polypeptide chain comprising a CH3 domain. Further, an antibody for use in the disclosure may lack at least a portion of a CH2 domain (*e.g.*, all or part of a CH2 domain). As set forth above, it will be understood by one of ordinary skill in the art that the heavy chain constant region may be modified such that they vary in amino acid sequence from the naturally occurring immunoglobulin molecule.

[0089] The heavy chain constant region of an antibody disclosed herein may be derived from different immunoglobulin molecules. For example, a heavy chain constant region of a polypeptide may comprise a CH1 domain derived from an IgG₁ molecule and a hinge region derived from an IgG₃ molecule. In another example, a heavy chain constant region can comprise a hinge region derived, in part, from an IgG₁ molecule and, in part, from an IgG₃ molecule. In another example, a heavy chain portion can comprise a chimeric hinge derived, in part, from an IgG₁ molecule and, in part, from an IgG₄ molecule.

[0090] As used herein, the term “light chain constant region” includes amino acid sequences derived from antibody light chain. Preferably, the light chain constant region comprises at least one of a constant kappa domain or constant lambda domain.

[0091] A “light chain-heavy chain pair” refers to the collection of a light chain and heavy chain that can form a dimer through a disulfide bond between the CL domain of the light chain and the CH1 domain of the heavy chain.

[0092] As previously indicated, the subunit structures and three-dimensional configuration of the constant regions of the various immunoglobulin classes are well known. As used herein, the term “VH domain” includes the amino terminal variable domain of an immunoglobulin heavy chain and the term “CH1 domain” includes the first (most amino terminal) constant

region domain of an immunoglobulin heavy chain. The CH1 domain is adjacent to the VH domain and is amino terminal to the hinge region of an immunoglobulin heavy chain molecule.

[0093] As used herein the term “CH2 domain” includes the portion of a heavy chain molecule that extends, *e.g.*, from about residue 244 to residue 360 of an antibody using conventional numbering schemes (residues 244 to 360, Kabat numbering system; and residues 231-340, EU numbering system; *see* Kabat *et al.*, U.S. Dept. of Health and Human Services, “Sequences of Proteins of Immunological Interest” (1983). The CH2 domain is unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains are interposed between the two CH2 domains of an intact native IgG molecule. It is also well documented that the CH3 domain extends from the CH2 domain to the C-terminal of the IgG molecule and comprises approximately 108 residues.

[0094] As used herein, the term “hinge region” includes the portion of a heavy chain molecule that joins the CH1 domain to the CH2 domain. This hinge region comprises approximately 25 residues and is flexible, thus allowing the two N-terminal antigen-binding regions to move independently. Hinge regions can be subdivided into three distinct domains: upper, middle, and lower hinge domains (Roux *et al.*, *J. Immunol* 161:4083 (1998)).

[0095] As used herein the term “disulfide bond” includes the covalent bond formed between two sulfur atoms. The amino acid cysteine comprises a thiol group that can form a disulfide bond or bridge with a second thiol group. In most naturally occurring IgG molecules, the CH1 and CK regions are linked by a disulfide bond and the two heavy chains are linked by two disulfide bonds at positions corresponding to 239 and 242 using the Kabat numbering system (position 226 or 229, EU numbering system).

[0096] As used herein, the term “chimeric antibody” will be held to mean any antibody wherein the immunoreactive region or site is obtained or derived from a first species and the constant region (which may be intact, partial or modified in accordance with the instant disclosure) is obtained from a second species. In certain embodiments the target binding region or site will be from a non-human source (*e.g.* mouse or primate) and the constant region is human.

[0097] As used herein, “percent humanization” is calculated by determining the number of framework amino acid differences (*i.e.*, non-CDR difference) between the humanized domain

and the germline domain, subtracting that number from the total number of amino acids, and then dividing that by the total number of amino acids and multiplying by 100.

[0098] By “specifically binds” or “has specificity to,” it is generally meant that an antibody binds to an epitope via its antigen-binding domain, and that the binding entails some complementarity between the antigen-binding domain and the epitope. According to this definition, an antibody is said to “specifically bind” to an epitope when it binds to that epitope, via its antigen-binding domain more readily than it would bind to a random, unrelated epitope. The term “specificity” is used herein to qualify the relative affinity by which a certain antibody binds to a certain epitope. For example, antibody “A” may be deemed to have a higher specificity for a given epitope than antibody “B,” or antibody “A” may be said to bind to epitope “C” with a higher specificity than it has for related epitope “D.”

[0099] As used herein, the terms “treat” or “treatment” refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the progression of cancer. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. “Treatment” can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

[0100] By “subject” or “individual” or “animal” or “patient” or “mammal,” is meant any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include humans, domestic animals, farm animals, and zoo, sport, or pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, cows, and so on.

[0101] As used herein, phrases such as “to a patient in need of treatment” or “a subject in need of treatment” includes subjects, such as mammalian subjects, that would benefit from

administration of an antibody or composition of the present disclosure used, *e.g.*, for detection, for a diagnostic procedure and/or for treatment.

Anti-CDH17 Antibodies

[0102] The anti-CDH17 antibodies and antigen-binding fragments thereof provided herein, in comparison with the two reference antibodies, BMK-BI-905711(a monospecific anti-CDH17 Ig molecule synthesized according to Boehringer Ingelheim’s bi-specific antibody BI 905711) and BMK-ARB202 (a monospecific anti-CDH17 Ig molecule which is synthesized according to Arbele’s bi-specific antibody ARB202), showed comparable binding affinity to the human CDH17 protein. While having a distinct epitope from the BMK-BI-905711, the anti-CDH17 antibodies and antigen-binding fragments thereof provided herein showed no non-specific binding to human CDH17 protein and are selective in binding to monkey CDH17 protein but not to rodent CDH17 protein.

[0103] Importantly, as demonstrated in **FIG. 10-11**, antibody-drug conjugates (ADC) based on the antibodies and fragments of the present disclosure exhibited potent cytotoxicity not only to CDH17-positive tumor cells, but also to adjacent bystander CDH17-negative cells. Subsequently, *in vivo* testing showed that these ADC had excellent anti-tumor efficacy and superior safety profile.

[0104] In accordance with one embodiment of the present disclosure, therefore, provided are antibodies and antigen-binding fragments thereof that are able to bind to CDH17. An example antibody is a humanized anti-CDH17 antibody 8G5G9.p1.z12, 296G7E10.p4.Z9, 312E10A9.p2.Z7, 135B2E11.p1.z1, 153A3D7.p3.z5, 35E7D6.p1.z9, and 155B9A5.p3.V2.z2 having sequences of CDRs and variable regions shown in **Tables A1-A4**. Also included are those that include the same CDRs as illustrated herein. In some embodiments, the disclosed antibodies and fragments include those that bind to the same epitope as those illustrated here, and those that compete with the instantly disclosed in binding to CDH17.

[0105] In accordance with one embodiment of the present disclosure, provided is an antibody or antigen-binding fragments thereof that includes the heavy chain and light chain variable domains with the CDR regions disclosed herein, as well as their biological equivalents.

Table A1. CDR sequences of 8G5G9.p1.z12

Region	Sequence	SEQ ID NO:
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VH CDR1	TSGMGVS	1
VH CDR2	HIYWDDDKRYNPSLKS	2
VH CDR3	RALITSPFEY	3
VL CDR1	RASQTIGTSIH	4
VL CDR2	YASESIY	5
VL CDR3	QQSNAWPLT	6

Table A2. Sequences of variable regions and constant regions of 8G5G9.p1.z12

Antibody chain	Sequence	SEQ ID NO:
VH	EVTLKESGPALVKPTQTLTLTCTFSGFSLSTSGMGVSWIRQPPGKALEWLA <u>HIYWDDDKRYNPSLKS</u> RLTISKDTSKNQVFLTMTNMDPVDATATYYCAR <u>RAL</u> <u>ITSPFEY</u> WGQGTITVTVSS	7
VL	EIVMTQSPATLSVSPGERATLSC <u>RASQTIGTSIH</u> HWYQQKPGQAPRLLIKYA <u>SESIY</u> GIPARFSGSGSGTEFTLTISSLQSEDAVYYC <u>QQSNAWPLT</u> FGQGT KLEIK	8
CH	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS CDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNAKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVF SCSMHEALHNHYTQKSLSLSPGK	9
CL	RTVAAPSVFIFPPSDEQLKSGTASVVCCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSF NRGEC	10

Table A3. CDR Sequences of 296G7E10.p4.Z9, 312E10A9.p2.Z7, 135B2E11.p1.z1, 153A3D7.p3.z5, 35E7D6.p1.z9, and 155B9A5.p3.V2.z2

Antibody	Sequence	SEQ ID NO:
296G7E10.p4.Z9VH	SNWMH AIYPGRSETSYNQNFKD HSGFDEFDY	11 12 13
296G7E10.p4.Z9 VL	RSSQTIVHSSGNAYLE KVSNRFS FQGSHVPFT	14 15 16
312E10A9.p2.Z7 VH	SYTMH YIYPTRDYTNYNQKFKD QGVYGNWDWYFDV	17 18 19
312E10A9.p2.Z7 VL	TSSKSLVHSNAKTYLY RMSNLAS MQHLEYPFT	20 21 22
135B2E11.p1.z1 VH	SYWIN NIYPSNAYTNYNQKFKD YYGSSYWYFDV	23 24 25
135B2E11.p1.z1 VL	KASENVGPYVS GASNRYT GQSYTYPYT	26 27 28

153A3D7.p3.z5 VH	SGYWN YISYSGSTYYNPSLRS RGYDVYWFDDV	29 30 31
153A3D7.p3.z5 VL	RSSQSLVLSNANTYLH KISNRFS SQSTHIPFT	32 33 34
35E7D6.p1.z9 VH	SYMH YIDPFNAGTDYNQQFKG GYRYPWFVY	35 36 37
35E7D6.p1.z9 VL	RASENIYSYLV NVKTLAE QHNYDIPLT	38 39 40
155B9A5.p3.V2.z2 VH	DYNMH YIYPYNAGTDYSQKFKS EEIWYGNYGFDY	41 42 43
155B9A5.p3.V2.z2 VL	RSSQSLVHSNANTYLH KVSNRFS SQSTHVPPT	44 45 46

Table A4. Sequences of the variable regions of the antibodies 296G7E10.p4.Z9, 312E10A9.p2.Z7, 135B2E11.p1.z1, 153A3D7.p3.z5, 35E7D6.p1.z9, and 155B9A5.p3.V2.z2

Antibody	Sequence	SEQ ID NO:
296G7E10.p4.Z9 VH	EVQLVQSGAEVKKPGASVKVSCASGYTFI <u>SNWMH</u> WVRQAPGQGLEWMGA <u>IYPGRSETSYNQNF</u> KDRVTMTADTSTSTAYMELRSLRSDDMAVYYCSR <u>HS</u> <u>GFDEFDY</u> WGQGT ² TVTVSS	47
296G7E10.p4.Z9 VL	DVLTQSPFLSLPVTGPGEPAISCR <u>SSQ</u> TIVHSSGNAYLEWYLQKPGQSPQLLIY <u>KVSNRFS</u> GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC <u>FQGS</u> HVP <u>FT</u> FGSGTKLEIK	48
312E10A9.p2.Z7 VH	EVQLVQSGAEVKKPGSSSVKVSCKASGYTFT <u>SYTMH</u> WVRQAPGQGLEWMGY <u>IYPTRDYTNYNQK</u> KDRVTITADKSTSTAYMELSSLRSEDTAVYYCGR <u>QG</u> <u>VYGN</u> YDWYFDVWGQGT ² TVTVSS	49
312E10A9.p2.Z7 VL	DIVMTQSPFLSLPVTGPGEPAISCT <u>SSKSLVHSNAKTYLY</u> WYLQKPGQSPQLLIY <u>RMSNLAS</u> GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC <u>MOHLEYP</u> <u>FT</u> FGGGTKVEIK	50
135B2E11.p1.z1 VH	EVQLVQSGAEVKKPGASVKVSCASGYTFT <u>SYWIN</u> WVRQAPGQGLEWMGN <u>IYPSNAYTNYNQK</u> KDRVTMTDRDTSTSTVYMESSLRSEDTAVYYCARY <u>Y</u> <u>GSSYWF</u> FDVWGQGT ² TVTVSS	51
135B2E11.p1.z1 VL	DIVMTQSPDSLAVSLGERATINCK <u>ASENVGPYVS</u> SWYQQKPGQPPKLLIYG <u>ASNRYT</u> GVPDRFSGSGSGTDFTLTISLQAEDVAVYYC <u>GQSYTYPYT</u> FGG GTKVEIK	52
153A3D7.p3.z5 VH	EVQLQESGPGLVKPSQTLSTCTVSGDSISS <u>SGYWN</u> WIRQPPGKGLEYIGY <u>ISYSGSTYYNPSLRS</u> RVTTISRDTSKNQFSLKSSVTAADTAVYYCARR <u>GY</u> <u>DVYWF</u> FDVWGQGT ² TVTVSS	53
153A3D7.p3.z5 VL	DVMTQTPLSLSVTPGQPASISCR <u>SSQSLVLSNANTYLH</u> WYLQKPGQSPQLLIY <u>KISNRFS</u> GVPDRFSGSGSGTDFTLKISRVEAEDVGVYFC <u>SQSTHIP</u> <u>FT</u> FGGGTKLEIK	54
35E7D6.p1.z9 VH	EIQLVQSGAEVKKPGASVKVSCASGYTFT <u>SYMH</u> WVRQAPGQRLEWMGY <u>IDPFNAGTDYNQQ</u> KGKATITVDKSASTAYMELSSLRSEDMAVYYCTSGY <u>RYP</u> AWFVYWGQGT ² LTVTVSS	55

35E7D6.p1.z9 VL	DIQMTQSPSSLSASVGDRVITITC RASENIYSYLV WYQQKPGKSPQLLV YN VKTTLAE GVPSRFSGSGSGTQFTLTIS SLQ PEDFATYYC QHNYDIPLT FGG GTKVEIK	56
155B9A5.p3. V2.z2 VH	EVQLVQSGAEVKKPGASVKV SCKASGYTFTDYNMH WVRQAPGQRLEW MGY IYPYNAGTDYSQKF KSRVTITRDT SASTAYMELSS LRSEDTAVYYC AREE IWYGNYGFDY WGQGT TVTVSS	57
155B9A5.p3. V2.z2 VL	DVVM TQTPLSLSVTPGQPASISCRSSQSLVHSNANTYLH WYLQKPGQSPQ LLIY KVSNRFS GV PD RFSGSGSGTDFTLKISRVEAEDVGVYFC SQSTHVP PT FGGGTKVEIK	58

[0106] It is appreciated that CDRs can be modified to include those having one, two or three amino acid addition, deletion and/or substitutions. In some embodiments, the substitutions can be conservative substitutions.

[0107] A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a nonessential amino acid residue in an immunoglobulin polypeptide is preferably replaced with another amino acid residue from the same side chain family. In another embodiment, a string of amino acids can be replaced with a structurally similar string that differs in order and/or composition of side chain family members.

[0108] Non-limiting examples of conservative amino acid substitutions are provided in the table below, where a similarity score of 0 or higher indicates conservative substitution between the two amino acids.

Table B. Amino Acid Similarity Matrix

	C	G	P	S	A	T	D	E	N	Q	H	K	R	V	M	I	L	F	Y	W
W	-8	-7	-6	-2	-6	-5	-7	-7	-4	-5	-3	-3	2	-6	-4	-5	-2	0	0	17
Y	0	-5	-5	-3	-3	-3	-4	-4	-2	-4	0	-4	-5	-2	-2	-1	-1	7	10	
F	-4	-5	-5	-3	-4	-3	-6	-5	-4	-5	-2	-5	-4	-1	0	1	2	9		
L	-6	-4	-3	-3	-2	-2	-4	-3	-3	-2	-2	-3	-3	2	4	2	6			
I	-2	-3	-2	-1	-1	0	-2	-2	-2	-2	-2	-2	-2	4	2	5				
M	-5	-3	-2	-2	-1	-1	-3	-2	0	-1	-2	0	0	2	6					
V	-2	-1	-1	-1	0	0	-2	-2	-2	-2	-2	-2	-2	4						
R	-4	-3	0	0	-2	-1	-1	-1	0	1	2	3	6							
K	-5	-2	-1	0	-1	0	0	0	1	1	0	5								
H	-3	-2	0	-1	-1	-1	1	1	2	3	6									
Q	-5	-1	0	-1	0	-1	2	2	1	4										
N	-4	0	-1	1	0	0	2	1	2											
E	-5	0	-1	0	0	0	3	4												
D	-5	1	-1	0	0	0	4													
T	-2	0	0	1	1	3														
A	-2	1	1	1	2															
S	0	1	1	1																
P	-3	-1	6																	
G	-3	5																		
C	12																			

Table C. Conservative Amino Acid Substitutions

For Amino Acid	Substitution With
Alanine	D-Ala, Gly, Aib, β -Ala, L-Cys, D-Cys
Arginine	D-Arg, Lys, D-Lys, Orn D-Orn
Asparagine	D-Asn, Asp, D-Asp, Glu, D-Glu Gln, D-Gln
Aspartic Acid	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Cysteine	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr, L-Ser, D-Ser
Glutamine	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	Ala, D-Ala, Pro, D-Pro, Aib, β -Ala
Isoleucine	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	Val, D-Val, Met, D-Met, D-Ile, D-Leu, Ile
Lysine	D-Lys, Arg, D-Arg, Orn, D-Orn
Methionine	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val
Phenylalanine	D-Phe, Tyr, D-Tyr, His, D-His, Trp, D-Trp
Proline	D-Pro
Serine	D-Ser, Thr, D-Thr, allo-Thr, L-Cys, D-Cys
Threonine	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Val, D-Val
Tyrosine	D-Tyr, Phe, D-Phe, His, D-His, Trp, D-Trp
Valine	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met

[0109] It will also be understood by one of ordinary skill in the art that antibodies as disclosed herein may be modified such that they vary in amino acid sequence from the naturally occurring binding polypeptide from which they were derived. For example, a polypeptide or amino acid sequence derived from a designated protein may be similar, *e.g.*, have a certain percent identity to the starting sequence, *e.g.*, it may be 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identical to the starting sequence.

[0110] According to specific embodiments, the antibody is a humanized antibody. Humanized forms of non-human (*e.g.*, murine) antibodies are chimeric molecules of immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues form a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin (Jones et al., *Nature*, 321: 522-525 (1986); Riechmann et al., *Nature*, 332: 323-329 (1988); and Presta, *Curr. Op. Struct. Biol.*, 2: 593-596 (1992)).

[0111] In one embodiment, the CDRs are those of 296G7E10.p4.Z9, as exemplified in **Table A3**. In one embodiment, the VH CDR1 includes the amino acid sequence of SEQ ID NO: 11 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VH CDR2 includes the amino acid sequence of SEQ ID NO: 12 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VH CDR3 includes the amino acid sequence of SEQ ID NO: 13 or a variant thereof having one, two, or three deletions, additions, substitutions or the

combinations thereof, the VL CDR1 includes the amino acid sequence of SEQ ID NO: 14 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VL CDR2 includes the amino acid sequence of SEQ ID NO: 15 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the VL CDR3 includes the amino acid sequence of SEQ ID NO: 16 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0112] Also provided, in some embodiments, are those that include the same CDRs as 296G7E10.p4.Z9. In some embodiments, the disclosed antibodies and fragments include those that bind to the same epitope as 296G7E10.p4.Z9, and those that compete with any of them in binding to CDH17.

[0113] In some embodiments, the heavy chain variable region includes an amino acid sequence selected from the group consisting of SEQ ID NO:47, or a peptide having at least 90% sequence identity to an amino acid sequence of SEQ ID NO:47.

[0114] In some embodiments, the light chain variable region includes an amino acid sequence selected from the group consisting of SEQ ID NO:48, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 48.

[0115] In some embodiments, the heavy chain variable region includes the amino acid sequence of SEQ ID NO:47 and the light chain variable region includes the amino acid sequence of any one of SEQ ID NO: 48.

[0116] In one embodiment, the CDRs are those of 312E10A9.p2.Z7, as exemplified in **Table A3**. In one embodiment, the VH CDR1 includes the amino acid sequence of SEQ ID NO: 17 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VH CDR2 includes the amino acid sequence of SEQ ID NO: 18 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VH CDR3 includes the amino acid sequence of SEQ ID NO: 19 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VL CDR1 includes the amino acid sequence of SEQ ID NO: 20 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VL CDR2 includes the amino acid sequence of SEQ ID NO: 21 or

a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the VL CDR3 includes the amino acid sequence of SEQ ID NO: 22 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0117] Also provided, in some embodiments, are those that include the same CDRs as 312E10A9.p2.Z7. In some embodiments, the disclosed antibodies and fragments include those that bind to the same epitope as 312E10A9.p2.Z7, and those that compete with any of them in binding to CDH17.

[0118] In some embodiments, the heavy chain variable region includes an amino acid sequence selected from the group consisting of SEQ ID NO:49, or a peptide having at least 90% sequence identity to an amino acid sequence of SEQ ID NO:49.

[0119] In some embodiments, the light chain variable region includes an amino acid sequence selected from the group consisting of SEQ ID NO:50, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 50.

[0120] In some embodiments, the heavy chain variable region includes the amino acid sequence of SEQ ID NO:49 and the light chain variable region includes the amino acid sequence of any one of SEQ ID NO: 50.

[0121] In one embodiment, the CDRs are those of 135B2E11.p1.z1, as exemplified in **Table A3**. In one embodiment, the VH CDR1 includes the amino acid sequence of SEQ ID NO: 23 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VH CDR2 includes the amino acid sequence of SEQ ID NO: 24 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VH CDR3 includes the amino acid sequence of SEQ ID NO: 25 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VL CDR1 includes the amino acid sequence of SEQ ID NO: 26 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VL CDR2 includes the amino acid sequence of SEQ ID NO: 27 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the VL CDR3 includes the amino acid sequence of SEQ ID NO:

28 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0122] Also provided, in some embodiments, are those that include the same CDRs as 135B2E11.p1.z1. In some embodiments, the disclosed antibodies and fragments include those that bind to the same epitope as 135B2E11.p1.z1, and those that compete with any of them in binding to CDH17.

[0123] In some embodiments, the heavy chain variable region includes an amino acid sequence selected from the group consisting of SEQ ID NO:51, or a peptide having at least 90% sequence identity to an amino acid sequence of SEQ ID NO:51.

[0124] In some embodiments, the light chain variable region includes an amino acid sequence selected from the group consisting of SEQ ID NO:52, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 52.

[0125] In some embodiments, the heavy chain variable region includes the amino acid sequence of SEQ ID NO:51 and the light chain variable region includes the amino acid sequence of any one of SEQ ID NO: 52.

[0126] In one embodiment, the CDRs are those of 153A3D7.p3.z5, as exemplified in **Table A3**. In one embodiment, the VH CDR1 includes the amino acid sequence of SEQ ID NO: 29 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VH CDR2 includes the amino acid sequence of SEQ ID NO: 30 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VH CDR3 includes the amino acid sequence of SEQ ID NO: 31 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VL CDR1 includes the amino acid sequence of SEQ ID NO: 32 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VL CDR2 includes the amino acid sequence of SEQ ID NO: 33 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the VL CDR3 includes the amino acid sequence of SEQ ID NO: 34 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0127] Also provided, in some embodiments, are those that include the same CDRs as 153A3D7.p3.z5. In some embodiments, the disclosed antibodies and fragments include those that bind to the same epitope as 153A3D7.p3.z5, and those that compete with any of them in binding to CDH17.

[0128] In some embodiments, the heavy chain variable region includes an amino acid sequence selected from the group consisting of SEQ ID NO:53, or a peptide having at least 90% sequence identity to an amino acid sequence of SEQ ID NO:53.

[0129] In some embodiments, the light chain variable region includes an amino acid sequence selected from the group consisting of SEQ ID NO:54, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 54.

[0130] In some embodiments, the heavy chain variable region includes the amino acid sequence of SEQ ID NO:53 and the light chain variable region includes the amino acid sequence of any one of SEQ ID NO: 54.

[0131] In one embodiment, the CDRs are those of 35E7D6.p1.z9, as exemplified in **Table A3**. In one embodiment, the VH CDR1 includes the amino acid sequence of SEQ ID NO: 35 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VH CDR2 includes the amino acid sequence of SEQ ID NO: 36 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VH CDR3 includes the amino acid sequence of SEQ ID NO: 37 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VL CDR1 includes the amino acid sequence of SEQ ID NO: 38 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VL CDR2 includes the amino acid sequence of SEQ ID NO: 39 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the VL CDR3 includes the amino acid sequence of SEQ ID NO: 40 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0132] Also provided, in some embodiments, are those that include the same CDRs as 35E7D6.p1.z9. In some embodiments, the disclosed antibodies and fragments include those

that bind to the same epitope as 35E7D6.p1.z9, and those that compete with any of them in binding to CDH17.

[0133] In some embodiments, the heavy chain variable region includes an amino acid sequence selected from the group consisting of SEQ ID NO:55, or a peptide having at least 90% sequence identity to an amino acid sequence of SEQ ID NO:55.

[0134] In some embodiments, the light chain variable region includes an amino acid sequence selected from the group consisting of SEQ ID NO:56, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 56.

[0135] In some embodiments, the heavy chain variable region includes the amino acid sequence of SEQ ID NO:55 and the light chain variable region includes the amino acid sequence of any one of SEQ ID NO: 56.

[0136] In one embodiment, the CDRs are those of 155B9A5.p3.V2.z2, as exemplified in **Table A3**. In one embodiment, the VH CDR1 includes the amino acid sequence of SEQ ID NO: 41 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VH CDR2 includes the amino acid sequence of SEQ ID NO: 42 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VH CDR3 includes the amino acid sequence of SEQ ID NO: 43 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VL CDR1 includes the amino acid sequence of SEQ ID NO: 44 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VL CDR2 includes the amino acid sequence of SEQ ID NO: 45 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the VL CDR3 includes the amino acid sequence of SEQ ID NO: 46 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0137] Also provided, in some embodiments, are those that include the same CDRs as 155B9A5.p3.V2.z2. In some embodiments, the disclosed antibodies and fragments include those that bind to the same epitope as 155B9A5.p3.V2.z2, and those that compete with any of them in binding to CDH17.

[0138] In some embodiments, the heavy chain variable region includes an amino acid sequence selected from the group consisting of SEQ ID NO:57, or a peptide having at least 90% sequence identity to an amino acid sequence of SEQ ID NO:57.

[0139] In some embodiments, the light chain variable region includes an amino acid sequence selected from the group consisting of SEQ ID NO:58, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 58.

[0140] In some embodiments, the heavy chain variable region includes the amino acid sequence of SEQ ID NO:57 and the light chain variable region includes the amino acid sequence of any one of SEQ ID NO: 58.

[0141] In one embodiment, the CDRs are those of 8G5G9.p1.z12, as exemplified in **A2**. In one embodiment, the VH CDR1 includes the amino acid sequence of SEQ ID NO: 1 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VH CDR2 includes the amino acid sequence of SEQ ID NO: 2 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VH CDR3 includes the amino acid sequence of SEQ ID NO: 3 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VL CDR1 includes the amino acid sequence of SEQ ID NO: 4 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VL CDR2 includes the amino acid sequence of SEQ ID NO: 5 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the VL CDR3 includes the amino acid sequence of SEQ ID NO: 6 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0142] Also provided, in some embodiments, are those that include the same CDRs as 8G5G9.p1.z12. In some embodiments, the disclosed antibodies and fragments include those that bind to the same epitope as 8G5G9.p1.z12, and those that compete with any of them in binding to CDH17.

[0143] In certain embodiments, the antibodies and antigen-binding fragment thereof are humanized and comprise a heavy chain variable region comprising an amino acid sequence

of SEQ ID NO: 7, or a peptide having at least 90%, at least 95%, or at least 98% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 7.

[0144] In certain embodiments, the antibodies and antigen-binding fragment thereof are humanized and comprise a light chain variable region comprising an amino acid sequence of SEQ ID NO: 8, or a peptide having at least 90%, at least 95%, or at least 98% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 8.

[0145] In certain embodiments, the antibody provided herein further comprises a heavy chain constant region, a light chain constant region, an Fc region, or the combination thereof.

[0146] In certain embodiments, the antibody provided herein further comprises a heavy chain constant region of SEQ ID NO: 9, or a peptide having at least 90%, at least 95%, or at least 98% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 9.

[0147] In certain embodiments, the antibody provided herein further comprises a light chain constant region of SEQ ID NO: 10, or a peptide having at least 90%, at least 95%, or at least 98% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 10.

[0148] The Fc region can be engineered to enhance effector function. IgG antibodies can induce direct anti-tumor effects by way of indirect anti-tumor effects via the Fc-mediated effector functions that engage other immune cells or killer mechanisms. “Effector functions” or “antibody effector functions” as used herein refer to biological activities attributable to the binding of Fc region of an antibody to its effectors such as C1 complex and Fc receptor (FcγRIIa or FcγRIIIa). Exemplary effector functions include: complement dependent cytotoxicity (CDC) induced by interaction of antibodies and C1q on the C1 complex; antibody-dependent cell-mediated cytotoxicity (ADCC) induced by binding of Fc region of an antibody to Fc receptor on an effector cell; and antibody dependent cell mediated phagocytosis (ADCP), where nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause phagocytosis of the target cell. Among the four IgG subclasses, IgG1 and IgG3 induce the strongest Fc-effector functions. However, since IgG1 has the longest half-life and is more stable than IgG3, most therapeutic antibodies with Fc-mediated functions are of IgG1 isotype.

[0149] In certain embodiments, the Fc region of the antibodies provided herein is engineered to enhance the effector function, such as ADCC or CDC. Various methods (mainly Fc mutations) can be found to enhance the Fc-mediated effector function, such as those described in the PCT publications WO2007024249A2 and WO2011044368A1, which are incorporated herein by reference in their entireties. In certain embodiments, the Fc region is engineered to include S298A/E333A/K334A mutations in human IgG1. Such Fc mutation combination is reported to enhance binding to FcγRIIIa, thus enhancing ADCC (See Shields R.L. et al, J. Biol. Chem 276:6591-6604 (2001)).

[0150] In certain embodiments, the antibody comprises an amino acid sequence or one or more moieties not normally associated with an antibody. Exemplary modifications are described in more detail below. For example, an antibody of the disclosure may comprise a flexible linker sequence, or may be modified to add a functional moiety (*e.g.*, PEG, a drug, a toxin, or a label).

[0151] Antibodies, variants, or derivatives thereof of the disclosure include derivatives that are modified, *i.e.*, by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from binding to the epitope. For example, but not by way of limitation, the antibodies can be modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the antibodies may contain one or more non-classical amino acids.

[0152] The antibodies can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antigen-binding polypeptide is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, thionin acridinium ester, imidazole, acridinium salt and oxalate ester.

[0153] The antibodies can also be detectably labeled using fluorescence emitting metals such as ¹⁵²Eu, or others of the lanthanide series. These metals can be attached to the antibody

using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA). Techniques for conjugating various moieties to an antibody are well known, *see, e.g.*, Arnon *et al.*, “Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy”, in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. (1985); Hellstrom *et al.*, “Antibodies For Drug Delivery”, in *Controlled Drug Delivery* (2nd Ed.), Robinson *et al.*, (eds.), Marcel Dekker, Inc., pp. 623- 53 (1987); Thorpe, “Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review”, in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera *et al.* (eds.), pp. 475-506 (1985); “Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy”, in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin *et al.* (eds.), and Academic Press pp. 303-16 (1985).

Bi-functional Molecules and Combination Therapies

[0154] CDH17 is overexpressed on tumor cells. As a tumor antigen targeting molecule, an antibody or antigen-binding fragment specific to CDH17 can be combined with a second antigen-binding fragment specific to an immune cell, or an antigen-binding fragment specific to an immune checkpoint to generate a combination therapy or a bispecific antibody.

[0155] In some embodiments, the immune cell is selected from the group consisting of a T cell, a B cell, a monocyte, a macrophage, a neutrophil, a dendritic cell, a phagocyte, a natural killer cell, an eosinophil, a basophil, and a mast cell. Molecules on the immune cell which can be targeted include, for example, CCL1, CD3, CD16, CD19, CD28, and CD64. Other examples include PD-1, PD-L1, CTLA-4, LAG-3 (also known as CD223), CD28, CD122, 4-1BB (also known as CD137), TIM3, OX-40 or OX40L, CD40 or CD40L, LIGHT, ICOS/ICOSL, GITR/GITRL, TIGIT, CD27, VISTA, B7H3, B7H4, HEVM or BTLA (also known as CD272), killer-cell immunoglobulin-like receptors (KIRs), and CD47.

[0156] Different format of bispecific antibodies are also provided. In some embodiments, each of the anti-CDH17 fragment and the second fragment each is independently selected from a Fab fragment, a single-chain variable fragment (scFv), or a single-domain antibody. In some embodiments, the bispecific antibody further includes a Fc fragment.

[0157] Bifunctional molecules that include not just antibody or antigen binding fragment are also provided. As a tumor antigen targeting molecule, an antibody or antigen-binding fragment specific to CDH17, such as those described here, can be combined with an immune cytokine or ligand optionally through a peptide linker. The linked immune cytokines or ligands include, but not limited to, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, IL-15, GM-CSF, TNF- α , CD40L, OX40L, CD27L, CD30L, 4-1BBL, LIGHT and GITRL. Such bi-functional molecules can combine the immune checkpoint blocking effect with tumor site local immune modulation.

Chimeric Antigen Receptors (CAR)

[0158] The present disclosure provides a chimeric antigen receptor (CAR) or an engineered T cell receptors (TCRs) comprising the anti-CDH17 antigen-binding fragment thereof provided herein.

[0159] CARs and TCRs are genetically engineered receptors. These engineered receptors can be readily inserted into and expressed by immune cells, such as T cells. A CAR can recognize a specific antigen and, when bound to that antigen, activate the immune cell to attack and destroy the cell bearing that antigen. When these antigens exist on tumor cells, an immune cell that expresses the CAR can target and kill the tumor cell.

[0160] CARs can be engineered to bind to an antigen (such as a cell-surface antigen) by incorporating an antigen binding molecule that interacts with that targeted antigen. Preferably, the antigen binding molecule is an antibody fragment thereof, and more preferably one or more single chain antibody fragment ("scFv"). An scFv is a single chain antibody fragment having the variable regions of the heavy and light chains of an antibody linked together. It will be appreciated that the antigen binding molecule is typically contained within the extracellular portion of the CAR such that it is capable of recognizing and binding to the antigen of interest. Bispecific and multispecific CARs are contemplated within the scope of the invention, with specificity to more than one target of interest.

[0161] An example CAR includes, in addition to the antigen binding molecule, a transmembrane domain and an activation domain. In some embodiments, the CAR can further include an extracellular domain between the antigen binding molecule and the

transmembrane domain. The extracellular domain may comprise a hinge domain. In some embodiments, the CAR can further include a co-stimulatory domain.

A. Antigen Binding Molecule

[0162] The antigen binding portion of the CAR of the instant disclosure can be an antibody or antigen binding fragment (in particular a scFv) targeting human CDH17, as disclosed herein.

B. Extracellular Domain and Transmembrane Domain

Extracellular Domains

[0163] Chimeric antigen receptors may comprise an extracellular domain. Extracellular domains of particular use in this invention may be derived from (*i.e.*, comprise) all or some of CD28, OX-40, 4-1BB/CD137, CD2, CD7, CD27, CD30, CD40, programmed death-1 (PD-1), inducible T cell costimulator (ICOS), lymphocyte function-associated antigen-1 (LFA-1, CD1-1a/CD18), CD3 gamma, CD3 delta, CD3 epsilon, CD247, CD276 (B7-H3), LIGHT, (TNFSF14), NKG2C, Ig alpha (CD79a), DAP-10, Fc gamma receptor, MHC class 1 molecule, TNF receptor proteins, an Immunoglobulin protein, IgG1, IgG2, IgG3, IgG4, IgA, IgD, IgE, IgM, cytokine receptor, integrins, Signaling Lymphocytic Activation Molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, ICAM-1, B7-H3, CDS, ICAM-1, GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL-2R beta, IL-2R gamma, IL-7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, a ligand that specifically binds with CD83, or any combination thereof. The extracellular domain may be derived either from a natural or from a synthetic source.

[0164] Extracellular domains often comprise the hinge portion, sometimes referred to as the “spacer” region. A variety of hinges can be employed in accordance with the invention,

including portions or derivatives of the molecules as listed above. In certain embodiments, the hinge portion is a hinge region of IgG1, IgG2, IgG3, IgG4, IgA, IgD, IgE, IgM, CD28, or CD8 alpha, any truncation thereof, or any combination thereof.

Transmembrane Domain

[0165] The CAR can be designed with a transmembrane domain that is fused to the extracellular domain of the CAR. It can similarly be fused to the intracellular domain of the CAR. In some instances, the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex. The transmembrane domain may be derived either from a natural or from a synthetic source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein.

[0166] Non-limiting examples of such proteins include CD28, CD8alpha, CD8beta, 4-1BB, B7-H3, BAFR, BLAME, BTLA, CD100, CD103, CD11a, CD11b, CD11c, CD11d, CD160, CD18, CD19, CD19a, CD2, CD247, CD27, CD276, CD29, CD3 delta, CD3 epsilon, CD3 gamma, CD30, CD4, CD40, CD49a, CD49D, CD49f, CD69, CD7, CD79a, CD84, CD96, CDS, CEACAM1, CRT AM, DAP-10, DNAM1, Fc gamma receptor, GADS, GITR, HVEM, IA4, ICAM-1, ICOS, IL-2R beta, IL-2R gamma, IL-7R alpha, ITGA4, ITGA6, ITGAD, ITGAE, ITGAL, ITGAM, ITGAX, ITGB1, ITGB2, ITGB7, KIRDS2, LAT, LFA-1, LIGHT, LTBR, Ly9, NKG2C, NKG2D, NKp30, NKp44, NKp46, NKp80, OX-40, PAG, PD-1, PSGL1, SELPLG, SLAM, SLAMF4, SLAMF6, SLAMF7, SLP-76, TNFR2, TNFSF14, TRANCE, VLA1, VLA-6, a cytokine receptor, a MHC class I molecule, a SLAM protein, a TNF receptor protein, a Toll ligand receptor, an activating NK cell receptor, an immunoglobulin protein, and an integrin.

C. Intracellular Domain---Costimulatory Domain and Activation Domain

[0167] The intracellular (cytoplasmic) domain of the engineered T cells of the invention can provide activation of at least one of the normal effector functions of the immune cell. Effector function of a T cell, for example, may refer to cytolytic activity or helper activity, including the secretion of cytokines. The intracellular domain may include at least an activation domain. The intracellular domain can also include one or more costimulatory domains.

Costimulatory Domains

[0168] A “costimulatory domain” as used herein refers to a molecule that provides a signal which mediates a T cell response, including, but not limited to, proliferation, activation, differentiation, and the like. Costimulatory domain can provide a signal in addition to the primary signal provided by an activating molecule as described herein.

[0169] Chimeric antigen receptors may incorporate costimulatory (signaling) domains to increase their potency. See U.S. Patent Nos. 7,741,465, and 6,319,494, as well as Krause *et al.* and Finney *et al.* (supra), Song *et al.*, Blood 119:696-706 (2012); Kalos *et al.*, Sci Transl. Med. 3:95 (2011); Porter *et al.*, N. Engl. J. Med. 365:725-33 (2011), and Gross *et al.*, Annu. Rev. Pharmacol. Toxicol. 56:59–83 (2016). For example, CD28 is a costimulatory protein found naturally on T-cells. A variety of costimulatory molecules are set forth herein, but it will be appreciated that additional costimulatory molecules are also included within the scope of this invention.

[0170] It will be appreciated that suitable costimulatory domains within the scope of the invention include the signaling domain (or other suitable portion) of CD28, OX-40, 4-1BB/CD137, CD2, CD7, CD27, CD30, CD40, Programmed Death-1 (PD-1), inducible T cell costimulator (ICOS), lymphocyte function-associated antigen-1 (LFA-1, CD1-1a/CD18), CD3 gamma, CD3 delta, CD3 epsilon, CD247, CD276 (B7-H3), LIGHT, (TNFSF14), NKG2C, Ig alpha (CD79a), DAP-10, Fc gamma receptor, MHC class 1 molecule, TNF receptor proteins, an Immunoglobulin protein, cytokine receptor, integrins, Signaling Lymphocytic Activation Molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, ICAM-1, B7-H3, CDS, ICAM-1, GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL-2R beta, IL-2R gamma, IL-7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD1 1d, ITGAE, CD103, ITGAL, CD1 1a, LFA-1, ITGAM, CD1 1b, ITGAX, CD1 1c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, a ligand that specifically binds with CD83, or fragments or any combination thereof. It will be

appreciated that additional costimulatory molecules, or fragments thereof, not listed above are within the scope of the invention.

Activating Domains

[0171] An “activation domain” refers to a molecule on a T cell, *e.g.*, the TCR/CD3 complex that specifically binds with a cognate stimulatory ligand present on an antigen present cell. Suitable activating molecules are described herein. The activation domain is part of the intracellular (cytoplasmic) portion of a CAR. The intracellular domain of a CAR can provide activation of at least one of the normal effector functions of the immune cell. Effector function of a T cell, for example, may refer to cytolytic activity or helper activity, including the secretion of cytokines.

[0172] CD3 is an element of the T cell receptor on native T cells, and has been shown to be an important intracellular activating element in CARs. In a preferred embodiment, the CD3 is CD3 zeta, CD3 epsilon, CD3 delta, or CD3 gamma.

CAR-Immune Cells

[0173] Immune cells enclosing a CAR of the present disclosure or one or more polynucleotides encoding the CAR are also provided.

[0174] As described, an engineered receptors can be inserted into and expressed by an immune cell, such as T cell, which can recognize a specific antigen and, when bound to that antigen, activate the immune cell to attack and destroy the cell bearing that antigen.

[0175] Various types of immune cells have been tested for expressing CARs. Non-limiting examples include T cells, NK cells, macrophages and monocytes. A T cell, in some embodiment, may be an alpha beta T cell. In some embodiments, the T cell is a gamma delta T cell.

Polynucleotides Encoding the Antibodies and Methods of Preparing the Antibodies

[0176] The present disclosure also provides isolated polynucleotides or nucleic acid molecules encoding the antibodies, variants or derivatives thereof of the disclosure. The polynucleotides of the present disclosure may encode the entire heavy and light chain variable regions of the antigen-binding polypeptides, variants or derivatives thereof on the

same polynucleotide molecule or on separate polynucleotide molecules. Additionally, the polynucleotides of the present disclosure may encode portions of the heavy and light chain variable regions of the antigen-binding polypeptides, variants or derivatives thereof on the same polynucleotide molecule or on separate polynucleotide molecules.

[0177] Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as import residues, which are typically taken from an import variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., Nature, 321: 522-525 (1986); Riechmann et al., Nature 332: 323-327 (1988); Verhoeven et al., Science, 239: 1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such humanized antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

Antibody-drug conjugates

[0178] The present disclosure provides antibody-drug conjugates (ADC) of the anti-CDH17 antibodies. The anti-CDH17 ADCs show comparable binding affinity to CDH17 as compared with the antibodies and have significant cytotoxic effect on CDH17-expressing tumor cells and prominent *in vivo* anti-tumor effect. In addition, the anti-CDH17 ADCs showed potent bystander effects to human CDH17-negative cell lines.

[0179] A “bystander effect” is usually used to describe the capability of the antibody-targeted cytotoxic agents in eradicating not only antigen-positive cells but also antigen-negative neighboring cells. The efficacy of an antibody-drug conjugate is generally compromised by the heterogeneous expression of the target antigen in some tumors, because targeted delivery of conjugated drug via antibody binding to the antigen-positive tumor cells will spare any antigen-negative tumor cells to which the antibody cannot bind. Furthermore, tumor vasculature is an attractive target that is attracting more and more attention as a way to shrink

tumors by starving them of their blood supply. Tumor vasculature is usually tumor antigen-negative.

[0180] The antibody-drug conjugate comprises antibody or the antigen-binding fragment thereof conjugated to a drug (or payload) via a linker. The drugs can be connected to the antibodies via a cysteine residue or a lysine residue on the antibodies. The ADC can be constructed via chemical approach such as stochastic conjugation on pre-existing lysine or cysteine residues via appropriate coupling reactions, such as amide coupling (of lysine) and sulfhydryl coupling (of cysteine). An active carboxylic acid ester (when available in the linker) is used to connect payloads to lysine residues on the antibody. The primary amine in Lys easily reacts with N-hydroxysuccinimide (NHS) esters introduced into the linker, forming a stable amide. A typical IgG1 antibody molecule has roughly 90 Lys residues, of which approximately 30 can be modified for conjugation, implying that between 1 and 30 payloads can be covalently coupled to the antibody. As for cysteine, after reduction, the disulfide bond could transform to expose free cysteine residues which are accessible for coupling reactions, such as Michael additions, disulfide formation, and α -halo carbonyl alkylations.

[0181] The ADC can also be constructed via enzymatic approach such as site-specific conjugation. The site-specific conjugation includes introduction of engineered reactive cysteine residues, disulfide re-bridging, unnatural amino acids, enzyme-assisted ligation, or glycan remodeling, glycoconjugation or click chemistry. Details of the methods of conjugation can be found in the art, such as Fu et al., *Signal Transduction and Targeted Therapy* 7:93 (2022).

[0182] The drug-antibody ratio (DAR) for an ADC can be more than 2, more than 4, more than 6, more than 8, more than 10, more than 16, more than 20, or more than 30. In certain embodiments, the drug-antibody ratio (DAR) can be from 1-30, from 1-20, from 1-10, from 1-8, such as, 4-8, or 2-4. In certain embodiments, the drug-antibody ratio (DAR) can be 2, 4, 6, 8, 10, 16, 20, or 30. In certain embodiments, the DAR is homogenous.

[0183] The linker contained in the drug-linker compound can be non-cleavable linker and cleavable linker. The cleavable linker mainly includes enzyme-cleavable linkers and chemically sensitive linkers.

[0184] Non-cleavable linkers consist of stable bonds that resist proteolytic degradation and ensure greater plasma stability. The mechanism of action of non-cleavable linkers is based on the internalization of the ADC complex followed by degradation of the mAb component in the lysosome, resulting in the release of a cytotoxic drug that kills tumor cells. They do not unleash cytotoxic agents at off-target sites and thus do not harm healthy cells. Non-cleavable linkers are divided into two groups, namely thioether or maleimidocaproyl (MC). Examples of the non-cleavable linkers include, but not limited to, 4-maleimidomethyl cyclohexane-1-carboxylate (MCC), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), maleimidecaproyl (MC), and p-carboxycyclo hexylmethylmaleimide.

[0185] Cleavable linkers mainly include chemically sensitive linkers those are usually cleaved by environmental differences (such as redox potential, pH) and enzyme-cleavable linkers which are cleaved by specific enzymes in response to extracellular and intracellular environments.

[0186] Chemically sensitive linkers include, but not limited to, types of pH-sensitive linkers, and glutathione-sensitive disulfide linkers. (Khongorzul et al., Mol Cancer Res; 18(1) (2020)).

[0187] PH-sensitive linkers are a group of linkers that are sensitive to the acidic environment but are stable in the alkaline environment such as systemic circulation, such as hydrozone based linker. One successful example of ADC design using pH-sensitive linker is the IMMU-110 which is composed of a humanized anti-CD74 mAb conjugated to doxorubicin via acid-labile hydrazone.

[0188] Glutathione-sensitive disulfide linkers utilize difference in reduction potential in the cytoplasm in contrast to plasma. A high concentration of glutathione can be found in cancer cells than normal cells. Glutathione-sensitive linkers are stable in the blood flow and particularly cleaved by the elevated intracellular concentration of glutathione in the tumor cell, releasing the active drugs at the tumor sites from the nontoxic prodrugs (see *supra*).

[0189] Enzyme-cleavable linkers include, but not limited to, peptide-based linkers, β -glucuronide based linkers and phosphate based linkers.

[0190] Peptide based linkers, also known as protease-sensitive linkers, are the most commonly used ADC linkers. These linkers can be cleaved by specific proteases extracellularly and/or intracellularly. For intracellular cleavage, as tumor cells exhibit high

expression of lysosomal proteases like cathepsin B compared with the normal cells, therefore, proteases-sensitive peptide linker ADCs are selectively bound to and transformed into cancerous cells through receptor mediated endocytosis. The peptide linkers are stable in the systemic circulation and only unleash the drug in the target cells (see *supra*). Examples are Valine-citrulline (VC or Val-Cit), Valine-alanine (VA), phenylalanine-lysine (PL), and glycine-glycine-phenylalanine-glycine (GGFG).

[0191] β -glucuronide based linkers are recognized and hydrolyzed by β -glucuronidase or β -galactosidase for the drug release, while the β -glucuronidase and β -galactosidase are enriched in lysosomes and tumor necrotic regions. β -glucuronidase is inactive at physiologic pH (blood circulation) and active at lysosomal pH. Such selective site of action allows for the cleavage of the glycosidic linkage of the β -glucuronidase-sensitive β -glucuronide linker, thereby enabling the selective release of cytotoxic payloads (see *supra*).

[0192] Phosphate based linkers are a class of enzyme-cleavable linkers expressed exclusively to target enzymes in the lysosomal compartment. These linkers target pyrophosphatase and acid phosphatase enzymes, which hydrolyze pyrophosphates and terminal monophosphates into their respective alcohols.

[0193] Moieties that can be introduced as part of the linkers to facilitate the drug-linker connection or antibody-linker connection include maleimidocaproyl, maleimide-caproyl (MC) moiety, maleimide-methylene-cyclohexyl carbonyl moiety spacer, maleimide propoyl (MP), para-aminobenzylcarbamate (PAB) spacer, para-aminobenzylloxycarbonyl (PABC) spacer, aminomethoxy methylenecarbonyl (-NH-CH₂-O-CH₂-CO-) spacer, 4-(4'-acetylphenoxy) butanoic acid moiety, acylhydrazide (-CO-NH-NH₂) and thiol (-SH) moieties, Lys-PABC, PEG chain and PEG8.

[0194] Examples of the linkers include, but not limited to succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), sulfo-SMCC, MC-VC-PABC, CL2A, MC- GGFG, MC, MC-PEG8-GGFG, MC-PEG8-GGFG-PAB, or MP-PEG8-VA-PABC.

[0195] The drug or payload can be a cytotoxin, a therapeutic peptide or polypeptide.

[0196] The peptide or polypeptide may be any peptide or polypeptide which has therapeutic properties, for example antinociceptive, antidiabetes, antitumor or antiviral activity. Additionally, or alternatively, the drug preferably comprises an amine group, a thiol group, or

a carboxylic acid group, as these types of groups provide ideal sites for conjugation of the drug with the linker of the present disclosure. Examples of biologics drug are pseudomonas aeruginosa exotoxin PE38, diphtheria toxin, staphylococcus aureus enterotoxin A/E-120, shigatoxin, ricin, and urease.

[0197] The cytotoxin are activated after release from ADC inside the cytoplasm of tumor cells and are able to destroy the tumor cells. There are two main classes of cytotoxins that can be used in the ADC designs: microtubule-disrupting agents (such as auristatins and maytansinoids) and DNA-damaging agents (such as calicheamicins, duocarmycin, and doxorubicin). Examples included, but not limited to includes auristatins, maytansinoids, benzodiazepines, tubulysins, duocarmycin, calicheamicins, exatecans, irinotecans (SN38), doxorubicin, anthracycline, pyrrolbenzodiazepenes (PBD), TLR agonist, and STING agonists.

[0198] Auristatins are synthetic antineoplastic agents derived from the natural product dolastatin 10. They block the tubulin polymerization process resulting in cell-cycle arrest and apoptosis. Examples include monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF).

[0199] Maytansinoids are isolated from the maytansine, a benzoansamacrolide. These drugs inhibit tubulin polymerization. Examples include DM1 and DM4.

[0200] Calicheamicins are a class of enediyne antitumor antibiotics derived from the bacterium *Micromonospora echinospora*. Calicheamicin recognizes the minor groove of DNA and halts DNA replication resulting in mitotic arrest and cell death. One example is N-acetyl-calicheamicin, a derivative of calicheamicin.

[0201] Duocarmycin is a natural product derivative extracted from the bacteria *Streptomyces* strains. Duocarmycins are another class of DNA minor groove-binding alkylating agents. This class of drugs shows its action by binding to the minor groove of DNA and subsequently cause irreparable alkylation of DNA that disrupts the nucleic acid architecture and structural integrity.

[0202] Doxorubicin shows its action by intercalation of DNA that inhibits DNA synthesis. One example is IMMU-110.

[0203] Exatecans are synthetic derivatives of the natural cytotoxin, camptothecin, isolated from the Chinese tree *Camptotheca acuminata*. Like camptothecin, exatecan binds to the topoisomerase 1-DNA complex, preventing DNA re-ligation which results in the accumulation of DNA strand breaks and ultimately leads to cell death. Examples of exatecans are DX-8951f and DXd. 7-ethyl-10-hydroxycamptothecin (SN-38) is also a derivative of camptothecin.

[0204] Further examples of the cytotoxin includes, without limitation, tublysin A, camptothecin, DGN462, Amberstatin269, anthramycin, SG3199/SCX, IRDye®700DX, TLR7/8 agonist, diABZI STING agonist-2.

[0205] The cytotoxin may be a chemotherapeutic agent which may be categorized by their mechanism of action into, for example, the following groups:

- anti-metabolites/anti-cancer agents such as pyrimidine analogs floxuridine, capecitabine, and cytarabine;
- purine analogs, folate antagonists, and related inhibitors;
- antiproliferative/antimitotic agents including natural products such as vinca alkaloid (vinblastine, vincristine) and microtubule such as taxane (paclitaxel, docetaxel), vinblastin, nocodazole, epothilones, vinorelbine (NAVELBINE®), and epipodophyllotoxins (etoposide, teniposide);
- DNA damaging agents such as actinomycin, amsacrine, busulfan, carboplatin, chlorambucil, cisplatin, cyclophosphamide (CYTOXAN®), dactinomycin, daunorubicin, doxorubicin, epirubicin, ifosfamide, melphalan, merchlorethamine, mitomycin, mitoxantrone, nitrosourea, procarbazine, taxol, taxotere, teniposide, etoposide, and triethylenethiophosphoramide;
- antibiotics such as dactinomycin, daunorubicin, doxorubicin, idarubicin, anthracyclines, mitoxantrone, bleomycins, plicamycin (mithramycin), and mitomycin;
- enzymes such as L-asparaginase which systemically metabolizes L-asparagine and deprives cells which do not have the capacity to synthesize their own asparagine;
- antiplatelet agents;

- antiproliferative/antimitotic alkylating agents such as nitrogen mustards cyclophosphamide and analogs (melphalan, chlorambucil, hexamethylmelamine, and thiotepa), alkyl nitrosoureas (carmustine) and analogs, streptozocin, and triazenes (dacarbazine);
- antiproliferative/antimitotic antimetabolites such as folic acid analogs (methotrexate);
- platinum coordination complexes (cisplatin, oxiloplatin, and carboplatin), procarbazine, hydroxyurea, mitotane, and aminoglutethimide;
- hormones, hormone analogs (estrogen, tamoxifen, goserelin, bicalutamide, and nilutamide), and aromatase inhibitors (letrozole and anastrozole);
- anticoagulants such as heparin, synthetic heparin salts, and other inhibitors of thrombin;
- fibrinolytic agents such as tissue plasminogen activator, streptokinase, urokinase, aspirin, dipyridamole, ticlopidine, and clopidogrel;
- antimigratory agents;
- antisecretory agents (breveldin);
- immunosuppressives tacrolimus, sirolimus, azathioprine, and mycophenolate;
- compounds (TNP-470, genistein) and growth factor inhibitors (vascular endothelial growth factor inhibitors and fibroblast growth factor inhibitors);
- angiotensin receptor blockers, nitric oxide donors;
- anti-sense oligonucleotides;
- antibodies such as trastuzumab and rituximab;
- cell cycle inhibitors and differentiation inducers such as tretinoin;
- inhibitors, topoisomerase inhibitors (doxorubicin, daunorubicin, dactinomycin, eniposide, epirubicin, etoposide, idarubicin, irinotecan, mitoxantrone, topotecan, and irinotecan), and corticosteroids (cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisone, and prednisolone);
- growth factor signal transduction kinase inhibitors;
- dysfunction inducers;

- toxins such as Cholera toxin, ricin, Pseudomonas exotoxin, Bordetella pertussis adenylate cyclase toxin, diphtheria toxin, and caspase activators;
- and chromatin.

[0206] Further examples of chemotherapeutic agents include:

- alkylating agents such as thiotepa and cyclophosphamide (CYTOXAN®);
- alkyl sulfonates such as busulfan, improsulfan, and piposulfan;
- aziridines such as benzodopa, carboquone, meturedopa, and uredopa;
- emylerumines and memylamelamines including alfretamine, triemylemelamine, triethylenephosphoramidate, triethylenethiophosphoramidate, and trimemylolomelamine;
- acetogenins, especially bullatacin and bullatacinone;
- a camptothecin, including synthetic analog topotecan;
- bryostatin;
- callistatin;
- CC-1065, including its adozelesin, carzelesin, and bizelesin synthetic analogs;
- cryptophycins, particularly cryptophycin 1 and cryptophycin 8;
- dolastatin;
- duocarmycin, including the synthetic analogs KW-2189 and CBI-TMI;
- eleutherobin;
- pancratistatin;
- a sarcodictyin;
- spongistatin;
- nitrogen mustards such as chlorambucil, chlornaphazine, cyclophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, and uracil mustard;
- nitrosoureas such as carmustine, chlorozotocin, foremustine, lomustine, nimustine, and ranimustine;
- antibiotics such as the enediyne antibiotics (*e.g.*, calicheamicin, especially calicheamicin gammaII and calicheamicin phiII), dynemicin including dynemicin A,

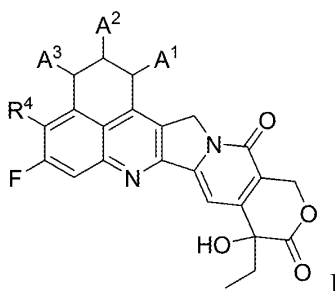
bisphosphonates such as clodronate, an esperamicin, neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromomophores, aclacinomycins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabycin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, and zorubicin;

- anti-metabolites such as methotrexate and 5-fluorouracil (5-FU);
- folic acid analogs such as demopterin, methotrexate, pteropterin, and trimetrexate;
- purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, and thioguanine;
- pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofer, cytarabine, dideoxyuridine, doxifluridine, enocitabine, and floxuridine;
- androgens such as calusterone, dromostanolone propionate, epitioestanol, mepitioestane, and testolactone;
- anti-adrenals such as aminoglutethimide, mitotane, and trilostane;
- folic acid replinishers such as frolinic acid;
- trichothecenes, especially T-2 toxin, verracurin A, roridin A, and anguidine;
- taxoids such as paclitaxel (TAXOL[®]) and docetaxel (TAXOTERE[®]);
- platinum analogs such as cisplatin and carboplatin;
- aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; hestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformthine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; leucovorin; lonidamine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; losoxantrone; fluoropyrimidine; folinic acid; podophyllinic acid; 2-ethylhydrazide; procarbazine; polysaccharide-K (PSK); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid;

triaziquone; 2,2',2''-tricyclohexylmethylamine; urethane; vindesine; dacarbazine; mannometrine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiopeta; chlorambucil; gemcitabine (GEMZAR®); 6-thioguanine; mercaptopurine; methotrexate; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vancristine; vinorelbine (NAVELBINE®); novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeoloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DFMO); retinoids such as retinoic acid; capecitabine; FOLFIRI (fluorouracil, leucovorin, and irinotecan);

- and pharmaceutically acceptable salts, acids, or derivatives of any of the above.

[0207] In certain embodiments, the drug and the linker form a drug-linker compound of Formula I:



or stereoisomer or pharmaceutically acceptable salt thereof, wherein:

A¹ is -NHR¹, where R¹ is hydrogen, -C(O)R⁵, -C₆ aryl-C₁₋₆ alkyl-NH₂, or -C₆ aryl-C₁₋₆ alkyl-NHC(O)R⁵; and A² and A³ are both hydrogen; or

A² is -NHR², where R² is hydrogen, -C(O)R⁵, -C₆ aryl-C₁₋₆ alkyl-NH₂, or -C₆ aryl-C₁₋₆ alkyl-NHC(O)R⁵; and A¹ and A³ are both hydrogen; or

A³ is -NHR³, where R³ is hydrogen, -C(O)R⁵, -C₆ aryl-C₁₋₆ alkyl-NH₂, or -C₆ aryl-C₁₋₆ alkyl-NHC(O)R⁵; and A¹ and A² are both hydrogen;

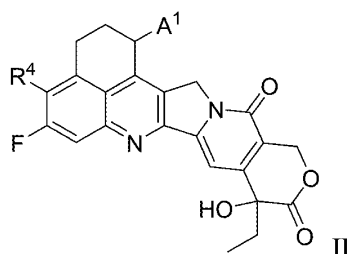
R⁴ is -C₁₋₆ alkyl; provided that when A¹ is -NHR¹, then R⁴ is other than methyl;

R⁵ is -L-R⁶;

L is a linker moiety; and

R⁶ is hydrogen or heterocyclyl, wherein said heterocyclyl is optionally covalently linked to an antibody or antigen-binding fragment. More details of these compounds and their synthesis can be seen in PCT patent application No: PCT/CN2023/123581.

[0208] In some embodiments, A^1 is $-NHR^1$, where R^1 is hydrogen, $-C(O)R^5$, $-C_6$ aryl- C_{1-6} alkyl- NH_2 , or $-C_6$ aryl- C_{1-6} alkyl- $NHC(O)R^5$; and A^2 and A^3 are both hydrogen. Accordingly, in some embodiments, provided is a compound of Formula II:



or stereoisomer or pharmaceutically acceptable salt thereof, wherein R^4 is $-C_{2-6}$ alkyl, and A^1 is independently as defined herein.

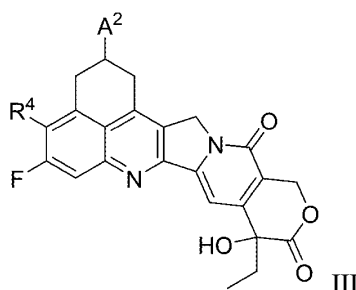
[0209] In some embodiments, A^1 is $-NH_2$; and A^2 and A^3 are both hydrogen.

[0210] In some embodiments, A^1 is $-NH-C(O)R^5$; and A^2 and A^3 are both hydrogen.

[0211] In some embodiments, A^1 is $-NH-C_6$ aryl- C_{1-6} alkyl- NH_2 ; and A^2 and A^3 are both hydrogen. In some embodiments, A^1 is $-NH-C_6$ aryl- CH_2-NH_2 ; and A^2 and A^3 are both hydrogen.

[0212] In some embodiments, A^1 is $-NH-C_6$ aryl- C_{1-6} alkyl- $NHC(O)R^5$; and A^2 and A^3 are both hydrogen. In some embodiments, A^1 is $-NH-C_6$ aryl- $CH_2-NHC(O)R^5$; and A^2 and A^3 are both hydrogen.

[0213] In some embodiments, A^2 is $-NHR^2$, where R^2 is hydrogen, $-C(O)R^5$, $-C_6$ aryl- C_{1-6} alkyl- NH_2 , or $-C_6$ aryl- C_{1-6} alkyl- $NHC(O)R^5$; and A^1 and A^3 are both hydrogen. Accordingly, in some embodiments, provided is a compound of Formula III:



or stereoisomer or pharmaceutically acceptable salt thereof, wherein A^2 and R^4 are each independently as defined herein.

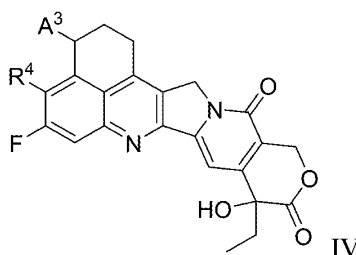
[0214] In some embodiments, A^2 is $-NH_2$; and A^1 and A^3 are both hydrogen.

[0215] In some embodiments, A^2 is $-NH-C(O)R^5$; and A^1 and A^3 are both hydrogen.

[0216] In some embodiments, A^2 is $-NH-C_6$ aryl- C_{1-6} alkyl- NH_2 ; and A^1 and A^3 are both hydrogen. In some embodiments, A^2 is $-NH-C_6$ aryl- CH_2-NH_2 ; and A^1 and A^3 are both hydrogen.

[0217] In some embodiments, A^2 is $-NH-C_6$ aryl- C_{1-6} alkyl- $NHC(O)R^5$; and A^1 and A^3 are both hydrogen. In some embodiments, A^2 is $-NH-C_6$ aryl- $CH_2-NHC(O)R^5$; and A^1 and A^3 are both hydrogen.

[0218] In some embodiments, A^3 is $-NHR^3$, where R^3 is hydrogen, $-C(O)R^5$, $-C_6$ aryl- C_{1-6} alkyl- NH_2 , or $-C_6$ aryl- C_{1-6} alkyl- $NHC(O)R^5$; and A^1 and A^2 are both hydrogen. Accordingly, in some embodiments, provided is a compound of Formula IV:



or stereoisomer or pharmaceutically acceptable salt thereof, wherein A^3 and R^4 are each independently as defined herein.

[0219] In some embodiments, A^3 is $-NH_2$; and A^1 and A^2 are both hydrogen.

[0220] In some embodiments, A^3 is $-NH-C(O)R^5$; and A^1 and A^2 are both hydrogen.

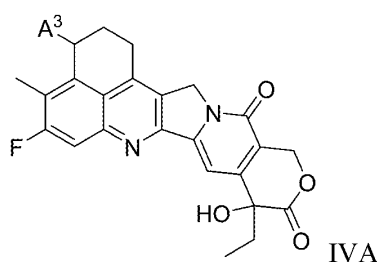
[0221] In some embodiments, A^3 is $-NH-C_6$ aryl- C_{1-6} alkyl- NH_2 ; and A^1 and A^2 are both hydrogen. In some embodiments, A^3 is $-NH-C_6$ aryl- CH_2-NH_2 ; and A^1 and A^2 are both hydrogen.

[0222] In some embodiments, A^3 is $-NH-C_6$ aryl- C_{1-6} alkyl- $NHC(O)R^5$; and A^1 and A^2 are both hydrogen. In some embodiments, A^3 is $-NH-C_6$ aryl- $CH_2-NHC(O)R^5$; and A^1 and A^2 are both hydrogen.

[0223] In some embodiments, R^4 is methyl, ethyl or isopropyl. In some embodiments, R^4 is ethyl or isopropyl. In some embodiments, R^4 is methyl. In some embodiments, R^4 is ethyl.

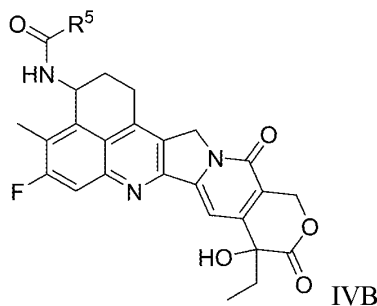
[0224] In some embodiments, R^4 is isopropyl.

[0225] In some embodiments, provided is a compound of Formula IVA:



or stereoisomer or pharmaceutically acceptable salt thereof, wherein A^3 is independently as defined herein.

[0226] In some embodiments, provided is a compound of Formula IVB:



or stereoisomer or pharmaceutically acceptable salt thereof, wherein R^5 is independently as defined herein.

[0227] In some embodiments, when R^1 , R^2 , or R^3 is other than hydrogen, the compound comprises a linking moiety, L, which covalently links R^6 to the remainder of the compound.

[0228] In some embodiments, L comprises one or more chain heteroatoms and one or more alkylene, alkenylene, alkynylene, arylene, heteroarylene, cycloalkylene, or heterocycloalkylene moieties; wherein each alkylene, alkenylene, alkynylene, arylene, heteroarylene, cycloalkylene, or heterocycloalkylene moiety, may be independently optionally substituted with one to five substituents independently selected from oxo, halo, C₁₋₄ alkyl, C₁₋₄ alkoxy, C₁₋₄ haloalkyl, and benzyl.

[0229] In some embodiments, L is an alkylene linker optionally comprising one or more -O-, -S-, amine, ester, amide, carbamate, carbonate, thio-succinimide, or ketone functional groups.

[0230] In some embodiments, L comprises one or more amino acids. In some embodiments, L comprises one or more linear or branched, natural or unnatural amino acids. In some embodiments, L comprises a polypeptide.

[0231] In some embodiments, L comprises one or more polyethylene glycol unit (e.g., PEG having an average molecular weight of from 300 g/mol to 10,000 g/mol).

[0232] In some embodiments, R⁵ is -L¹-(AA)_n-L²-R⁶, where each L¹, AA, n, L², and R⁶ are independently as defined herein.

[0233] In some embodiments, L¹ is C₁₋₂₀ alkylene or C₂₋₂₀ heteroalkylene;
n is 0, 1, 2, 3, 4, 5, or 6;
each AA is independently an amino acid; and
L² is C₁₋₄₀ alkylene or C₂₋₄₀ heteroalkylene, wherein the C₁₋₄₀ alkylene or C₂₋₄₀ heteroalkylene optionally comprises a phenylene within the C₁₋₄₀ alkylene or C₂₋₄₀ heteroalkylene chain, and the C₁₋₄₀ alkylene or C₂₋₄₀ heteroalkylene is optionally substituted with one or more (e.g., one or two) oxo.

[0234] In some embodiments, L¹ is C₁₋₂₀ alkylene or C₂₋₂₀ heteroalkylene;
n is 0, 1, 2, 3, 4, 5, or 6;
each AA is independently an amino acid; and
L² is C₁₋₄₀ alkylene or C₂₋₄₀ heteroalkylene, wherein the C₁₋₄₀ alkylene or C₂₋₄₀ heteroalkylene is optionally substituted with one or more (e.g., one or two) oxo.

[0235] In some embodiments, n is 0. In some embodiments, n is 1, 2, 3, 4, 5, 6, or 7. In some embodiments, n is 2, 3, 4, 5, 6, or 7. In some embodiments, n is 1. In some

embodiments, n is 2. In some embodiments, n is 3. In some embodiments, n is 4. In some embodiments, n is 5. In some embodiments, n is 6. In some embodiments, n is 7.

[0236] In some embodiments, L^1 is C_{1-20} alkylene. In some embodiments, L^1 is C_{1-10} alkylene. In some embodiments, L^1 is C_{2-6} alkylene.

[0237] In some embodiments, L^1 is C_{2-20} heteroalkylene. In some embodiments, L^1 is C_{2-10} heteroalkylene. In some embodiments, L^1 is C_{2-6} heteroalkylene.

[0238] In some embodiments, L^1 is $-(CH_2)_p-X^1-(CH_2)_q-X^2-*$ or $-X^1-(CH_2)_p$ -phenylene- $(CH_2)_q-X^2-*$; wherein the * bond is attached to the $-(AA)_n-L^2-R^6$; and

X^1 is a bond, -O-, -S-, or -NH-;

X^2 is a bond, -O-, -S-, or -NH-;

p is 1, 2, 3, or 4; and

q is 1, 2, 3, or 4.

[0239] In some embodiments, L^1 is $-CH_2-X^1-CH_2-X^2-*$. In some embodiments, L^1 is $-(CH_2)_2-X^1-CH_2-X^2-*$. In some embodiments, L^1 is $-(CH_2)_3-X^1-CH_2-X^2-*$. In some embodiments, L^1 is $-(CH_2)_4-X^1-CH_2-X^2-*$.

[0240] In some embodiments, L^1 is $-CH_2-X^1-(CH_2)_2-X^2-*$. In some embodiments, L^1 is $-(CH_2)_2-X^1-(CH_2)_2-X^2-*$. In some embodiments, L^1 is $-(CH_2)_3-X^1-(CH_2)_2-X^2-*$. In some embodiments, L^1 is $-(CH_2)_4-X^1-(CH_2)_2-X^2-*$.

[0241] In some embodiments, L^1 is $-CH_2-X^1-(CH_2)_3-X^2-*$. In some embodiments, L^1 is $-(CH_2)_2-X^1-(CH_2)_3-X^2-*$. In some embodiments, L^1 is $-(CH_2)_3-X^1-(CH_2)_3-X^2-*$. In some embodiments, L^1 is $-(CH_2)_4-X^1-(CH_2)_3-X^2-*$.

[0242] In some embodiments, L^1 is $-CH_2-X^1-(CH_2)_4-X^2-*$. In some embodiments, L^1 is $-(CH_2)_2-X^1-(CH_2)_4-X^2-*$. In some embodiments, L^1 is $-(CH_2)_3-X^1-(CH_2)_4-X^2-*$. In some embodiments, L^1 is $-(CH_2)_4-X^1-(CH_2)_4-X^2-*$.

[0243] In some embodiments, X^1 is a bond. In some embodiments, X^1 is -O-. In some embodiments, X^1 is -S-. In some embodiments, X^1 is -NH-.

[0244] In some embodiments, X^2 is a bond. In some embodiments, X^2 is -O-. In some embodiments, X^2 is -S-. In some embodiments, X^2 is -NH-.

[0245] In some embodiments, L^1 is $-\text{CH}_2-\text{X}^1-\text{CH}_2-\text{NH}-^*$. In some embodiments, L^1 is $-(\text{CH}_2)_2-\text{X}^1-\text{CH}_2-\text{NH}-^*$. In some embodiments, L^1 is $-(\text{CH}_2)_3-\text{X}^1-\text{CH}_2-\text{NH}-^*$. In some embodiments, L^1 is $-(\text{CH}_2)_4-\text{X}^1-\text{CH}_2-\text{NH}-^*$.

[0246] In some embodiments, L^1 is $-\text{CH}_2-\text{X}^1-(\text{CH}_2)_2-\text{NH}-^*$. In some embodiments, L^1 is $-(\text{CH}_2)_2-\text{X}^1-(\text{CH}_2)_2-\text{NH}-^*$. In some embodiments, L^1 is $-(\text{CH}_2)_3-\text{X}^1-(\text{CH}_2)_2-\text{NH}-^*$. In some embodiments, L^1 is $-(\text{CH}_2)_4-\text{X}^1-(\text{CH}_2)_2-\text{NH}-^*$.

[0247] In some embodiments, L^1 is $-\text{CH}_2-\text{X}^1-(\text{CH}_2)_3-\text{NH}-^*$. In some embodiments, L^1 is $-(\text{CH}_2)_2-\text{X}^1-(\text{CH}_2)_3-\text{NH}-^*$. In some embodiments, L^1 is $-(\text{CH}_2)_3-\text{X}^1-(\text{CH}_2)_3-\text{NH}-^*$. In some embodiments, L^1 is $-(\text{CH}_2)_4-\text{X}^1-(\text{CH}_2)_3-\text{NH}-^*$.

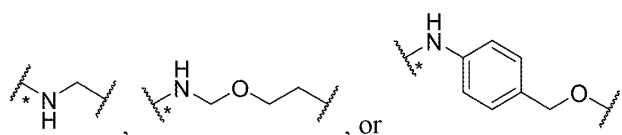
[0248] In some embodiments, L^1 is $-\text{CH}_2-\text{X}^1-(\text{CH}_2)_4-\text{NH}-^*$. In some embodiments, L^1 is $-(\text{CH}_2)_2-\text{X}^1-(\text{CH}_2)_4-\text{NH}-^*$. In some embodiments, L^1 is $-(\text{CH}_2)_3-\text{X}^1-(\text{CH}_2)_4-\text{NH}-^*$. In some embodiments, L^1 is $-(\text{CH}_2)_4-\text{X}^1-(\text{CH}_2)_4-\text{NH}-^*$.

[0249] In some embodiments, L^1 is $-\text{CH}_2-\text{O}-\text{CH}_2-\text{X}^2-^*$. In some embodiments, L^1 is $-(\text{CH}_2)_2-\text{O}-\text{CH}_2-\text{X}^2-^*$. In some embodiments, L^1 is $-(\text{CH}_2)_3-\text{O}-\text{CH}_2-\text{X}^2-^*$. In some embodiments, L^1 is $-(\text{CH}_2)_4-\text{O}-\text{CH}_2-\text{X}^2-^*$.

[0250] In some embodiments, L^1 is $-\text{CH}_2-\text{O}-(\text{CH}_2)_2-\text{X}^2-^*$. In some embodiments, L^1 is $-(\text{CH}_2)_2-\text{O}-(\text{CH}_2)_2-\text{X}^2-^*$. In some embodiments, L^1 is $-(\text{CH}_2)_3-\text{O}-(\text{CH}_2)_2-\text{X}^2-^*$. In some embodiments, L^1 is $-(\text{CH}_2)_4-\text{O}-(\text{CH}_2)_2-\text{X}^2-^*$.

[0251] In some embodiments, L^1 is $-\text{CH}_2-\text{O}-(\text{CH}_2)_3-\text{X}^2-^*$. In some embodiments, L^1 is $-(\text{CH}_2)_2-\text{O}-(\text{CH}_2)_3-\text{X}^2-^*$. In some embodiments, L^1 is $-(\text{CH}_2)_3-\text{O}-(\text{CH}_2)_3-\text{X}^2-^*$. In some embodiments, L^1 is $-(\text{CH}_2)_4-\text{O}-(\text{CH}_2)_3-\text{X}^2-^*$.

[0252] In some embodiments, L^1 is $-\text{CH}_2-\text{O}-(\text{CH}_2)_4-\text{X}^2-^*$. In some embodiments, L^1 is $-(\text{CH}_2)_2-\text{O}-(\text{CH}_2)_4-\text{X}^2-^*$. In some embodiments, L^1 is $-(\text{CH}_2)_3-\text{O}-(\text{CH}_2)_4-\text{X}^2-^*$. In some embodiments, L^1 is $-(\text{CH}_2)_4-\text{O}-(\text{CH}_2)_4-\text{X}^2-^*$.

[0253] In some embodiments, L^1 is , or
wherein the * bond is attached to the $-(\text{AA})_n-\text{L}^2-\text{R}^6$.

[0254] In some embodiments, L^1 is $-(CH_2)_p-O-(CH_2)_q-NH^*$.

[0255] In some embodiments, L^1 is $-CH_2-O-CH_2-NH^*$. In some embodiments, L^1 is $-(CH_2)_2-O-CH_2-NH^*$. In some embodiments, L^1 is $-(CH_2)_3-O-CH_2-NH^*$. In some embodiments, L^1 is $-(CH_2)_4-O-CH_2-NH^*$.

[0256] In some embodiments, L^1 is $-CH_2-O-(CH_2)_2-NH^*$. In some embodiments, L^1 is $-(CH_2)_2-O-(CH_2)_2-NH^*$. In some embodiments, L^1 is $-(CH_2)_3-O-(CH_2)_2-NH^*$. In some embodiments, L^1 is $-(CH_2)_4-O-(CH_2)_2-NH^*$.

[0257] In some embodiments, L^1 is $-CH_2-O-(CH_2)_3-NH^*$. In some embodiments, L^1 is $-(CH_2)_2-O-(CH_2)_3-NH^*$. In some embodiments, L^1 is $-(CH_2)_3-O-(CH_2)_3-NH^*$. In some embodiments, L^1 is $-(CH_2)_4-O-(CH_2)_3-NH^*$.

[0258] In some embodiments, L^1 is $-CH_2-O-(CH_2)_4-NH^*$. In some embodiments, L^1 is $-(CH_2)_2-O-(CH_2)_4-NH^*$. In some embodiments, L^1 is $-(CH_2)_3-O-(CH_2)_4-NH^*$. In some embodiments, L^1 is $-(CH_2)_4-O-(CH_2)_4-NH^*$.

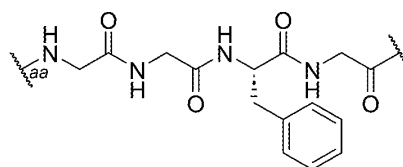
[0259] In certain embodiments, L comprises more than one sequential amino acid to form a peptide unit of formula $-(AA)_n-$. In certain embodiments, the peptide unit of formula $-(AA)_n-$ consists of 2 to 7 amino acids, where the amino acids are linked by a peptide (i.e., amide) bonding. In certain embodiments, the peptide unit of formula $-(AA)_n-$, the linker L^1 is bonded to the AA at its C-terminus, and to the $-L^2-R^6$ moiety at its N-terminus.

[0260] The amino acid sequence of the peptide unit $-(AA)_n-$ is not particularly limited, but examples thereof include an L- or a D-amino acid, and can comprise an amino acid having a structure such as β -alanine, ϵ -aminocaproic acid, γ -aminobutyric acid, an α -amino acid, or a non-natural type amino acid, such as N-methylated amino acid. Specific examples include phenylalanine (Phe; F), tyrosine (Tyr; Y), leucine (Leu; L), glycine (Gly; G), alanine (Ala; A), valine (Val; V), lysine (Lys; K), citrulline (Cit), serine (Ser; S), glutamic acid (Glu; E), and aspartic acid (Asp; D). In certain embodiments, the peptide unit $-(AA)_n-$ comprises one or more (e.g., 2 to 7, or 2 to 5, or 3 to 5, or 4) amino acid residues independently selected from phenylalanine, glycine, valine, lysine, citrulline, serine, glutamic acid, and aspartic acid.

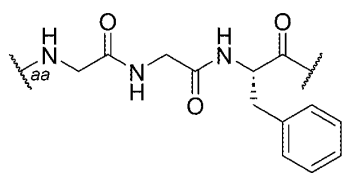
[0261] In certain embodiments, $-(AA)_n-$ is selected from -FGG-, -FGGD-, -FGG-(D-)D-, -FGGE-, -GFGG-, -FGGS-, -FGGK-, -GFGGD-, -GGFGG-, -GFGGDD-, -GFGGDK-, -FGGGFGG-, wherein (D-)D refers to D-aspartic acid.

[0262] In some embodiments, n is 2, 3, 4, 5, 6, or 7, and each AA is independently selected from Gly and Phe.

[0263] In some embodiments, $-(AA)_n-$ is -Gly-Phe-Gly-Gly- (-GFGG-).



[0264] In some embodiments, $-(AA)_n-$ is



, wherein bond aa is attached to L^2 .

[0265] In some embodiments, L^2 is C_{1-40} alkylene optionally substituted with one or more oxo. In some embodiments, L^2 is C_{1-30} alkylene optionally substituted with one or more oxo. In some embodiments, L^2 is C_{1-20} alkylene optionally substituted with one or more oxo. In some embodiments, L^2 is C_{1-10} alkylene optionally substituted with one or more oxo. In some embodiments, L^2 is C_{5-40} alkylene optionally substituted with one or more oxo. In some embodiments, L^2 is C_{5-30} alkylene optionally substituted with one or more oxo. In some embodiments, L^2 is C_{5-20} alkylene optionally substituted with one or more oxo. In some embodiments, L^2 is C_{5-10} alkylene optionally substituted with one or more oxo.

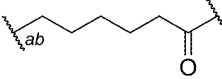
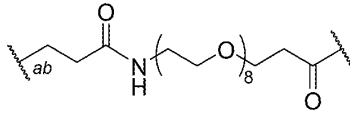
[0266] In some embodiments, L^2 is $-C(O)-C_{0-39}$ alkylene. In some embodiments, L^2 is $-C(O)-C_{1-15}$ alkylene. In some embodiments, L^2 is $-C(O)-C_{1-10}$ alkylene. In some embodiments, L^2 is $-C(O)-C_{5-10}$ alkylene.

[0267] In some embodiments, L^2 is C_{2-40} heteroalkylene optionally substituted with one or more oxo. In some embodiments, L^2 is $-C(O)-C_{1-39}$ heteroalkylene.

[0268] In some embodiments, L^2 is $-C(O)-(CH_2CH_2O)_{1-10}-CH_2CH_2-$. In some embodiments, L^2 is $-C(O)-(CH_2CH_2O)_{1-8}-CH_2CH_2-$. In some embodiments, L^2 is $-C(O)-(CH_2CH_2O)_{1-5}-CH_2CH_2-$. In some embodiments, L^2 is $-C(O)-(CH_2CH_2O)_{2-10}-CH_2CH_2-$. In some

embodiments, L^2 is $-\text{C}(\text{O})-(\text{CH}_2\text{CH}_2\text{O})_{2-8}-\text{CH}_2\text{CH}_2-$. In some embodiments, L^2 is $-\text{C}(\text{O})-(\text{CH}_2\text{CH}_2\text{O})_{2-5}-\text{CH}_2\text{CH}_2-$.

[0269] In some embodiments, L^2 is $-\text{C}(\text{O})-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2-$. In some embodiments, L^2 is $-\text{C}(\text{O})-(\text{CH}_2\text{CH}_2\text{O})_2-\text{CH}_2\text{CH}_2-$. In some embodiments, L^2 is $-\text{C}(\text{O})-(\text{CH}_2\text{CH}_2\text{O})_3-\text{CH}_2\text{CH}_2-$. In some embodiments, L^2 is $-\text{C}(\text{O})-(\text{CH}_2\text{CH}_2\text{O})_4-\text{CH}_2\text{CH}_2-$. In some embodiments, L^2 is $-\text{C}(\text{O})-(\text{CH}_2\text{CH}_2\text{O})_5-\text{CH}_2\text{CH}_2-$. In some embodiments, L^2 is $-\text{C}(\text{O})-(\text{CH}_2\text{CH}_2\text{O})_6-\text{CH}_2\text{CH}_2-$. In some embodiments, L^2 is $-\text{C}(\text{O})-(\text{CH}_2\text{CH}_2\text{O})_7-\text{CH}_2\text{CH}_2-$. In some embodiments, L^2 is $-\text{C}(\text{O})-(\text{CH}_2\text{CH}_2\text{O})_8-\text{CH}_2\text{CH}_2-$. In some embodiments, L^2 is $-\text{C}(\text{O})-(\text{CH}_2\text{CH}_2\text{O})_9-\text{CH}_2\text{CH}_2-$. In some embodiments, L^2 is $-\text{C}(\text{O})-(\text{CH}_2\text{CH}_2\text{O})_{10}-\text{CH}_2\text{CH}_2-$.

[0270] In some embodiments, L^2 is  or , wherein bond ab is attached to R^6 .

[0271] In some embodiments, R^5 is $-(\text{CH}_2)_p-\text{O}-(\text{CH}_2)_q-\text{NH}-(\text{AA})_n-\text{C}(\text{O})-\text{C}_{1-10}$ alkylene- R^6 . In some embodiments, R^5 is $-(\text{CH}_2)_p-\text{O}-(\text{CH}_2)_q-\text{NH}-(\text{AA})_n-\text{C}(\text{O})-(\text{CH}_2)_{1-10}-\text{CH}_2\text{CH}_2-R^6$.

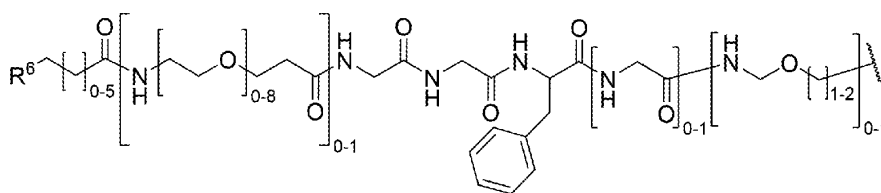
[0272] In some embodiments, R^5 is $-(\text{CH}_2)_p-\text{O}-(\text{CH}_2)_q-\text{NH}-\text{GFGG}-\text{C}(\text{O})-\text{C}_{1-10}$ alkylene- R^6 . In some embodiments, R^5 is $-(\text{CH}_2)_p-\text{O}-(\text{CH}_2)_q-\text{NH}-\text{GFGG}-\text{C}(\text{O})-(\text{CH}_2)_{1-10}-\text{CH}_2\text{CH}_2-R^6$.

[0273] In some embodiments, R^5 is $-(\text{CH}_2)_p-\text{O}-(\text{CH}_2)_q-\text{NH}-(\text{AA})_n-\text{C}(\text{O})-\text{C}_{1-30}$ heteroalkylene- R^6 . In some embodiments, R^5 is $-(\text{CH}_2)_p-\text{O}-(\text{CH}_2)_q-\text{NH}-(\text{AA})_n-\text{C}(\text{O})-(\text{CH}_2\text{CH}_2\text{O})_{1-10}-\text{CH}_2\text{CH}_2-R^6$.

[0274] In some embodiments, R^5 is $-(\text{CH}_2)_p-\text{O}-(\text{CH}_2)_q-\text{NH}-\text{GFGG}-\text{C}(\text{O})-\text{C}_{1-35}$ heteroalkylene- R^6 . In some embodiments, R^5 is $-(\text{CH}_2)_p-\text{O}-(\text{CH}_2)_q-\text{NH}-\text{GFGG}-\text{C}(\text{O})-\text{C}_{1-30}$ heteroalkylene- R^6 . In some embodiments, R^5 is $-(\text{CH}_2)_p-\text{O}-(\text{CH}_2)_q-\text{NH}-\text{GFGG}-\text{C}(\text{O})-(\text{CH}_2\text{CH}_2\text{O})_{1-10}-\text{CH}_2\text{CH}_2-R^6$.

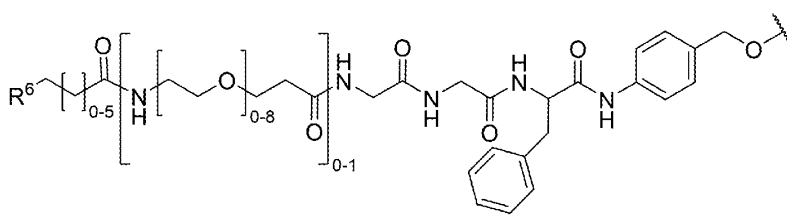
[0275] In some embodiments, R^5 is $-(\text{CH}_2)_p-\text{O}-(\text{CH}_2)_q-\text{NH}-\text{GFGG}-\text{C}(\text{O})-\text{C}_{1-35}$ heteroalkylene- R^6 . In some embodiments, R^5 is $-(\text{CH}_2)_p-\text{O}-(\text{CH}_2)_q-\text{NH}-\text{GFGG}-\text{C}(\text{O})-\text{C}_{1-30}$ heteroalkylene- R^6 . In some embodiments, R^5 is $-(\text{CH}_2)_p-\text{O}-(\text{CH}_2)_q-\text{NH}-\text{GFGG}-\text{C}(\text{O})-(\text{CH}_2\text{CH}_2\text{O})_{1-10}-\text{CH}_2\text{CH}_2-R^6$.

[0276] In some embodiments, R^5 is:



where R^6 is heterocyclyl, wherein said heterocyclyl is optionally covalently linked to an antibody or antigen-binding fragment.

[0277] In some embodiments, R^5 is:



where R^6 is heterocyclyl, wherein said heterocyclyl is optionally covalently linked to an antibody or antigen-binding fragment.

[0278] In some embodiments, p is 1. In some embodiments, p is 2. In some embodiments, p is 3. In some embodiments, p is 4.

[0279] In some embodiments, q is 1. In some embodiments, q is 2. In some embodiments, q is 3. In some embodiments, q is 4.

[0280] In some embodiments, p is 1 and q is 1. In some embodiments, p is 2 and q is 1. In some embodiments, p is 3 and q is 1. In some embodiments, p is 4 and q is 1. In some embodiments, p is 1 and q is 2. In some embodiments, p is 2 and q is 2. In some embodiments, p is 3 and q is 2. In some embodiments, p is 4 and q is 2. In some embodiments, p is 1 and q is 3. In some embodiments, p is 2 and q is 3. In some embodiments, p is 3 and q is 3. In some embodiments, p is 4 and q is 3. In some embodiments, p is 1 and q is 4. In some embodiments, p is 2 and q is 4. In some embodiments, p is 3 and q is 4. In some embodiments, p is 4 and q is 4.

[0281] In some embodiments, A^3 is $-NH-C(O)R^5$; and A^1 and A^2 are both hydrogen.

[0282] In some embodiments, A^3 is $-NH-C(O)-L^1-(AA)_n-L^2-R^6$; and A^1 and A^2 are both hydrogen.

[0283] In some embodiments, A^3 is $-NH-C(O)-L^1-(AA)_n-L^2-R^6$; and A^1 and A^2 are both hydrogen;

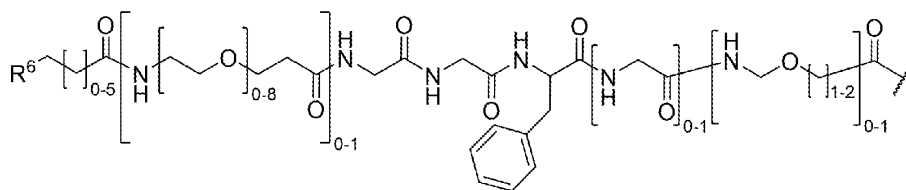
L^1 is $-(CH_2)_p-X^1-(CH_2)_q-X^2-*$ or $-X^1-(CH_2)_p$ -phenylene- $(CH_2)_q-X^2-*$; wherein the * bond is attached to the $-(AA)_n-L^2-R^6$;

n is 0, 1, 2, 3, 4, 5, or 6;

each AA is independently an amino acid; and

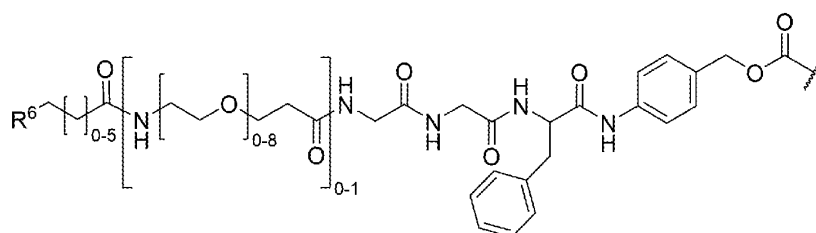
L^2 is C_{1-40} alkylene or C_{2-40} heteroalkylene, wherein the C_{1-40} alkylene or C_{2-40} heteroalkylene optionally comprises a phenylene within the C_{1-40} alkylene or C_{2-40} heteroalkylene chain, and the C_{1-40} alkylene or C_{2-40} heteroalkylene is optionally substituted with one or more (e.g., one or two) oxo.

[0284] In some embodiments, R^1 , R^2 , or R^3 is:



where R^6 is heterocyclyl, wherein said heterocyclyl is optionally covalently linked to an antibody or antigen-binding fragment.

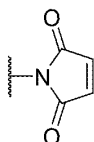
[0285] In some embodiments, R^1 , R^2 , or R^3 is:



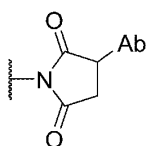
where R^6 is heterocyclyl, wherein said heterocyclyl is optionally covalently linked to an antibody or antigen-binding fragment.

[0286] In some embodiments, R^6 is heterocyclyl, which heterocyclyl is covalently linked to an antibody or antigen-binding fragment.

[0287] In some embodiments, R^6 is heterocyclyl, wherein said heterocyclyl is optionally covalently linked to an antibody or antigen-binding fragment via a cystine or lysine residue on the antibody or antigen-binding fragment.



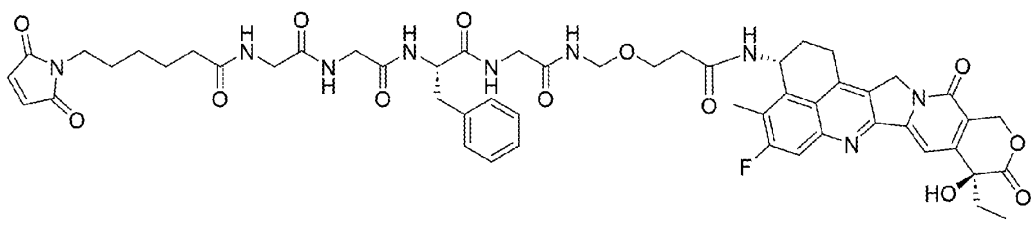
[0288] In some embodiments, R^6 is



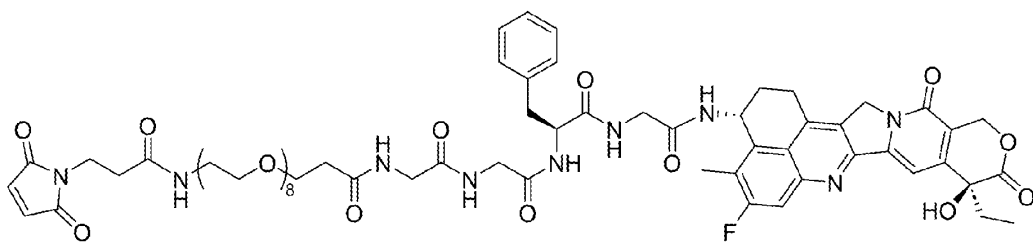
[0289] In some embodiments, R^6 is , wherein Ab is an antibody or an antigen-binding fragment provided herein.

[0290] In some embodiments, provided is a drug-linker compound selected from:

(a) Formula VIII (LM-D01)

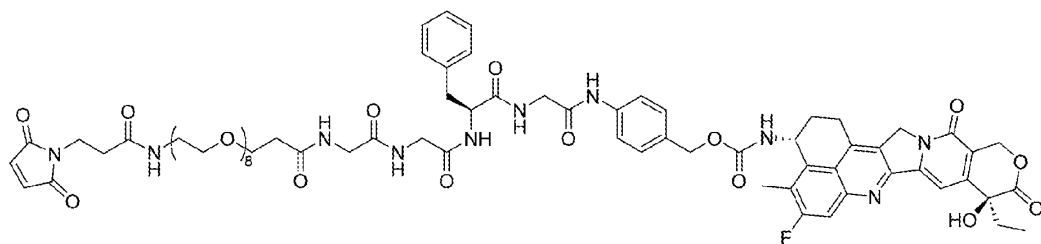


(b) Formula IX (LM-D02)

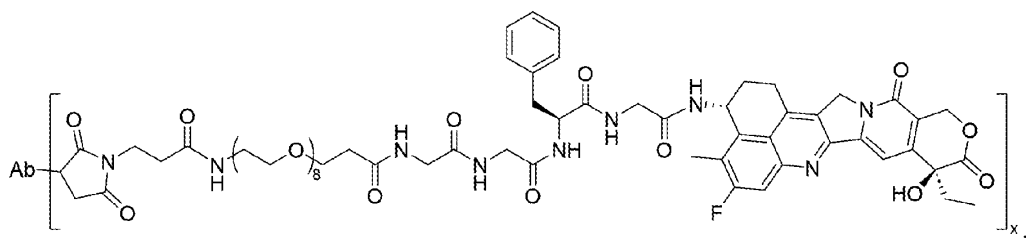
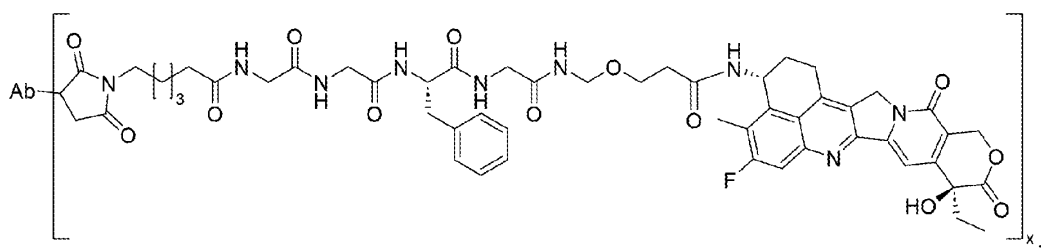


and

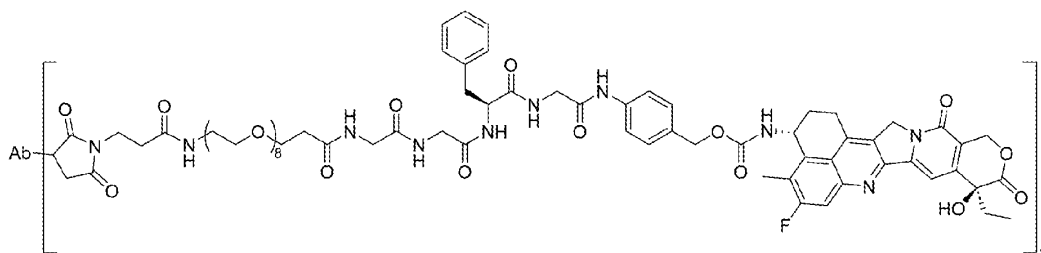
(c) Formula X (LM-D03)



[0291] In some embodiments, provided is an ADC selected from:



and



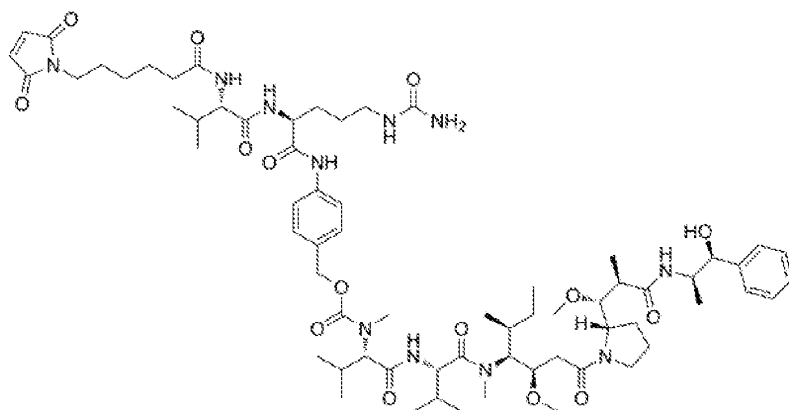
wherein Ab is an antibody or an antigen-binding fragment provided herein.

[0292] In some embodiments, x is 4. In some embodiments, x is 5. In some embodiments, x is 6. In some embodiments, x is 7. In some embodiments, x is 8. In some embodiments, x is 4, 6, or 8. In some embodiments, x is 4-8.

[0293] In certain embodiments of the present disclosure, the drug and the linker form a drug-linker compound of ozogamicin (derivatives of calicheamicin, see U.S. Pat. No. 5773001), vedotin (MC-VC-PABC-MMAE, see U.S. Pat. No. 7659241), mafodotin (MC-MMAF, see U.S. Pat. No. 7498298), emtansine (SMCC-DM1, U.S. Pat. No. 5208020), deruxtecan (MC-GGFG-DXd, see U.S. Pat. No. 10195288), govitecan (CL2A-SN38, see U.S. Pat. No. 8420086), or tesirine (MP-PEG8-VA-PABC-SG3199/SCX, see U.S. Pat. No. 9889207), which are hereby incorporated by reference in their entirety.

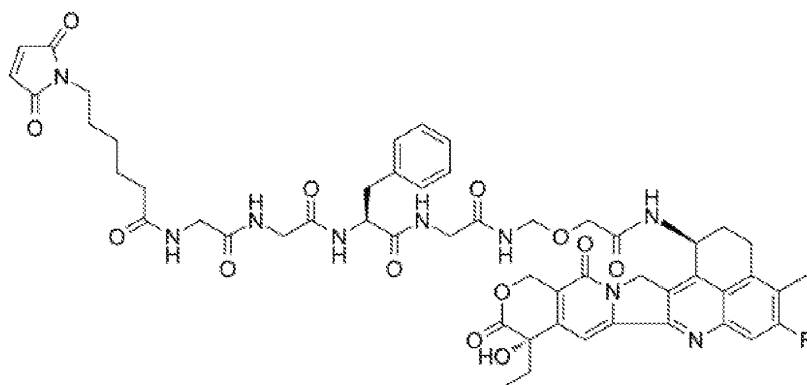
[0294] Further examples of the drug-linker compound includes pasudotox (PE38), soravtansine (DM4), ravtansine (DM4), and mertansine (DM1).

[0295] In certain embodiments of the present disclosure, the drug-linker compound is vedotin having a Formula VI, or the derivatives thereof:



(Mc-vc-PAB-MMAE).

[0296] In certain embodiments of the present disclosure, the drug-linker compound is deruxtecan having a Formula VII, or the derivatives thereof:



(Mc-GGFG-DXd).

Treatment Methods

[0297] As described herein, the antibodies, ADCs, variants or derivatives of the present disclosure may be used in certain treatment and diagnostic methods.

[0298] The present disclosure is further directed to antibody-based therapies which involve administering the antibodies or ADCs of the disclosure to a patient such as an animal, a mammal, and a human for treating one or more of the disorders or conditions described herein. Therapeutic compounds of the disclosure include, but are not limited to, antibodies or ADCs of the disclosure (including variants and derivatives thereof as described herein) and nucleic acids or polynucleotides encoding antibodies of the disclosure (including variants and derivatives thereof as described herein).

[0299] The antibodies or ADCs of the disclosure can also be used to treat or inhibit cancer. In some embodiments, the cancer cells in the patient express or overexpress CDH17. As provided above, CDH17 can be overexpressed in tumor cells, in particular gastric, pancreatic, colorectal, hepatocarcinoma, and neuroendocrine tumors. Inhibition of CDH17 has been shown to be useful for treating the tumors.

[0300] Accordingly, in some embodiments, provided are methods for treating a cancer in a patient in need thereof. The method, in one embodiment, entails administering to the patient an effective amount of an antibody or an ADC of the present disclosure. In some embodiments, at least one of the cancer cells (e.g., stromal cells) in the patient over-express CDH17.

[0301] Cellular therapies, such as chimeric antigen receptor (CAR) T-cell therapies, are also provided in the present disclosure. A suitable cell can be used, that is put in contact with an anti-CDH17 antibody of the present disclosure (or alternatively engineered to express an anti-CDH17 antibody of the present disclosure). In some embodiments, the antibody is presented in a chimeric antigen receptor (CAR). Upon such contact or engineering, the cell can then be introduced to a cancer patient in need of a treatment. The cancer patient may have a cancer of any of the types as disclosed herein. The cell (e.g., T cell) can be, for instance, a tumor-infiltrating T lymphocyte, a CD4⁺ T cell, a CD8⁺ T cell, or the combination thereof, without limitation.

[0302] In some embodiments, the cell was isolated from the cancer patient him- or her-self. In some embodiments, the cell was provided by a donor or from a cell bank. When the cell is isolated from the cancer patient, undesired immune reactions can be minimized.

[0303] Non-limiting examples of cancers include gastric cancer, colorectal cancer, hepatocarcinoma, bladder cancer, liver cancer, endometrial cancer, leukemia, lymphoma, pancreatic cancer, neuroendocrine cancer, small cell lung cancer, non-small cell lung cancer, breast cancer, urethral cancer, head and neck cancer, gastrointestinal cancer, oesophageal cancer, ovarian cancer, renal cancer, melanoma, prostate cancer and thyroid cancer.

[0304] Additional diseases or conditions associated with increased cell survival, that may be treated, prevented, diagnosed and/or prognosed with the antibodies or variants, or derivatives thereof of the disclosure include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (*e.g.*, acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (*e.g.*, chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (*e.g.*, Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyo sarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma,

papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma and retinoblastoma.

[0305] A specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the particular antibodies, variant or derivative thereof used, the patient's age, body weight, general health, sex, and diet, and the time of administration, rate of excretion, drug combination, and the severity of the particular disease being treated. Judgment of such factors by medical caregivers is within the ordinary skill in the art. The amount will also depend on the individual patient to be treated, the route of administration, the type of formulation, the characteristics of the compound used, the severity of the disease, and the desired effect. The amount used can be determined by pharmacological and pharmacokinetic principles well known in the art.

[0306] Methods of administration of the antibodies, ADCs, variants or derivatives include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The antigen-binding polypeptides, ADCs or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Thus, pharmaceutical compositions containing the antigen-binding polypeptides or ADCs of the disclosure may be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), buccally, or as an oral or nasal spray.

[0307] The term “parenteral” as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intra-articular injection and infusion.

[0308] Administration can be systemic or local. In addition, it may be desirable to introduce the antibodies or ADCs of the disclosure into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be

facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0309] It may be desirable to administer the antigen-binding polypeptides, ADCs or compositions of the disclosure locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, *e.g.*, in conjunction, with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody or an ADC, of the disclosure, care must be taken to use materials to which the protein does not absorb.

[0310] The amount of the antibodies or ADCs of the disclosure which will be effective in the treatment, inhibition and prevention of an immune or malignant disease, disorder or condition can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease, disorder or condition, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[0311] As a general proposition, the dosage administered to a patient of the antigen-binding polypeptides or ADCs of the present disclosure is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight, between 0.1 mg/kg and 20 mg/kg of the patient's body weight, or 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies or ADCs of the disclosure may be reduced by enhancing uptake and tissue penetration (*e.g.*, into the brain) of the antibodies by modifications such as, for example, lipidation.

[0312] In an additional embodiment, the compositions of the disclosure are administered in combination with cytokines. Cytokines that may be administered with the compositions of the disclosure include, but are not limited to, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, IL-15, anti-CD40, CD40L, and TNF- α .

[0313] In additional embodiments, the compositions of the disclosure are administered in combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

Diagnostic Methods

[0314] Over-expression of CDH17 is observed in certain tumor samples, and patients having CDH17-over-expressing cells are likely responsive to treatments with the anti-CDH17 antibodies of the present disclosure. Accordingly, the antibodies of the present disclosure can also be used for diagnostic and prognostic purposes.

[0315] A sample that preferably includes a cell can be obtained from a patient, which can be a cancer patient or a patient desiring diagnosis. The cell be a cell of a tumor tissue or a tumor block, a blood sample, a urine sample or any sample from the patient. Upon optional pre-treatment of the sample, the sample can be incubated with an antibody of the present disclosure under conditions allowing the antibody to interact with an CDH17 protein potentially present in the sample. Methods such as ELISA can be used, taking advantage of the anti-CDH17 antibody, to detect the presence of the CDH17 protein in the sample.

[0316] Presence of the CDH17 protein in the sample (optionally with the amount or concentration) can be used for diagnosis of cancer, as an indication that the patient is suitable for a treatment with the antibody, or as an indication that the patient has (or has not) responded to a cancer treatment. For a prognostic method, the detection can be done at once, twice or more, at certain stages, upon initiation of a cancer treatment to indicate the progress of the treatment.

Compositions

[0317] The present disclosure also provides pharmaceutical compositions. Such compositions comprise an effective amount of an antibody or an ADC, and an acceptable

carrier. In some embodiments, the composition further includes a second anticancer agent (e.g., an immune checkpoint inhibitor).

[0318] In a specific embodiment, the term “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. Further, a “pharmaceutically acceptable carrier” will generally be a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

[0319] The term “carrier” refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents such as acetates, citrates or phosphates. Antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; and agents for the adjustment of tonicity such as sodium chloride or dextrose are also envisioned. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences by E. W. Martin, incorporated herein by reference. Such compositions will contain a therapeutically effective amount of the antigen-binding polypeptide, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration. The parental preparation

can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0320] In an embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0321] The compounds of the disclosure can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

EXAMPLES

Example 1: Binding to human CDH17-overexpressing cell lines

[0322] This example examined the specific binding of a newly developed humanized antibody 8G5G9.p1.z12.

[0323] Cell-based binding of 8G5G9.p1.z12 and reference Abs BMK-BI-905711 (a monospecific version of the bispecific antibody BI-905711 having specificity to TRAILR2 and CDH17) and BMK-ARB202 (monospecific version of the bispecific antibody ARB202 which has specificity to CDH17 and CD3), and human IgG1 isotype control to human CDH17 overexpressing cell lines were assessed using flow cytometry.

[0324] Briefly, cell lines HEK-293/H_CDH17 and DLD-1/H_CDH17 were incubated with titrated testing mAbs (starting from 200 nM, 3-fold dilution, 12 points) respectively at 4°C for 60 minutes. Then cells were washed twice with 2% FBS/PBS buffer and stained with fluorescent conjugated anti-human Fc secondary antibody at 4°C for 30 minutes. Cells were washed twice again and then analyzed by flow cytometry for fluorescent signal. The graphs were made and EC50s were calculated in GraphPad Prism Software (LaJolla, CA).

[0325] The results in **FIG. 1A-1B** show that 8G5G9.p1.z12 efficiently bound to the human CDH17 overexpressing cells in dose-dependent manner.

Example 2. Non-specific binding test to blank HEK-293 cell line

[0326] This example examined the non-specific binding of 8G5G9.p1.z12.

[0327] Non-specific binding of 8G5G9.p1.z12, with reference Abs BMK-BI-905711 and BMK-ARB202, and human IgG1 isotype control to blank HEK-293 cell line were assessed using flow cytometry.

[0328] Briefly, HEK-293 cells were incubated with titrated testing mAbs (starting from 200 nM, 3-fold dilution, 12 points) at 4°C for 60 minutes. Then cells were washed twice with 2% FBS/PBS buffer and stained with fluorescent conjugated anti-human Fc secondary antibody at 4°C for 30 minutes. Cells were washed twice again and then analyzed by flow cytometry for fluorescent signal. The graphs were made and EC50s were calculated in GraphPad Prism Software (LaJolla, CA).

[0329] As shown in **FIG. 2**, unlike BMK-BI-905711, 8G5G9.p1.z12 had no non-specific binding to blank HEK-293 cells.

Example 3. Specific binding to endogenous human CDH17 on tumor cells

[0330] This example examined the binding of the 8G5G9.p1.z12 to endogenously expressed human CDH17.

[0331] Cell based binding of 8G5G9.p1.z12, reference Abs BMK-BI-905711 and BMK-ARB202, and human IgG1 isotype control to endogenous human CDH17 expressing tumor cell lines were assessed using flow cytometry. Briefly, gastric cancer (GC) cell line SNU-16 and colorectal cancer (CRC) cell line LoVo were incubated with titrated testing mAbs

(starting from 200 nM, 3-fold dilution, 12 points) respectively at 4°C for 60 minutes. Then cells were washed twice with 2% FBS/PBS buffer and stained with fluorescent conjugated anti-human Fc secondary antibody at 4°C for 30 minutes. Cells were washed twice again and then analyzed by flow cytometry for fluorescent signal. The graphs were made and EC50s were calculated in GraphPad Prism Software (LaJolla, CA).

[0332] As shown in **FIG. 3**, 8G5G9.p1.z12 effectively bound to the human tumor cells expressing endogenous CDH17 in dose-dependent manner.

Example 4. Cross-reactivity to monkey and rodent CDH17-overexpressing cell lines

[0333] This example examined the cross-species reactivity of 8G5G9.p1.z12.

[0334] Cell based binding of 8G5G9.p1.z12, reference Abs BMK-BI-905711 and BMK-ARB202, and human IgG1 isotype control to cynomolgus monkey, rhesus monkey, rat and mouse CDH17 overexpressing cell lines were assessed using flow cytometry. Briefly, engineered cell lines HEK-293/Cyno_CDH17, HEK-293/Rhesus_CDH17, HEK-293/Rat_CDH17, and HEK-293/Mouse_CDH17 were incubated with titrated testing mAbs (starting from 200 nM, 3-fold dilution, 12 points) respectively at 4°C for 60 minutes. Then cells were washed twice with 2% FBS/PBS buffer and stained with fluorescent conjugated anti-human Fc secondary antibody at 4°C for 30 minutes. Cells were washed twice again and then analyzed by flow cytometry for fluorescent signal. The graphs were made and EC50s were calculated in GraphPad Prism Software (LaJolla, CA).

[0335] As shown **FIG. 4A-4D**, 8G5G9.p1.z12 cross-reacted with monkey CDH17 but did not bind rodent CDH17.

Example 5. Epitope binning

[0336] This example conducted the epitope binning for 8G5G9.p1.z12.

[0337] Cell based Epitope binning assay of 8G5G9.p1.z12, BMK-BI-905711 and human IgG1 isotype control against BMK-BI-905711 with mouse IgG2a Fc fragment were assessed using flow cytometry on HEK-293/H_CDH17 cells. Briefly, HEK-293/H_CDH17 cells were incubated with the mixtures of BMK-BI-905711 (Anti CDH17)-mFc dilution (1 nM) and titrated testing mAbs (starting from 200 nM, 3-fold dilution, 12 points) at 4°C for 60 minutes.

Then cells were washed twice with 2% FBS/PBS buffer and stained with fluorescent conjugated anti-mouse Fc secondary antibody at 4°C for 30 minutes. Cells were washed twice again and then analyzed by flow cytometry for fluorescent signal. The graphs were made and IC50s were calculated in GraphPad Prism Software (LaJolla, CA).

[0338] The results (**FIG. 5** and **Table 1**) show that 8G5G9.p1.z12 did not compete with the reference Ab BMK-BI-905711 in binding to CDH17, and thus they do not bind to the same epitope.

Table 1. Inhibition Rates

Inhibition Rate	BMK-BI-905711	8G5G9.p1.z12
BMK-BI-905711	96.81%	3.18%

Example 6. Internalization potency on human CDH17-overexpressing cells

[0339] This example examined the internalization potency of 8G5G9.p1.z12.

[0340] Internalization potency of 8G5G9.p1.z12 was assessed using secondary antibodies labeled with pH sensor dyes (pHAb dyes) that becomes highly fluorescent at acidic pH. Briefly, testing mAbs 8G5G9.p1.z12, reference Abs and human IgG1 isotype control (100 nM) were incubated with pHAb-conjugated secondary antibodies (100 nM) at room temperature for 30 minutes before 3-fold serial dilution. Then the titrated mixtures were added into HEK-293/H_CDH17 or DLD-1/H_CDH17 cells seeded in 96-well plates one day before. After incubation at 37°C overnight, cells were washed twice gently with DPBS and detected by Tecan microplate reader for fluorescence density. The graphs were made and EC50s were calculated in GraphPad Prism Software (LaJolla, CA).

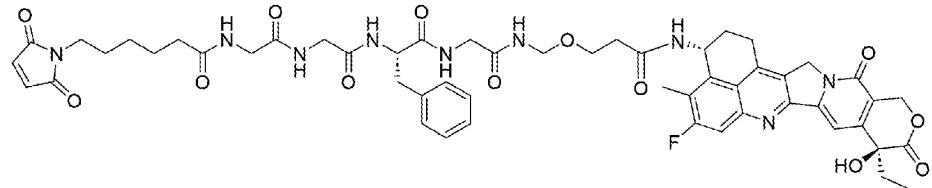
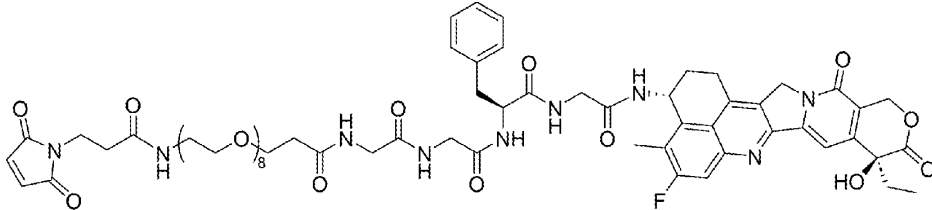
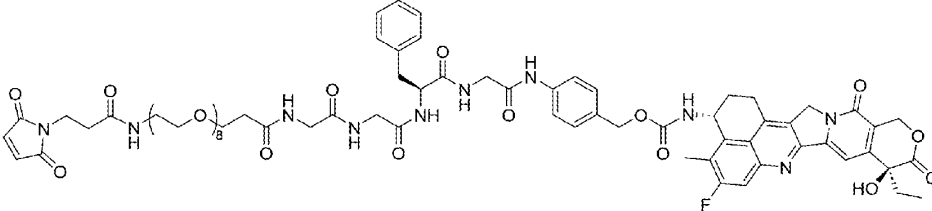
[0341] The results (**FIG. 6A-6B**) show that 8G5G9.p1.z12 had potent internalization activity on CDH17-overexpressing cell line.

Example 7. Specific binding of Anti-CDH17 ADCs to human CDH17 overexpressing cell lines

[0342] This example prepared an antibody-drug conjugate (ADC) with 8G5G9.p1.z12 and tested its binding to human CDH17-overexpressing cell lines.

[0343] Cell based binding of 8G5G9.p1.z12, reference Abs, human IgG1 isotype control and the corresponding ADCs (conjugated with MMAE, DXd, LM-D01, LM-D02, and LM-D03 at indicated DAR) to human CDH17 overexpressing cell lines were assessed using flow cytometry. The structures of LM-D01, LM-D02, and LM-D03 are shown in **Table 2**.

Table 2. Structures of LM-D01, LM-D02, and LM-D03

Name	Structure
LM-D01	
LM-D02	
LM-D03	

[0344] Briefly, HCT116/H_CDH17 and DLD-1/H_CDH17 cells were incubated with titrated testing samples (starting from 50 nM, 5-fold dilution, 8 points) respectively at 4°C for 60 minutes. Then cells were washed twice with 2% FBS/PBS buffer and stained with fluorescent conjugated anti-human Fc secondary antibody at 4°C for 30 minutes. Cells were washed twice again and then analyzed using flow cytometer Agilent NovoCyte for fluorescent density. The graphs were made and EC50s were calculated in GraphPad Prism Software (LaJolla, CA).

[0345] The results of the study (**FIG. 7A-7B** and **Table 3**) show that the anti-CDH17 ADCs effectively bound to the human CDH17-overexpressing cells in a dose-dependent manner with comparable affinity to the bare antibodies.

Table 3. Binding to CDH17 overexpressing cell lines

Name	HCT116/H CDH17		DLD-1/H CDH17	
	EC50 (nM)	Top (MFI)	EC50 (nM)	Top (MFI)
8G5G9.p1.z12	0.21	4538	0.22	5478
8G5G9.p1.z12-MMAE (Dar4)	0.21	4619	0.24	5399
8G5G9.p1.z12-DXd (Dar8)	0.25	4580	0.29	5346
8G5G9.p1.z12-LM-D01 (Dar8)	0.24	4263	0.27	5454
8G5G9.p1.z12-LM-D02 (Dar8)	0.27	4649	0.24	4935
8G5G9.p1.z12-LM-D03 (Dar4)	0.26	4188	0.23	4575
BMK-BI-905711	0.81	8723	0.05	5616
BMK-BI-905711-DXd (Dar8)	0.09	4290	0.05	4813
BMK-ARB202	0.33	2973	0.23	3987

Dar: Drug-antibody ratio

Example 8. Specific binding of Anti-CDH17 ADCs to endogenous human CDH17 on tumor cells

[0346] This example measured the binding of the anti-CDH17 ADCs to endogenous human CDH17 on tumor cells.

[0347] Cell based binding of 8G5G9.p1.z12, reference Abs, human IgG1 isotype control and the corresponding ADCs (conjugated with MMAE, DXd, LM-D01, LM-D02 or LM-D03 at indicated DAR) to tumor cell lines with endogenous human CDH17 expression were assessed using flow cytometry. Briefly, human colorectal cancer (CRC) cell line DLD-1 and gastric cancer (GC) cell line SNU-16 were incubated with titrated testing samples (starting from 50 nM, 5-fold dilution, 8 points) respectively at 4°C for 60 minutes. Then cells were washed twice with 2% FBS/PBS buffer, and stained with fluorescent conjugated anti-human Fc secondary antibody at 4°C for 30 minutes. Cells were washed twice again and then analyzed using flow cytometer Agilent NovoCyte for fluorescent density. The graphs were made and EC50s were calculated in GraphPad Prism Software (LaJolla, CA).

[0348] The results of the study (**FIG. 8A-8B and Table 4**) show that the anti-CDH17 ADCs could effectively bind to the endogenous human CDH17 expressing tumor cells in a dose dependent way with comparable affinity with the anti-CDH17 mAb.

Table 4. Binding to endogenous human CDH17 on tumor cells

Name	DLD-1		SNU-16	
	EC50 (nM)	Top (MFI)	EC50 (nM)	Top (MFI)
8G5G9.p1.z12	0.25	842	1.06	102945
8G5G9.p1.z12-MMAE (Dar4)	0.33	854	0.96	99093
8G5G9.p1.z12-DXd (Dar8)	0.32	850	1.04	93835

8G5G9.p1.z12-LM-D01 (Dar8)	0.33	831	1.29	98613
8G5G9.p1.z12-LM-D02 (Dar8)	0.26	762	0.93	90783
8G5G9.p1.z12-LM-D03 (Dar4)	0.30	801	0.77	88996
BMK-BI-905711 (Anti CDH17)	0.89	1941	0.23	116127
BMK-BI-905711 (Anti CDH17)-DXd (Dar8)	0.08	871	0.22	84687
BMK-ARB202 (Anti CDH17)	0.96	394	0.61	93378

Example 9. Cytotoxic effect of Anti-CDH17 ADCs towards human CDH17 expressing tumor cells

[0349] This example measured the cytotoxic effect of the anti-CDH17 ADCs.

[0350] The human CDH17 overexpressing HCT116/H_CDH17 and DLD-1/H_CDH17 cells were seeded into 96-well tissue culture plates and incubated with titrated testing ADCs (starting from 50 nM, 5-fold dilution, 8 points) respectively in a 37 °C, 5% CO₂ incubator. Simultaneously, cells without ADC treatment were set up as control groups. After 6 days of treatment, Cell Titer-Glo reagent (Promega) was added and the relative luminescence unit (RLU) was detected by EnVision. Cell viability index was determined as the percentage of sample well RLU in average control RLU. The graphs were made and IC₅₀s were calculated in GraphPad Prism Software (LaJolla, CA).

[0351] The results are shown in **FIG. 9** and **Table 5**. The tested anti-CDH17 ADCs had significant cytotoxic effect towards human CDH17-expressing cells.

Table 5. Cytotoxic effect of Anti CDH17 ADCs

Name	HCT116/H_CDH17		DLD-1/H_CDH17	
	IC ₅₀ (nM)	Bottom (%viability)	IC ₅₀ (nM)	Bottom (%viability)
8G5G9.p1.z12-MMAE (Dar4)	0.042	7.2	0.35	40.1
8G5G9.p1.z12-DXd (Dar8)	0.040	20.4	0.09	12.4
8G5G9.p1.z12-LM-D01 (Dar8)	0.017	13.8	0.07	14.3
8G5G9.p1.z12-LM-D02 (Dar8)	0.016	16.3	0.12	15.0
8G5G9.p1.z12-LM-D03 (Dar4)	0.066	20.6	0.43	7.2
BMK-BI-905711-DXd (Dar8)	0.023	15.5	0.07	13.4

Example 10. Bystander effect of Anti-CDH17 ADCs towards human CDH17 negative cell lines

[0352] This example measured the bystander effect of the anti-CDH17 ADCs.

[0353] The human CDH17 overexpressing CRC cell lines DLD-1/H_CDH17 and HCT116/H_CDH17 were incubated with ADC dilutions respectively in a 37 °C, 5% CO₂ incubator for 6 days to produce free payload-containing conditional medium (CM). Simultaneously, cells without ADC treatment were set up as control groups. The conditional medium was collected at day 6 and added into the CDH17 negative human tumor cells MDA-MB-231 and HCT116 seeded in black 96-well plates respectively at the volume ratio of 1:1. After 6 days of incubation at 37 °C, Cell Titer-Glo reagent (Promega) was added and the relative luminescence unit (RLU) was detected by EnVision. Cell viability index was determined as the percentage of sample well RLU in average control group RLU. The graphs were made in GraphPad Prism Software (LaJolla, CA).

[0354] The results are shown in **FIG. 10A-10F**, which demonstrate that the anti-CDH17 ADCs conjugated with DXd and LM-D03 showed marked bystander effect on CDH17 negative MDA-MB-231 and HCT116 cells, while the ADCs with MMAE showed marked bystander effect on MDA-MB-231 cells.

Example 11. Antitumor activity of anti-CDH17 ADCs in colorectal cancer (CRC) CDX (Cell line-derived xenograft) model

[0355] This example measured the anti-tumor effect of the anti-CDH17 ADCs.

[0356] The CRC CDX models were established by injecting 5 million of DLD-1/H_CDH17 cells per mouse s.c. into the right flank of the Balb/C nude mice. The tumor-bearing mice were randomized into treatment and control groups (5 mice/group) before the tumor volume reached 100 mm³, and dosing initiated (day 0). Each ADC was given i.v. to the mice at a dose of 3 or 6 mg/kg, and a frequency of q.w. × 3 weeks. As a vehicle, DPBS buffer was given at the same volume as the ADCs.

[0357] Most of the tested ADSc induced potent tumor regression at indicated doses (**FIG. 11A-11B**) and were safe.

Example 12. Characterization of the anti-CDH17 antibodies

[0358] The VH/VL sequences of additional 6 lead humanized antibodies are further provided in **Table A3** and the CDR sequences are provided in **Table A4**. The property of the anti-

CDH17 antibodies were evaluated in cell-based binding assay and antibody internalization assay.

Cell based binding assay

[0359] The cells were incubated with titrated antibodies or antibody drug conjugates (top concentrations for different cell lines ranges from 200nM to 40nM, all were done as 5-fold dilution) at 4°C for 60 minutes. Then the cells were washed with FACS buffer twice and stained with fluorescent conjugated secondary antibody (Alexa Fluor® 647 Goat Anti-Human IgG, Jackson, 109-605-098) at 4°C for 60 minutes. After that the cells were washed twice and analyzed by flow cytometry.

[0360] Both anti-CDH17 antibodies 296G7E10.p4.Z9 and 312E10A9.p2.Z7 bind to human CDH17 overexpressing HE293 cells (**FIG. 12**, with panel A-B) and rhesus CDH17 overexpressing HE293 cells (**FIG. 13**, with panel A-B).

[0361] Cell based binding of anti-CDH17 antibodies (such as 35E7D6.p1.z9, 135B2E11.p1.z1, 153A3D7.p3.z5, and 155B9A5.p3.V2.z2) and human IgG1 isotype control to human CDH17 overexpressing cell lines were assessed using flow cytometry. Briefly, HEK-293/H_CDH17 and DLD-1/H_CDH17 cells were incubated with titrated testing mAbs (starting from 200 nM, 3-fold dilution, 12 points) respectively at 4°C for 60 minutes. Then cells were washed twice with 2% FBS/PBS buffer, and stained with fluorescent conjugated anti-human Fc secondary antibody at 4°C for 30 minutes. Cells were washed twice again and then analyzed by flow cytometry for fluorescent signal. The graphs were made and EC50s were calculated in GraphPad Prism Software (LaJolla, CA).

[0362] From the results shown in **FIG. 12** (panel C-D) and **Table 6**, anti-CDH17 antibodies could efficiently bind to the human CDH17 overexpressing cells in dose-dependent way.

Table 6. Binding of anti-CDH17 antibodies to human CDH17 overexpressing cell lines

Sample	HEK-293/H_CDH17		DLD-1/H_CDH17	
	EC50 (nM)	Top (MFI)	EC50 (nM)	Top (MFI)
35E7D6.p1.z9	0.55	41294	0.57	10310
135B2E11.p1.z1	0.96	38265	0.96	9422
153A3D7.p3.z5	0.68	196238	1.14	11762
155B9A5.p3.V2.z2	0.66	149924	1.73	9796

[0363] Cell based binding of anti-CDH17 antibodies and human IgG1 isotype control to endogenous CDH17 expressing human tumor cell lines were assessed using flow cytometry. Briefly, gastric cancer (GC) cell line SNU-16 and colorectal cancer (CRC) cell line LoVo were incubated with titrated testing mAbs (starting from 200 nM, 3-fold dilution, 12 points) respectively at 4°C for 60 minutes. Then cells were washed twice with 2% FBS/PBS buffer, and stained with fluorescent conjugated anti-human Fc secondary antibody at 4°C for 30 minutes. Cells were washed twice again and then analyzed by flow cytometry for fluorescent signal. The graphs were made and EC50s were calculated in GraphPad Prism Software (LaJolla, CA).

[0364] As shown in **FIG. 14** (panel A-D) and **Table 7**, anti-CDH17 antibodies could effectively bind to the human tumor cell lines with endogenous CDH17 expression in dose dependent way.

Table 7. Binding of anti-CDH17 antibodies to endogenous human CDH17 on tumor cells

Sample	SNU-16		LoVo	
	EC50 (nM)	Top (MFI)	EC50 (nM)	Top (MFI)
35E7D6.p1.z9	2.60	170230	6.37	2759
135B2E11.p1.z1	3.92	143678	1.61	1608
153A3D7.p3.z5	2.24	109781	16.41	621
155B9A5.p3.V2.z2	1.10	119549	1.49	1160

Antibody internalization assay

CDH17 endogenously expressing cell line-based internalization

[0365] Internalization of 296G7E10.p4.Z9 and 312E10A9.p2.Z7 on human CDH17 endogenously expressing cells (SNU16 and LoVo) were assessed using flow cytometry. Briefly, the second antibody(AffiniPure F(ab')₂ Fragment Goat Anti-Human IgG, Fcγ fragment specific, Jackson) conjugated with pHAb Amine Reactive Dye(Promega) was mixed with 296G7E10.p4.Z9, 312E10A9.p2.Z7 and the isotype control at 37°C for 30 minutes. Then the first and second antibody mixture was titrated to incubate with SNU16 or LoVo cells at 37°C overnight. The next day, the cells were collected and analyzed by assessing fluorescence value using flow cytometer.

[0366] The cancer cell line-based internalization were shown in **FIG. 15** with panel A-B.

CDH17 overexpressing cell line-based internalization

[0367] Internalization potency of anti-CDH17 antibodies was assessed using secondary antibodies labeled with pH sensor dyes (pHAb dyes) that becomes highly fluorescent at acidic pH. Briefly, testing mAbs Anti-CDH17 antibodies or human IgG1 isotype control (33.3333 nM) were incubated with pHAb-conjugated secondary antibodies (33.3333 nM) at room temperature for 30 minutes before 3-fold serial dilution. Then the titrated mixtures were added into HEK-293/H_CDH17 or DLD-1/H_CDH17 cells seeded in 96-well plates one day before. After incubation at 37°C overnight, cells were washed twice gently with DPBS and detected by Tecan microplate reader for fluorescence density. The graphs were made and EC50s were calculated in GraphPad Prism Software (LaJolla, CA).

[0368] As shown in **FIG. 16** (A-B) and **Table 8**, anti-CDH17 antibodies showed potent internalization activity on CDH17 overexpressing cell line.

Table 8. Internalization activity of the anti-CDH17 antibodies on CDH17 overexpressing cell line

Sample	HEK-293/H_CDH17		DLD-1/H_CDH17	
	EC50 (nM)	Top (RFU)	EC50 (nM)	Top (RFU)
35E7D6.p1.z9	4.39	36486	6.00	27545
135B2E11.p1.z1	3.06	34337	6.40	28009
153A3D7.p3.z5	3.17	37268	40.64	57489
155B9A5.p3.V2.z2	3.36	35716	3.06	23017

Example 13. Cytotoxic effect of CDH17-targeted ADCs towards human CDH17 expressing cells

[0369] The human CDH17 overexpressing HEK-293/H_CDH17, DLD-1/H_CDH17 or CDH17 endogenously expressing LoVo cells were seeded into 96-well tissue culture plates and incubated with titrated testing ADCs (starting from 10 nM, 3-fold dilution, 9 points) respectively in a 37 °C, 5% CO₂ incubator. Simultaneously, cells without ADC treatment were set up as control groups. After 6 days of treatment, Cell Titer-Glo reagent (Promega) was added and the relative luminescence unit (RLU) was detected by EnVision. Cell viability index was determined as the percentage of sample well RLU in average control RLU. The graphs were made and IC50s were calculated in GraphPad Prism Software (LaJolla, CA).

[0370] As shown in **FIG. 17** (panel A-C) and **Table 9**, anti-CDH17 ADCs showed significant cytotoxic effect towards human CDH17 expressing cells.

Table 9. Cytotoxic effect of CDH17-targeted ADCs towards human CDH17 expressing cells

Sample	HEK-293/H_CDH17		DLD-1/H_CDH17		LoVo	
	IC50 (nM)	Min Viability	IC50 (nM)	Min Viability	IC50 (nM)	Min Viability
35E7D6.p1.z9-LM-D03	0.06	2.8	0.03	4.5	0.07	~ 0
135B2E11.p1.z1-LM-D03	0.07	4.1	0.11	3.5	0.21	11.3
153A3D7.p3.z5-LM-D03	0.07	5.6	0.06	5.3	NA	NA
155B9A5.p3.V2.z2-LM-D03	0.08	2.3	0.03	4.2	NA	27.9

Example 14. Cytotoxic effect of CDH17-targeted ADCs towards human CDH17 expressing cells

[0371] The viability of human CDH17-expressing colorectal cancer (CRC) or gastric cancer (GC) cells were accessed using CellTiter-Glo after ADC treatment for 6 days. As shown in **FIG. 18** (panel A-C) and **Table 10**, anti-CDH17 ADCs showed significant cytotoxic effect towards SNU-16, HCT116/H_CDH17 and DLD-1/H_CDH17 cells, with IC50 values ranging from 0.016 to 0.35 nM.

Table 10. Cytotoxic effect of CDH17-targeted ADCs towards human CDH17 expressing cells

Antibody	SNU-16		HCT116/CDH17		DLD-1/CDH17	
	IC50 (nM)	Bottom (%viability)	IC50 (nM)	Bottom (%viability)	IC50 (nM)	Bottom (%viability)
8G5G9.p1.z12-MMAE (Dar4)	0.016	1.9	0.042	7.2	0.35	40.1
8G5G9.p1.z12-DXd (Dar8)	0.056	37.5	0.040	20.4	0.09	12.4
8G5G9.p1.z12-LM-D01 (Dar8)	0.016	8.8	0.017	13.8	0.07	14.3

Example 15. *In vivo* anti-tumor effect of CDH17-targeted ADCs

1. Antitumor activity of Anti-CDH17 ADCs in CRC CDX (Cell line-derived xenograft) model

[0372] The CRC CDX models were established by injecting 5 million of DLD-1/H_CDH17 cells per mouse s.c. into the right flank of the Balb/C nude mice. The tumor-bearing mice were randomized into treatment and control groups (5 mice/group) before the tumor volume reached 100 mm³, and dosing initiated (day 0). Each ADC was given i.v. to the mice at a dose of 3 or 6 mg/kg, and at a frequency of q.w. × 3 weeks. As a vehicle, DPBS buffer was given at the same volume as the ADCs.

[0373] As a result, MMAE-conjugated ADCs at the dose of 3 mg/kg showed moderate tumor growth inhibition activity, while 8G5G9.p1.z12-LM-D01 at 6mg/kg induced potent tumor regression (**FIG. 19** with panel A and B and **Table 11**). TORL was TORL-3-600, generated by TORL BioTherapeutic from a fully humanized CDH17 mAb by MMAE conjugation.

Table 11. Tumor growth inhibition (%) and complete response (CR)

Group	%TGI	CR
BMK-MMAE (TORL)_3mg/kg	48.62	/
8G5G9.p1.z12-MMAE_3mg/kg	56.15	/
8G5G9.p1.z12- LM-D01 _6mg/kg	89.08	/

2. Antitumor activity of CDH17-targeted ADCs in SNU-16 (GC) xenograft model

[0374] SNU-16 models were established by injecting 10 million cells suspended in a Matrigel matrix s.c. into the right flank of the CB17-SCID mice. The tumor-bearing mice were randomized into treatment and control groups (5 mice/group) when the tumor volume reached 100-150 mm³, and given a single dose of 3 or 6 mg/kg i.v. (day 0). As a vehicle, DPBS buffer was given at the same volume as the ADCs.

[0375] As shown in **FIG. 20** (panel A and B) and **Table 12**, a single dose of 3 or 6 mg/kg of 8G5G9.p1.z12-LM-D01(Dar8) induced potent tumor regression in GC CDX model.

Table 12. Tumor growth inhibition (%) and complete response (CR)

Group	%TGI	CR
8G5G9.p1.z12-LM-D01 3 mg/kg	129	3/5
8G5G9.p1.z12-LM-D01 6 mg/kg	129	4/5
Human IgG1-LM-D01 6 mg/kg	18	/

* * *

[0376] The present disclosure is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the disclosure, and any compositions or methods which are functionally equivalent are within the scope of this disclosure. It will be apparent to those skilled in the art that various modifications and variations can be made in the methods and compositions of the present disclosure without departing from the spirit or scope of the disclosure. Thus, it is intended that the present disclosure cover the modifications and variations of this disclosure provided they come within the scope of the appended claims and their equivalents.

[0377] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

CLAIMS

What is claimed is:

1. An antibody or antigen-binding fragment thereof that has binding specificity to a human Cadherin-17 (CDH17) protein, wherein the antibody or the fragment thereof comprises a heavy chain variable region (VH) comprising heavy chain complementarity determining regions (CDR) VH CDR1, VH CDR2, and VH CDR3, and a light chain variable region (VL) comprising VL CDR1, VL CDR2, and VL CDR3, and wherein the VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3, respectively, comprise:
 - (a) the amino acid sequences of SEQ ID NO:11-16;
 - (b) the amino acid sequences of SEQ ID NO:17-22;
 - (c) the amino acid sequences of SEQ ID NO:23-28;
 - (d) the amino acid sequences of SEQ ID NO:29-34;
 - (e) the amino acid sequences of SEQ ID NO:35-40;
 - (f) the amino acid sequences of SEQ ID NO:41-46; or
 - (g) the amino acid sequences of SEQ ID NO:1-6.
2. The antibody or antigen-binding fragment thereof of claim 1, wherein the VH CDR1 comprises the amino acid sequence of SEQ ID NO:11, the VH CDR2 comprises the amino acid sequence of SEQ ID NO:12, the VH CDR3 comprises the amino acid sequence of SEQ ID NO:13, the VL CDR1 comprises the amino acid sequence of SEQ ID NO:14, the VL CDR2 comprises the amino acid sequence of SEQ ID NO:15, and the VL CDR3 comprises the amino acid sequence of SEQ ID NO:16.
3. The antibody or antigen-binding fragment thereof of claim 2, wherein the VH comprises the amino acid sequence of SEQ ID NO:47, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:47, and the VL comprises the amino acid sequence of SEQ ID NO:48, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:48.
4. The antibody or antigen-binding fragment thereof of claim 1, wherein the VH CDR1 comprises the amino acid sequence of SEQ ID NO:17, the VH CDR2 comprises the amino acid sequence of SEQ ID NO:18, the VH CDR3 comprises the amino acid sequence of SEQ ID NO:19,

the VL CDR1 comprises the amino acid sequence of SEQ ID NO:20,
the VL CDR2 comprises the amino acid sequence of SEQ ID NO:21, and
the VL CDR3 comprises the amino acid sequence of SEQ ID NO:22.

5. The antibody or antigen-binding fragment thereof of claim 4, wherein the VH comprises the amino acid sequence of SEQ ID NO:49, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:49, and the VL comprises the amino acid sequence of SEQ ID NO:50, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:50.

6. The antibody or antigen-binding fragment thereof of claim 1, wherein
the VH CDR1 comprises the amino acid sequence of SEQ ID NO:23,
the VH CDR2 comprises the amino acid sequence of SEQ ID NO:24,
the VH CDR3 comprises the amino acid sequence of SEQ ID NO:25,
the VL CDR1 comprises the amino acid sequence of SEQ ID NO:26,
the VL CDR2 comprises the amino acid sequence of SEQ ID NO:27, and
the VL CDR3 comprises the amino acid sequence of SEQ ID NO:28.

7. The antibody or antigen-binding fragment thereof of claim 6, wherein the VH comprises the amino acid sequence of SEQ ID NO:51, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:51, and the VL comprises the amino acid sequence of SEQ ID NO:52, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:52.

8. The antibody or antigen-binding fragment thereof of claim 1, wherein
the VH CDR1 comprises the amino acid sequence of SEQ ID NO:29,
the VH CDR2 comprises the amino acid sequence of SEQ ID NO:30,
the VH CDR3 comprises the amino acid sequence of SEQ ID NO:31,
the VL CDR1 comprises the amino acid sequence of SEQ ID NO:32,
the VL CDR2 comprises the amino acid sequence of SEQ ID NO:33, and
the VL CDR3 comprises the amino acid sequence of SEQ ID NO:34.

9. The antibody or antigen-binding fragment thereof of claim 8, wherein the VH comprises the amino acid sequence of SEQ ID NO:53, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:53, and the VL comprises the

amino acid sequence of SEQ ID NO:54, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:54.

10. The antibody or antigen-binding fragment thereof of claim 1, wherein the VH CDR1 comprises the amino acid sequence of SEQ ID NO:35, the VH CDR2 comprises the amino acid sequence of SEQ ID NO:36, the VH CDR3 comprises the amino acid sequence of SEQ ID NO:37, the VL CDR1 comprises the amino acid sequence of SEQ ID NO:38, the VL CDR2 comprises the amino acid sequence of SEQ ID NO:39, and the VL CDR3 comprises the amino acid sequence of SEQ ID NO:40.
11. The antibody or antigen-binding fragment thereof of claim 10, wherein the VH comprises the amino acid sequence of SEQ ID NO:55, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:55, and the VL comprises the amino acid sequence of SEQ ID NO:56, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:56.
12. The antibody or antigen-binding fragment thereof of claim 1, wherein the VH CDR1 comprises the amino acid sequence of SEQ ID NO:41, the VH CDR2 comprises the amino acid sequence of SEQ ID NO:42, the VH CDR3 comprises the amino acid sequence of SEQ ID NO:43, the VL CDR1 comprises the amino acid sequence of SEQ ID NO:44, the VL CDR2 comprises the amino acid sequence of SEQ ID NO:45, and the VL CDR3 comprises the amino acid sequence of SEQ ID NO:46.
13. The antibody or antigen-binding fragment thereof of claim 12, wherein the VH comprises the amino acid sequence of SEQ ID NO:57, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:57, and the VL comprises the amino acid sequence of SEQ ID NO:58, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:58.
14. The antibody or antigen-binding fragment thereof of claim 1, wherein the VH CDR1 comprises the amino acid sequence of SEQ ID NO:1, the VH CDR2 comprises the amino acid sequence of SEQ ID NO:2, the VH CDR3 comprises the amino acid sequence of SEQ ID NO:3, the VL CDR1 comprises the amino acid sequence of SEQ ID NO:4,

the VL CDR2 comprises the amino acid sequence of SEQ ID NO:5, and
the VL CDR3 comprises the amino acid sequence of SEQ ID NO:6.

15. The antibody or antigen-binding fragment thereof of claim 14, wherein the VH comprises the amino acid sequence of SEQ ID NO:7, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:7, and the VL comprises the amino acid sequence of SEQ ID NO:8, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:8.
16. An antibody or antigen-binding fragment thereof having specificity to a human CDH17 protein, wherein the antibody or antigen-binding fragment thereof competes with the antibody or antigen-binding fragment thereof of any one of the preceding claims in binding to the CDH17 protein.
17. The antibody or antigen-binding fragment thereof of any one of claims 1-16, which is humanized.
18. The antibody or antigen-binding fragment thereof of any one of claims 1-17, which is a F(ab')₂, a F(ab)₂, a Fab', a Fab, a Fv, or a scFv.
19. The antibody or antigen-binding fragment thereof of any one of claims 1-18, wherein the antibody or antigen-binding fragment thereof further comprises a heavy chain constant region, a light chain constant region, an Fc region, or the combination thereof.
20. The antibody or antigen-binding fragment thereof of any one of claims 1-19, wherein the antibody or antigen-binding fragment thereof is of an isotype of IgG1, IgG2, IgG3 or IgG4.
21. The antibody or antigen-binding fragment thereof of claim 20, wherein the antibody or antigen-binding fragment thereof is of an isotype of human IgG1.
22. The antibody or antigen-binding fragment thereof of any one of claims 1-21, which is conjugated.
23. A bifunctional molecule, comprising a first antigen-binding portion having specificity to a human CDH17 protein and a second portion having specificity to a second antigen,

wherein the first antigen-binding portion comprises the antibody or antigen-binding fragment thereof of any one of claims 1-22.

24. An antibody-drug conjugate, comprising the antibody or antigen-binding fragment thereof of any one of claims 1-22 or the bifunctional molecule of claim 23, and a drug moiety.

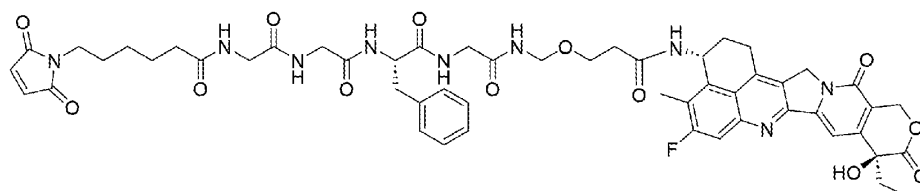
25. The antibody-drug conjugate of claim 24, wherein the drug moiety is a cytotoxin, an immunosuppressive agent, a radioisotope, or a toxin.

26. The antibody-drug conjugate of claim 25, wherein the drug moiety is a cytotoxin selected from the group consisting of auristatins, maytansinoids, macrocyclic ketone analogues, topoisomerase inhibitors, benzodiazepines, tubulysins, duocarmycin, camptothecin, calicheamicins, exatecans, irinotecans (SN38), doxorubicin, anthracycline, the pyrrolobenzodiazepenes (PBD), TLR agonist, STING agonists, pseudomonas aeruginosa exotoxin PE38, diphtheria toxin, staphylococcus aureus enterotoxin A/E-120, antibacterial antibiotic, shigatoxin, ricin, and urease.

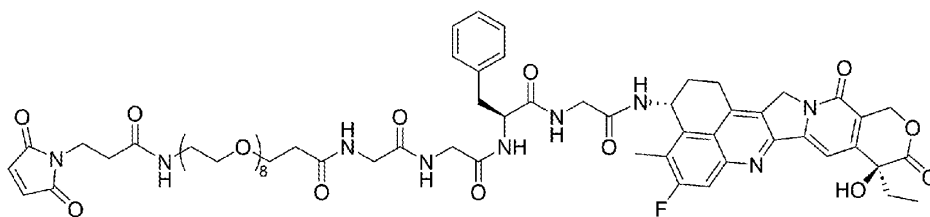
27. The antibody-drug conjugate of claim 25, wherein the drug moiety is a cytotoxin selected from the group consisting of monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF), maytansine, mertansine (DM1), ravtansine (DM4), tublysin A, DXd, 7-ethyl-10-hydroxycamptothecin (SN-38), DGN462, Amberstatin269, anthramycin, SG3199/SCX, IRDye®700DX, TLR7/8 agonist, diABZI STING agonist-2, or any derivative thereof.

28. The antibody-drug conjugate of any one of claims 25-27, wherein the drug moiety comprises MMAE, MMAF, or DXd.

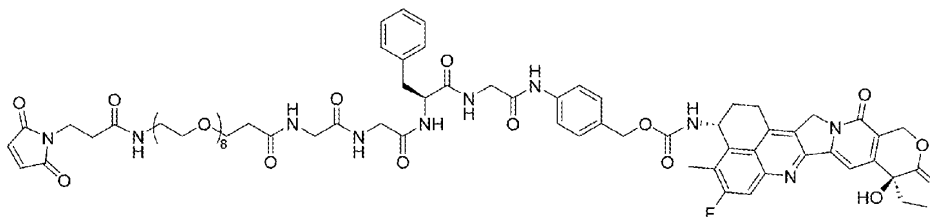
29. The antibody-drug conjugate of claim 26, wherein the drug moiety comprises a compound selected from the group consisting of:



(LM-D01),



(LM-D02), and



(LM-D03).

30. The antibody-drug conjugate of any one of claims 24-29, wherein the antibody or antigen-binding fragment thereof is conjugated to the drug moiety via a linker.

31. The antibody-drug conjugate of claim 30, wherein the linker is hydrolyzable under acidic conditions.

32. The antibody-drug conjugate of any one of claims 30-31, wherein the linker comprises succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), sulfo-SMCC, p-carboxycyclohexylmethylmaleimide, maleimide-caproyl (MC)-Valine-citrulline (VC)-para-aminobenzoyloxycarbonyl (PABC), CL2A, maleimide-caproyl (MC), MC-glycine-glycine-phenylalanine-glycine (GGFG), MC-PEG8-GGFG, MC-PEG8-GGFG-PAB, or maleimide propoyl (MP)-PEG8-Valine-alanine (VA)-PABC.

33. The antibody-drug conjugate of any one of claims 24-32, comprising ozogamicin, vedotin, mafodotin, emtansine, deruxtecan, govitecan, or tesirine.

34. The antibody-drug conjugate of any one of claims 24-33, wherein the drug-antibody ratio (DAR) is 1-20.

35. The antibody-drug conjugate of any one of claims 24-33, wherein the drug-antibody ratio (DAR) is 4-8.

36. A chimeric antigen receptor (CAR) having specificity to the human CDH17 protein, comprising an antigen-binding fragment of any one of claims 1-22 or a bifunctional molecule of claim 23, a transmembrane domain, and an intracellular activating domain.
37. The CAR of claim 36, wherein the antigen-binding fragment is a single chain fragment (scFv).
38. The CAR of claim 36 or 37, wherein the transmembrane domain is a transmembrane domain of 4-1BB, an alpha chain of a T cell receptor, a beta chain of a T cell receptor, a zeta chain of a T cell receptor, CD3 epsilon, CD4, CD5, CD8 alpha, CD9, CD16, CD19, CD22, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD134, CD137, or CD154.
39. The CAR of any one of claims 36-38, wherein the chimeric antigen receptor comprises an intracellular activation domain that is a signaling domain derived from CD3 zeta, CD3 epsilon, CD3 delta, and CD3 gamma.
40. The CAR of any one of claims 36-39, further comprising one or more costimulatory domains selected from a signaling domain (or other suitable portion) of CD28, OX-40, 4-1BB/CD137, CD2, CD7, CD27, CD30, CD40, Programmed Death-1 (PD-1), inducible T cell costimulator (ICOS), lymphocyte function-associated antigen-1 (LFA-1, CD1-1a/CD18), CD3 gamma, CD3 delta, CD3 epsilon, CD247, CD276 (B7-H3), LIGHT, (TNFSF14), NKG2C, Ig alpha (CD79a), DAP-10, Fc gamma receptor, MHC class 1 molecule, TNF receptor proteins, an Immunoglobulin protein, cytokine receptor, integrins, Signaling Lymphocytic Activation Molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, ICAM-1, B7-H3, CDS, ICAM-1, GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL-2R beta, IL-2R gamma, IL-7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, a ligand that specifically binds with CD83, or any combination thereof.

41. The CAR of any one of claims 36-40, further comprising a hinge domain selected from a hinge domain of IgG1, IgG2, IgG3, IgG4, IgA, IgD, IgE, IgM, CD28, or CD8 alpha, any truncation thereof, or any combination thereof.
42. A nucleic acid encoding the antibody or antigen-binding fragment thereof of any one of claims 1-22, the bifunctional molecule of claim 23, or the CAR of any one of claims 34-39.
43. A vector comprising the nucleic acid of claim 42.
44. An isolated cell comprising the nucleic acid of claim 42, or the vector of claim 43.
45. A pharmaceutical composition comprising the antibody or antigen-binding fragment thereof of any one of claims 1-22, the bifunctional molecule of claim 23, the antibody-drug conjugate of any one of claims 24-35, or the CAR of any one of claims 36-41, and a pharmaceutically acceptable carrier.
46. A method of treating cancer in a patient in need thereof, comprising administering to the patient the antibody or antigen-binding fragment thereof of any one of claims 1-22, the bifunctional molecule of claim 23, the antibody-drug conjugate of any one of claims 24-35, or the CAR of any one of claims 36-41, or the pharmaceutical composition of claim 45.
47. Use of the antibody or antigen-binding fragment thereof of any one of claims 1-22, the bifunctional molecule of claim 23, the antibody-drug conjugate of any one of claims 24-35, or the CAR of any one of claims 36-41, or the pharmaceutical composition of claim 45 for the preparation of a medicament for treating cancer.
48. The antibody or antigen-binding fragment thereof of any one of claims 1-22, the bifunctional molecule of claim 23, the antibody-drug conjugate of any one of claims 24-35, or the CAR of any one of claims 36-41, or the pharmaceutical composition of claim 45 for use in the treatment of cancer in a patient in need thereof.
49. The method of claim 46 or the use of claim 47 or the antibody or antigen-binding fragment thereof, bispecific antibody or antigen-binding fragment thereof, antibody-drug conjugate, CAR or pharmaceutical composition for use of claim 46, wherein the cancer is selected from the group consisting of gastric cancer, colorectal cancer, hepatocarcinoma, bladder cancer, liver cancer, endometrial cancer, leukemia, lymphoma, pancreatic cancer, neuroendocrine cancer, small cell lung cancer, non-small cell lung cancer, breast cancer,

urethral cancer, head and neck cancer, gastrointestinal cancer, oesophageal cancer, ovarian cancer, renal cancer, melanoma, prostate cancer and thyroid cancer.