ANTI-CEACAM5 ANTIBODIES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 63/558,509, filed February 27, 2024, the contents of which are incorporated herein by reference in their entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0002] The content of the electronically submitted sequence listing (Name: 3338_334PC02_Sequencelisting_ST26.xml; Size: 112,410 bytes; and Date of Creation: February 8, 2025), filed with the application, is incorporated herein by reference in its entirety.

FIELD OF DISCLOSURE

[0003] The disclosure provides novel antibodies or antigen binding portions that specifically binds to carcinoembryonic antigen-related cell adhesion molecule-5 for use in a therapy.

BACKGROUND

[0004] Carcinoembryonic antigen-related cell adhesion molecule-5 (CEACAM5) is a cell surface protein that is weakly expressed in normal epithelial tissues, including colon, esophagus, head and neck, stomach, and cervix tissue, but is highly expressed in several tumor types including colorectal, gastrointestinal, lung, and breast, with highest prevalence and expression occuring in greater than 80% of colorectal cancers. In normal tissue, CEACAM5 protects luminal organs from microbial invasion. In tumor cells, CEACAM5 is functionally associated with cell differentiation, cell adhesion, tumor invasion and metastasis.

[0005] CEACAM5 overexpression is often associated with poor prognosis. For example, in patients with stage I, II, and III colorectal cancer, 5-year survival was found to be inversely correlated with tissue expression of CEACAM5, and in patients with stage III disease, increased serum levels of CEACAM5 are associated with poor prognosis (Gazzah *et al.*, Ann Oncol.,

33(4):416-425). The consistent overexpression of CEACAM5 in many cancers has made it an accepted tumor biomarker and indicator of recurrence in patients with cancer, especially those with colorectal cancer.

[0006] Multiple therapeutic approaches to target CEACAM5 in cancer are in development. However, to date, there are no FDA-approved CEACAM5-targeted therapies for cancer. Therefore, there remains an urgent need for effective treatments for CEACAM5-associated cancers, including antibodies specifically directed to CEACAM5 that do not cross-react with other molecules of the CEACAM family, as well as antibody drug conjugates (ADCs) which specifically kill CEACAM5-expressing cancer cells.

SUMMARY

[0007] The present disclosure provides an isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising: (a) a heavy chain variable region (VH) comprising complementarity determining region (CDR)1, CDR2, and CDR3 regions comprising the amino acid sequences set forth in SEQ ID NOs: 14, 15, and 16, respectively, and a light chain variable region (VL) comprising CDR1, CDR2, and CDR3 regions comprising the amino acid sequences set forth in SEQ ID NOs: 19, 20, and 21, or; (b) a VH comprising CDR1, CDR2, and CDR3 regions which have at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to the amino acid sequences set forth in SEQ ID NOs: 14, 15, and 16, respectively, and a VL comprising CDR1, CDR2, and CDR3 regions which have at least 85%, 90%, 95%, 98%, or 99% sequence identity to the amino acid sequences set forth in SEQ ID NOs: 19, 20, and 21, respectively.

[0008] In some aspects, the VH comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 38, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, or SEQ ID NO: 93.

[0009] In some aspects, the VH comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 17.

[0010] In some aspects, the VH comprises the amino acid sequence set forth in SEQ ID NO: 38, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71,

SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, or SEQ ID NO: 93.

[0011] In some aspects, the VH comprises the amino acid sequence set forth in SEQ ID NO: 17.

[0012] In some aspects, the VL comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to an amino acid sequence set forth in SEQ ID NO: 43, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 92, or SEQ ID NO: 94.

[0013] In some aspects, the VL comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to an amino acid sequence set forth in SEQ ID NO: 22.

[0014] In some aspects, the VL comprises the amino acid sequence set forth in SEQ ID NO: 43, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 92, or SEQ ID NO: 94.

[0015] In some aspects, the VL comprises the amino acid sequence set forth in SEQ ID NO: 22.

[0016] In some aspects, the VH and the VL have at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to: (a) the amino acid sequence set forth in SEQ ID NOs: 38 and the amino acid sequence set forth in SEQ ID NO 43, respectively; (b) the amino acid sequence set forth in SEQ ID NO: 49 and the amino acid sequence set forth in SEQ ID NO: 50; respectively; (c) the amino acid sequence set forth in SEQ ID NO: 51 and the amino acid sequence set forth in SEQ ID NO: 52, respectively; (d) the amino acid sequence set forth in SEQ ID NO: 67 and the amino acid sequence set forth in SEQ ID NO: 68, respectively; (e) the amino acid sequence set forth in SEQ ID NO: 69 and the amino acid sequence set forth in SEQ ID NO: 70, respectively; (f) the amino acid sequence set forth in SEQ ID NO: 71 and the amino acid sequence set forth in SEQ ID NO: 72, respectively; (g) the amino acid sequence set forth in SEQ ID NO: 73 and the amino acid sequence set forth in SEQ ID NO: 74, respectively; (h) the amino acid sequence set forth in SEQ ID NO: 75 and the amino acid sequence set forth in SEQ ID NO: 76, respectively; (i) the amino acid sequence set forth in SEQ ID NO: 77 and the amino acid sequence set forth in SEQ ID NO: 78, respectively; (j) the amino acid sequence set forth in SEQ ID NO: 79 and the amino acid

sequence set forth in SEQ ID NO: 80, respectively; (k) the amino acid sequence set forth in SEQ ID NO: 81 and the amino acid sequence set forth in SEQ ID NO: 82, respectively; (l) the amino acid sequence set forth in SEQ ID NO: 83 and the amino acid sequence of SEQ ID NO: 84, respectively; (m) the amino acid sequence set forth in SEQ ID NO: 85 and the amino acid sequence set forth in SEQ ID NO: 86, respectively; (n) the amino acid sequence set forth in SEQ ID NO: 87 and the amino acid sequence set forth in SEQ ID NO: 88, respectively; (o) the amino acid sequence set forth in SEQ ID NO: 89 and the amino acid sequence set forth in SEQ ID NO: 90, respectively; (p) the amino acid sequence set forth in SEQ ID NO: 91 and the amino acid sequence set forth in SEQ ID NO: 92, respectively; or (q) the amino acid sequence set forth in SEQ ID NO: 93 and the amino acid sequence set forth in SEQ ID NO: 94, respectively.

[0017] In some aspects, the VH and the VL have at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to: the amino acid sequence set forth in SEQ ID NOs: 17 and the amino acid sequence set forth in SEQ ID NO:22, respectively.

[0018] In some aspects, the VH and the VL comprises: (a) the amino acid sequence set forth in SEQ ID NOs: 38 and the amino acid sequence set forth in SEQ ID NO 43, respectively; (b) the amino acid sequence set forth in SEQ ID NO: 49 and the amino acid sequence set forth in SEQ ID NO: 50; respectively; (c) the amino acid sequence set forth in SEQ ID NO: 51 and the amino acid sequence set forth in SEQ ID NO: 52, respectively; (d) the amino acid sequence set forth in SEQ ID NO: 67 and the amino acid sequence set forth in SEQ ID NO: 68, respectively; (e) the amino acid sequence set forth in SEQ ID NO: 69 and the amino acid sequence set forth in SEQ ID NO: 70, respectively; (f) the amino acid sequence set forth in SEQ ID NO: 71 and the amino acid sequence set forth in SEQ ID NO: 72, respectively; (g) the amino acid sequence set forth in SEQ ID NO: 73 and the amino acid sequence set forth in SEQ ID NO: 74, respectively; (h) the amino acid sequence set forth in SEQ ID NO: 75 and the amino acid sequence set forth in SEQ ID NO: 76, respectively; (i) the amino acid sequence set forth in SEQ ID NO: 77 and the amino acid sequence set forth in SEQ ID NO: 78, respectively; (j) the amino acid sequence set forth in SEQ ID NO: 79 and the amino acid sequence set forth in SEQ ID NO: 80, respectively; (k) the amino acid sequence set forth in SEQ ID NO: 81 and the amino acid sequence set forth in SEQ ID NO: 82, respectively; (l) the amino acid sequence set forth in SEQ ID NO: 83 and the amino acid sequence set forth in SEQ ID NO: 84, respectively; (m) the amino acid sequence set forth in SEQ ID NO: 85 and the amino acid sequence set forth in SEQ ID NO: 86, respectively; (n) the amino acid sequence set forth in SEQ ID NO: 87 and the amino acid sequence set forth in

SEQ ID NO: 88, respectively; (o) the amino acid sequence set forth in SEQ ID NO: 89 and the amino acid sequence set forth in SEQ ID NO: 90, respectively; (p) the amino acid sequence set forth in SEQ ID NO: 91 and the amino acid sequence set forth in SEQ ID NO: 92, respectively; or (q) the amino acid sequence set forth in SEQ ID NO: 93 and the amino acid sequence set forth in SEQ ID NO: 94, respectively.

[0019] Certain aspects of the disclosure are directed to an isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NOs: 38 and the amino acid sequence set forth in SEQ ID NO 43, respectively.

[0020] Certain aspects of the disclosure are directed to an isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 49 and the amino acid sequence set forth in SEQ ID NO: 50; respectively.

[0021] Certain aspects of the disclosure are directed to an isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 51 and the amino acid sequence set forth in SEQ ID NO: 52, respectively.

[0022] Certain aspects of the disclosure are directed to an isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 67 and the amino acid sequence set forth in SEQ ID NO: 68, respectively.

[0023] Certain aspects of the disclosure are directed to an isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 69 and the amino acid sequence sest forth in SEQ ID NO: 70, respectively.

[0024] Certain aspects of the disclosure are directed to an isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 71 and the amino acid sequence set forth in SEQ ID NO: 72, respectively.

[0025] Certain aspects of the disclosure are directed to an isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which

comprise the amino acid sequence set forth in SEQ ID NO: 73 and the amino acid sequence set forth in SEQ ID NO: 74, respectively.

[0026] Certain aspects of the disclosure are directed to an isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 75 and the amino acid sequence set forth in SEQ ID NO: 76, respectively.

[0027] Certain aspects of the disclosure are directed to an isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 77 and the amino acid sequence set forth in SEQ ID NO: 78, respectively.

[0028] Certain aspects of the disclosure are directed to an isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 79 and the amino acid sequence set forth in SEQ ID NO: 80, respectively.

[0029] Certain aspects of the disclosure are directed to an isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 81 and the amino acid sequence set forth in SEQ ID NO: 82, respectively.

[0030] Certain aspects of the disclosure are directed to an isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 83 and the amino acid sequence set forth in SEQ ID NO: 84, respectively.

[0031] Certain aspects of the disclosure are directed to an isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 85 and the amino acid sequence set forth in SEQ ID NO: 86, respectively.

[0032] Certain aspects of the disclosure are directed to an isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 87 and the amino acid sequence set forth in SEQ ID NO: 88, respectively.

[0033] Certain aspects of the disclosure are directed to an isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which

comprise the amino acid sequence set forth in SEQ ID NO: 89 and the amino acid sequence set forth in SEQ ID NO: 90, respectively.

[0034] Certain aspects of the disclosure are directed to an isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 91 and the amino acid sequence set forth in SEQ ID NO: 92, respectively.

[0035] Certain aspects of the disclosure are directed to an isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 93 and the amino acid sequence set forth in SEQ ID NO: 94, respectively.

[0036] Certain aspects of the disclosure are directed to an antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a light chain variable region which comprise the amino acid sequence set forth in SEQ ID NO: 17 and the amino acid sequence set forth in SEQ ID NO: 22, respectively.

[0037] Certain aspects of the disclosure are directed to an isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5, comprising a heavy chain comprising the amino acid sequence as set forth in SEQ ID NO: 45 and a light chain comprising the amino acid sequence as set forth in SEQ ID NO: 46.

[0038] Certain aspects of the disclosure are directed to antibody, or antigen binding portion thereof which binds human CEACAM5 and has at least one amino acid mutation described in Table 17.

[0039] In some aspects, the anti-CEACAM5 antibody or antigen binding portion thereof is described in Table 16 and Table 17. In some aspects, the CDRs, VH, VL, heavy chain and/or light chain is described in Table 16 and Table 17. For example, the anti-CEACAM5 antibody or antigen binding portion thereof comprises a heavy chain and the light chain comprise the amino acid sequence set forth in SEQ ID NO: 45 and the amino acid sequence set forth in SEQ ID NO: 46, respectively.

[0040] In some aspects, the antibody, or antigen binding portion thereof specifically binds to CEACAM5 with a K_D less than $1x10^{-7}$ M, less than $1x10^{-8}$ M, less than $1x10^{-9}$ M, or a K_D described in Table 8, Table 9, Table 10, Table 11, Table 18A, or Table 18B.

[0041] In some aspects, the antibody, or antigen binding portion thereof specifically binds to CEACAM5 with a K_D with about 100 nM or less, about 90 nM or less, about 80 nM or less,

about 70 nM or less, about 60 nM or less, about 50 nM or less, about 40 nM or less, about 30 nM or less, about 20 nM or less, about 10 nM or less, about 5 nM or less, about 3 nM or less, about 1 nM or less, about 0.9 nM or less, about 0.8 nM or less, about 0.7 nM or less, about 0.6 nM or less, about 0.5 nM or less, about 0.4 nM or less, about 0.3 nM or less, about 0.2 nM or less, or about 0.1 nM or less.

[0042] In some aspects, the antibody, or antigen binding portion thereof which internalizes into CEACAM5-expressing cells, for example the antibody, or antigen binding portion thereof internalizes with an AUC described in Table 14, Table 19A, or Table 19B.

[0043] In some aspects, the antibody, or antigen binding portion thereof which binds to the A3-B3 domain of human CEACAM5 and in addition does not bind to human CEACAM1, human CEACAM6, human CEACAM7, human CEACAM8, or any combination thereof.

[0044] In some aspects, the antibody, or antigen binding portion thereof which binds to an epitope of the A3-B3 domain of human CEACAM5 identified by HDX analysis in Example 19 and FIGs. 13A, 13B and 13C, for example, the epitope includes all or a portion of amino acids L^{590}, Y^{591}, G^{592}, D^{594}, T^{595}, I^{597}, I^{598}, S^{599}, D^{602}, S^{603}, S^{604}, Y^{605}, L^{606}, K^{670}SITVSASGTSPGLSA^{685} of human CEACAM5 (SEQ ID NO: 24).

[0045] In some aspects, the antibody, or antigen binding portion thereof which binds to an epitope of the A3-B3 domain of human CEACAM5 identified by Cryo-EM analysis in Example 19 and FIGs. 14A, 14B and 14C, for example, the epitope includes all or a portion of amino acids residues 505-529, 532-574, 579-676 of human CEACAM5 (SEQ ID NO: 96) and N-acetylglucosamine residues attached to Asn 508, Asn 529, Asn 553, Asn 560, Asn 612, Asn 650, and Asn 665.

[0046] In some aspects, the antibody, or antigen binding portion thereof which comprises an IgG1 constant region, IgG2 constant region, IgG3 constant region, IgG4 constant region, or a variant thereof. In some aspects, the antibody, or antigen binding portion thereof comprises an IgG1 antibody. In some aspects, the antibody, or antigen binding portion thereof comprises an IgG1.3f constant region.

[0047] In some aspects, the antibody, or antigen binding portion thereof which is a human, humanized, or chimeric antibody.

[0048] In some aspects, the antibody, or antigen binding portion thereof comprises a Fab, Fab', (Fab')2, Fv, or scFv fragment.

[0049] Certain apects of the disclosure are directed to an isolated antibody, or antigen binding portion thereof, which binds to the same epitope on human CEACAM5 as the antibody, or antigen binding portion thereof.

[0050] Certain aspects of the disclosure are directed to an isolated antibody, or antigen binding portion thereof, which competes for binding to human CEACAM5 with the antibody, or antigen binding portion thereof.

[0051] In some aspects, the antibody, or antigen binding portion thereof competes for binding to human CEACAM 5 with a Bin 1 mAb. In some aspects, the antibody, or antigen binding portion thereof competes for binding with MBP001, MBP002, MBP003, MBP004, MBP005, MBP006, MBP007, MBP008, MBP009, MBP010, MBP011, or any combination thereof.

[0052] In some aspects, the progeny mab of the antibody, or antigen binding portion thereof is mAb MBP001.

[0053] Certain aspects of the disclosure are directed to a bispecific molecule comprising the antibody, or antigen binding portion thereof, that binds human CEACAM5, and a second binding region that binds another antigen.

[0054] Certain aspects of the disclosure are directed to a multispecific molecule comprising the antibody, or antigen binding portion thereof, and at least two binding regions, each of which binds other antigens.

[0055] Certain aspects of the disclosure are directed to a conjugate comprising the antibody, antigen binding portion thereof disclosed herein, the bispecific molecule disclosed herein, or the multispecific molecule disclosed herein. In some aspects, the conjugate is linked to a detectable moiety, a binding moiety, a labeling moiety, or a biologically active moiety. In some aspects, the biologically active moiety comprises a cytotoxic moiety.

[0056] Certain aspects of the disclosure are directed to an antibody drug conjugate (ADC) comprising the antibody or antigen-binding fragment thereof disclosed herein, the bispecific molecule disclosed herein, or the multispecific molecule disclosed herein, and a cytotoxic moiety, which is conjugated to the antibody or antigen binding portion thereof.

[0057] In some aspects, the cytotoxic moiety comprises an alkylating agent, an alkylating-like agent, an angiogenesis inhibitor, an antimetabolite agent, an antimitotic agent, an antitumor antibiotic, an apoptosis regulator, a DNA intercalating agent, a DNA replication and repair inhibitor, a histone deacetylase inhibitor, a hormonal agent, a kinase inhibitor, a mitochondria

inhibitor, a nuclear export inhibitor, a proteasome inhibitor, a protein synthesis inhibitor, a radionuclide, a topoisomerase I inhibitor, a topoisomerase II inhibitor, or any combination thereof.

[0058] In some aspects, the cytotoxic moiety comprises an anti-tubulin agent, which comprises dolastatins (*e.g.*, auristatin E (dolastatin-10); auristatin F (MMAE); and monomethyl auristatin (MMAF)); AFP; AEB; and AEVB); maytansinoids; taxanes (e.g., paclitaxel, docetaxel); T67 (Tularik); baccatin derivatives; taxane analogs (*e.g.*, epothilone A and B); nocodazole; colchicine and colcimid; estramustine; cryptophysins; cemadotin; combretastatins; discodermolide; or eleutherobin.

[0059] In some aspects, the cytotoxic moiety comprises an apoptosis regulator, which comprises AT-101 ((−)gossypol); G3139 or oblimersen (Bcl-2-targeting antisense oligonucleotide); IPI-194; IPI-565; N-(4-(4-((4′-chloro(1,1′-biphenyl)-2-yl)methyl)piperazin-1-ylbenzoyl)-4-(((1R)-3-(dimethylamino)-1-((phenylsulfanyl)methyl)propyl)amino)-3-nitrobenzenesulfonamide); N-(4-(4-((2-(4-chlorophenyl)-5,5-dimethyl-1-cyclohex-1-en-1-yl)methyl)piperazin-1-yl)benzoyl)-4-(((1R)-3-(morpholin-4-yl)-1-((phenylsulfanyl)methyl)propyl)amino)-3-((trifluoromethyl)sulfonyl)benzenesulfonamide; GX-070 (Obatoclax®; 1H-Indole, 2-(2-((3,5-dimethyl-1H-pyrrol-2-yl)methylene)-3-methoxy-2H-pyrrol-5-yl)−)); HGS1029; GDC-0145; GDC-0152; LCL-161; LBW-242; venetoclax; agents that target TRAIL or death receptors (*e.g.*, DR4 and DR5) such as ETR2-ST01, GDC0145, HGS-1029, LBY-135, PRO-1762; drugs that target caspases, caspase-regulators, BCL-2 family members, death domain proteins, TNF family members, Toll family members, NF-kappa-B proteins, or any combination thereof.

[0060] In some aspects, the cytotoxic moiety comprises a DNA intercalating agent, which comprises anthramycin; chicamycin A; enediynes; lexitropsins; tomaymycin; DC-81; sibiromycin; pyrrolobenzodiazepine derivative; SGD-1882 ((S)-2-(4-aminophenyl)-7-methoxy-8-(3 S)-7-methoxy-2-(4-methoxyphenyl)-5-oxo-5,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5(11aH)-one); or SG2000 (SJG-136; (11aS,11a′S)-8,8′-(propane-1,3-diylbis(oxy))bis(7-methoxy-2-methylene-2,3-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5(11 aH)-one)).

[0061] In some aspects, the cytotoxic moiety comprises a DNA replication and repair inhibitor, which comprises Altretamine; Bleomycin; Dacarbazine; Dactinomycin; Mitobronitol; Mitomycin; Pingyangmycin; Plicamycin; Procarbazine; Temozolomide; ABT-888 (veliparib);

olaparib; KU-59436; AZD-2281; AG-014699; BSI-201; BGP-15; INO-1001; ONO-2231; or any combination thereof.

[0062] In some aspects, the cytotoxic moiety comprises a histone deacetylase inhibitor, which comprises vorinostat; romidepsin; chidamide; panobinostat; valproic acid; belinostat; mocetinostat; abexinostat; entinostat; SB939 (pracinostat); resminostat; givinostat; quisinostat; thioureidobutyronitrile (KEVETRIN™); CUDC-10; CHR-2845 (tefinostat); CHR-3996; 4SC-202; CG200745; ACY-1215 (rocilinostat); ME-344; sulforaphane, or any combination thereof.

[0063] In some aspects, the cytotoxic moiety comprises a hormonal agent, which comprises anastrozole; exemestane; arzoxifene; bicalutamide; cetrorelix; degarelix; deslorelin; trilostane; dexamethasone; flutamide; raloxifene; fadrozole; toremifene; fulvestrant; letrozole; formestane; glucocorticoids; doxercalciferol; sevelamer carbonate; lasofoxifene; leuprolide acetate; megesterol; mifepristone; nilutamide; tamoxifen citrate; abarelix; prednisone; finasteride; rilostane; buserelin; luteinizing hormone releasing hormone (LHRH); histrelin; trilostane or modrastane; fosrelin; goserelin, or any combination thereof.

[0064] In some aspects, the cytotoxic moiety comprises a kinase inhibitor, which comprises afantinib; axitinib; binimetinib; bosutinib; crizotinib; dasatinib; erlotinib; fostamatinib; gefitinib; ibrutinib; imatinib; lapatinib; lestaurtinib; lenvatinib; mubritinib; nilotinib; pazopanib; pegaptanib; ruxolitinib; selemetinib; semaxanib; sildenib; sorafenib; SU6656; sunitinib; vandetanib; vemurafenib; PI-103; PP242; PP30; Torin 1; XL019; INCB018424; ARRY-142886; ARRY-438162; PD-325901; PD-98059; AP-2357; CCI-779; everolimus; RAD-001; rapamycin; temsirolimus; LY294002; XL-147; CAL-120; ONC-21; AEZS-127; ETP-45658; PX-866; GDC-0941; BGT226; BEZ235; XL765; or any combination thereof.

[0065] In some aspects, the cytotoxic moiety comprises a mitochondria inhibitor, which comprises pancratistatin; phenpanstatin; rhodamine-123; edelfosine; d-alpha-tocopherol succinate; compound 11β; aspirin; ellipticine; berberine; cerulenin; GX015-070 (Obatoclax®; 1H-Indole, 2-(2-((3,5-dimethyl-1H-pyrrol-2-yl)methylene)-3-methoxy-2H-pyrrol-5-yl)−); celastrol (tripterine); metformin; Brilliant green; ME-344; or any combination thereof.

[0066] In some aspects, the cytotoxic moiety comprises a nuclear export inhibitor, which comprises callystatin A; delactonmycin; KPT-185 (propan-2-yl (Z)-3-[3-[3-methoxy-5-(trifluoromethyl)phenyl]-1,2,4-triazol-1-yl]prop-2-enoate); kazusamycin A; leptolstatin; leptofuranin A; leptomycin B; ratjadone; Verdinexor ((Z)-3-[3-[3,5-bis(trifluoromethyl)phenyl]-1,2,4-triazol-1-yl]-N-pyridin-2-ylprop-2-enehydrazide), or any combination thereof.

[0067] In some aspects, the cytotoxic moiety comprises a proteasome inhibitor, which comprises Bortezomib; Carfilzomib; Epoxomicin; Ixazomib; Salinosporamide A, or any combination thereof.

[0068] In some aspects, the cytotoxic moiety comprises a radionuclide agent, which comprises s I^{131}, In^{11}, Y^{90}, Lu^{177}, or any combination thereof.

[0069] In some aspects, the cytotoxic moiety comprises a protein synthesis inhibitor, which comprises Streptomycin; Dihydrostreptomycin; Neomycin; Framycetin; Paromomycin; Ribostamycin; Kanamycin; Amikacin; Arbekacin; Bekanamycin; Dibekacin; Tobramycin; Spectinomycin; Hygromycin B; Paromomycin; Gentamicin; Netilmicin; Sisomicin; Isepamicin; Verdamicin; Astromicin; Tetracycline; Doxycycline; Chlortetracycline; Clomocycline; Demeclocycline; Lymecycline; Meclocycline; Metacycline; Minocycline; Oxytetracycline; Penimepicycline; Rolitetracycline; Tetracycline; Glycylcyclines; Tigecycline; Oxazolidinone; Eperezolid; Linezolid; Posizolid; Radezolid; Ranbezolid; Sutezolid; Tedizolid; Peptidyl transferase inhibitors; Chloramphenicol; Azidamfenicol; Thiamphenicol; Florfenicol; Pleuromutilins; Retapamulin; Tiamulin; Valnemulin; Azithromycin; Clarithromycin; Dirithromycin; Erythromycin; Flurithromycin; Josamycin; Midecamycin; Miocamycin; Oleandomycin; Rokitamycin; Roxithromycin; Spiramycin; Troleandomycin; Tylosin; Ketolides; Telithromycin; Cethromycin; Solithromycin; Clindamycin; Lincomycin; Pirlimycin; Streptogramins; Pristinamycin; Quinupristin/dalfopristin; Virginiamycin, or any combination thereof.

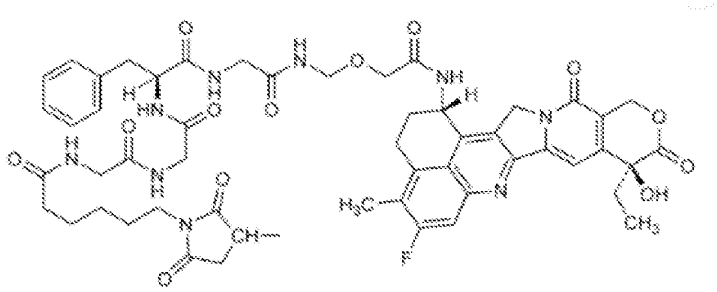
[0070] In some aspects, the cytotoxic moiety comprises a topoisomerase I inhibitor, which comprises camptothecin, derivatives thereof, or analogs thereof. In some aspects, the topoisomerase I inhibitor comprises topotecan, irinotecan, silatecan, cositecan, exatecan, lurtotecan, gimatecan, belotecan, rubitecan, NSC 100880, NSC 603071, NSC 107124, NSC 643833, NSC 629971, NSC 295500, NSC 249910, NSC 606985, NSC 74028, NSC 176323, NSC 295501, NSC 606172, NSC 606173, NSC 610458, NSC 618939, NSC 610457, NSC 610459, NSC 606499, NSC 610456, NSC 364830, NSC 606497, morpholinisoxorubicin, or SN-38, or derivative or analog thereof.

[0071] In some aspects, the cytotoxic moiety comprises exatecan or derivative or analog thereof.

[0072] In some aspects, the cytotoxic moiety is conjugated to the antibody or antigen binding portion thereof by a linker. In some aspects, the linker comprises a cleavable linker. In

some aspects, the cleavable linker comprises a peptidyl linker, a disulfide linker, an acid labile linker, a photolabile linker, a peptidase labile linker, or any combination thereof. In some aspects, the peptidyl linker comprises Val-Cit, Ala-Val, Val-Ala-Val, Lys-Lys, Pro-Val-Gly-Val-Val (SEQ ID NO: 99), Ala-Asn-Val, Val-Leu-Lys, Ala-Ala-Asn, Cit-Cit, Val-Lys, Lys, Cit, Ser, Glu, or any combination thereof. In some aspects, the linker comprises a non-cleavable linker.

[0073] Some aspects of the disclosure are directed to an antibody drug conjugate (ADC) comprising the antibody or antigen-binding fragment thereof as disclosed herein, which is linked to the following linker-cytotoxic moiety:



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an isomer thereof, a solvate thereof, or any pharmaceutical salt thereof.

[0074] Some aspects of the disclosure are directed to a nucleic acid, or set of nucleic acids, comprising a nucleotide sequence that encodes the antibody, or antigen binding portion thereof, disclosed herein, bispecific molecule disclosed herein, or multispecific molecule disclosed herein.

[0075] In some aspects, the nucleic acid, or set of nucleic acids, comprise a pair nucleotide sequences that comprise: (1) SEQ ID NO: 18 and SEQ ID NO: 23; (2) SEQ ID NO: 39 and SEQ ID NO: 44; (3) SEQ ID NO: 53 and SEQ ID NO: 54; or (4) SEQ ID NO: 55 and SEQ ID NO: 56.

[0076] Some aspects of the disclosure are directed to an expression vector comprising the nucleic acid, or set of nucleic acids, disclosed herein.

[0077] Some aspects of the disclosure are directed to a host cell cell comprising the nucleic acid, or set of nucleic acids, or the expression vector disclosed herein.

[0078] Some aspects of the disclosure are directed to an engineered cell comprising the nucleic acid, or set of nucleic acids, or the expression vector disclosed herein. In some aspects, the engineered cell is engineered ex vivo to expresss the antibody, or antigen binding portion thereof, disclosed herein. In some aspects, the engineered cell expresses a chimeric antigen receptor comprising the antibody, or antigen binding portion thereof disclosed herein.

[0079] Some aspects of the disclosure are directed to a pharmaceutical composition comprising the antibody, or antigen binding portion thereof, bispecific molecule, multispecific molecule, conjugate, ADC, nucleic acid or set of nucleic acids, expression vector, or engineered cell disclosed herein, and a pharmaceutically acceptable carrier.

[0080] In some aspects, the pharmaceutical composition further comprieses one or more additional therapeutic agents.

[0081] Some aspects of the disclosure are directed to a kit comprising the antibody, or antigen binding portion thereof, bispecific molecule, multispecific molecule, conjugate, ADC, nucleic acid or set of nucleic acids, expression vector, or engineered cell disclosed herein, and instructions for use.

[0082] Some aspects of the disclosure are directed to means for treating cancer in a patient in need thereof comprising a combination of an anti-CEACAM5 antibody, or antigen binding portion thereof and a cytotoxic moiety.

[0083] Some aspects of the disclosure are directed to means for treating cancer in a subject in need thereof comprising a combination of (i) an anti-CEACAM antibody, or antigen binding portion thereof, bispecific molecule, multispecific molecule, conjugate, ADC, nucleic acid or a set of nucleic acids, expression vector, or engineered cell, and (ii) a pharmaceutically acceptable carrier.

[0084] Some aspects of the disclosure are directed to a method of producing an anti-CEACAM5 antibody, or antigen binding portion thereof, a bispecific molecule, or a multispecific molecule comprising transfecting a cell with the nucleic acid or the expression vector as disclosed herein.

[0085] Some aspects of the disclosure are directed to a method of treating cancer that expresses CEACAM5 in a subject in need thereof comprising administering to the subject a therapeutically effective amount of the antibody, or antigen binding portion thereof, bispecific molecule, multispecific molecule, conjugate, ADC, nucleic acid or a set of nucleic acids, expression vector, or engineered cell disclosed herein.

[0086] In some aspects, the method further comprising administering one or more additional therapies.

[0087] In some aspects, the one or more additional therapies comprises radiation therapy, chemotherapy, immune checkpoint inhibitor therapy, CAR-T therapy, immunosuppressive therapy, immunostimulatory therapy, cell therapy, or any combination thereof.

[0088] In some aspects, the one or more additional therapies comprise an immune checkpoint inhibitor.

[0089] In some aspects, the immune checkpoint inhibitor comprises an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-LAG-3 antibody, an anti-CTLA-4 antibody, an anti-TIGIT antibody, an anti-TIM3 antibody, or any combination thereof.

[0090] Some aspects of the disclosure are directed to a method of detecting the absence or presence of human CEACAM5 in a sample comprising contacting the sample with the antibody, or antigen binding portion thereof, bispecific molecule, multispecific molecule, conjugate, ADC, nucleic acid or set of nucleic acids, expression vector, or engineered cell disclosed herein, under conditions that allow for formation of a complex between the antibody and human CEACAM5; and detecting the formation of the complex.

[0091] Some aspects of the disclosure are directed to a method of diagnosing a cancer that expresses human CEACAM5 comprising contacting a biological sample from a patient afflicted with or suspected to have the cancer with the antibody, or antigen binding portion thereof, bispecific molecule, multispecific molecule, conjugate, ADC, nucleic acid or set of nucleic acids, expression vector, or engineered cell disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0092] **FIG. 1A** and **FIG. 1B** are tables showing competition and binning of selected anti-CEACAM5 binding antibodies.

[0093] **FIGs. 2A-2D** are a set of graphs showing cell-based binding of anti-CEACAM5 antibodies. The anti-CEACAM5 antibodies were assayed to test their binding to the cell lines expressing different levels of human CEACAM5: BXPC-3 (**FIG. 2A**), Ls174T (**FIG. 2B**), MKN-45 (**FIG. 2C**), and HCT-116 (**FIG. 2D**), respectively.

[0094] **FIGs. 3A-3C** are graphs depicting the percentage of cell death (% inhibition) induced by anti-CEACAM5 antibodies delivering the cytotoxic agent MMAE which has been conjugated to secondary VHH (**FIG. 3A**), internalization (Red Area/ Phase Area) rates (**FIG. 3B**), and cytotoxicity levels of selected anti-CEACAM5 antibodies (Cytotoxicity AUC) as a function of internalization (Internalization AUC) (**FIG. 3C)** in MKN45 cells.

[0095] **FIGs. 4A-4B** are graphs that show percent growth inhibition of anti-CEACAM5 antibodies delivering cytotoxic agent MMAE conjugated to a VHH secondary antibody (**FIG. 4A**)

internalization (Red Area/ Phase Area) rates (**FIG. 4B**) of selected anti-CEACAM5 antibodies in Ls174T cells.

[0096] **FIGS. 5A-5H** are a set of graphs and tables that show the cytotoxic effects of the indicated anti-CEACAM5 antibodies conjugated to the topoisomerase 1 inhibitor (i.e., exatecan) across a panel of CEACAM5-expressing cell lines. The graphs show the percent of growth inhibition for selected antibody-drug-conjugate-treated cells compared to isotype controls at the indicated antibody concentrations in: a negative (HCT-116; **FIG. 5A**), a medium-expressing CEACAM5 cell line (BxPC-3; **FIG. 5B**), and high-expressing CEACAM5 cell lines (HCT-CEA, **FIG. 5E**; and MKN45, **FIG. 5F**). **FIG. 5C, FIG. 5D**, **FIG. 5G,** and **FIG. 5H** are tables that show IC50 and Amax values for the indicated antibody-drug-conjugates as determined. All antibody-conjugates used a DXd (deruxtecan) topoisomerase 1 inhibitor having a DAR of 8 (interchain disulfides).

[0097] **FIG. 6A** is a general description of the mutation analysis that was performed to generate single mutant libraries and for selection of progeny antibodies that bind human CEACAM5 and cynomolgus. **FIG. 6B** shows the mutational scan MBN001 CDR positions (by Kabat) analyzed. **FIG. 6C, FIG. 6D**, **FIG. 6E**, **FIG. 6F**, **FIG. 6G**, **FIG. 6H**, **FIG. 6I**, **FIG. 6J**, **FIG. 6K**, **FIG. 6L**, **FIG. 6M**, and **FIG. 6N** are the heat maps for human CEACAM5 and cynomolgus CEACAM5 that were generated using the mutational scan analysis of MBN001 antibody for LCDR1 substitutions (SEQ ID NO: 64, germline and parental) (**FIG. 6I** and **FIG. 6J**); LCDR2 substitutions (SEQ ID NO: 65, germline and parental) (**FIG. 6K** and **FIG. 6L**) LCDR3 substitutions (SEQ ID NO: 66, germline and parental) (**FIG. 6M** and **FIG. 6N**); HCDR1 substitutions (SEQ ID NO: 61, germline and parental) (**FIG. 6C** and **FIG. 6D**); HCDR2 substitutions (SEQ ID NO: 62, germline and parental) (**FIG. 6E** and **FIG. 6F**); and HCDR3 substitutions (SEQ ID NO: 63, germline and parental, (**FIG. 6G** and **FIG. 6H**). Note that in the graphs described herein that ND indicates that enrichment ratios could not be determined because NGS counts in the starting library were too low.

[0098] **FIG.7A** and **FIG. 7B** are isoaffinity plots showing improvements to the on-rate and off-rate to human CEACAM5 (**FIG. 7A**) and cyno CEACAM5 (**FIG. 7B**) for parental antibody MBN001 compared to MBN001 optimized progeny.

[0099] **FIGs. 8A-8C** are a set of graphs that shows percent inhibition of anti-CEACAM5 antibodies delivering cytotoxic agent MMAE conjugated to a VHH secondary antibody (**FIG. 8A**), internalization rates (**FIG. 8B**), and cytotoxicity levels of MBN001 antibody and selected progeny

(MBP004, MBP005, MBP007, MBP008, MBP009, MBP010, MBP006, and MBP011) as a function of internalization (**FIG. 8C)** in MKN45 cells.

[0100] **FIG. 9A** and **FIG. 9B** are a set of graphs that show the absence of non-specific binding to engineered cells lines that express human CEACAM1 (CHO-S; **FIG. 9A**) and human CEACAM6 (HCT-116; **FIG. 9B**) by MBN001 and progeny mAbs MBP003, MBP001 and MBP002.

[0101] **FIG. 10A**, **FIG. 10B**, and **FIG. 10C** are a set of graphs showing binding to CEACAM5-low expressing LS174T cells (**FIG. 10A**), CEACAM-medium expressing BxPC-3 cells (**FIG. 10B**), and CEACAM5-high expressing MKN45 cells (**FIG. 10C**) of anti-CEACAM5 mAbs MBN001, MBP001, MBP003, and MBP002 by FACS. EC50 values are shown in **FIG. 10D**.

[0102] **FIG. 11A** and **FIG. 11B** are graphs showing the ADCC activity of anti-human CEACAM5 mAbs in Jurkat-NFAT-FcγRIIIa (Promega) cell assays across CEACAM5-medium expressing BxPC3 (**FIG. 11A**) and CEACAM5-high expressing MKN45 (**FIG. 11B**) cells.

[0103] **FIG. 12** is a graph showing in vivo efficacy of bin 1 lead MBN001 (hIgG1.3)-DXd and bin 2 lead MBN002 (hIgG1.3)-DXd in the MKN45 CDX model after a single intravenous injection with 3 mg/kg dose of the ADCs.

[0104] **FIG. 13A** is a ribbon representation of the 3D model of human CEACAM5 along with the separate structural domains. A box is added to this figure to highlight the A3 and B3 domains of human CEACAM5. **FIG. 13B** shows an amino acid sequence for hCEACAM5 (UniProt entry: P06731) with shading identifying the separate structural domains shown in **FIG. 13A** (SEQ ID NO: 25). **FIG. 13C** is a drawing showing peptic sequence coverage for hCEACAM5-A3-B3 construct (SEQ ID NO: 24)**. FIG. 13D** is a graph showing differential deuterium uptake between hCEACAM5 bound to MBN001 vs. unbound hCEACAM5. Regions in hCEACAM5 with significant HDX reduction upon MBN001 binding are boxed. **FIG. 13E** is a drawing showing annotation on linear sequence of hCEACAM5, of HDX effects of MBN001 binding. Bold residues exhibit slower exchange, no information is available for residues in italics, no difference detected on straight type residues.

[0105] **FIG. 14A** is a map of the final cryoEM and **FIG. 14B** and **FIG. 14C** are ribbon diagrams of the final structure-based model derived from cryoEM showing the epitope and paratope interaction between human CEACAM5 and Fab construct of MBP001) and a Fab of a bin 2 mAb.

DETAILED DESCRIPTION

[0106] The present disclosure comprises a novel anti-CEACAM5 antibody, or antigen binding portion thereof. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, that is conjugated to a cytotoxic moiety.

Definitions

[0107] In order for the following detailed description to be readily understood, certain terms are first defined. Additional definitions are provided throughout.

[0108] The term "antibody" as used to herein includes whole antibodies and any antigen binding portions (*i.e.*, "antigen-binding portions") or single chains thereof. An "antibody" refers, in one aspect, to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen binding portion thereof. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. In certain naturally occurring antibodies, the heavy chain constant region is comprised of three domains, CH1, CH2, and CH3. In certain naturally occurring antibodies, each light chain is comprised of a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (Clq) of the classical complement system.

[0109] Antibodies typically bind specifically to their cognate antigen with high affinity, reflected by a dissociation constant (K_D) of 10^{-5} to 10^{-11} M or less. Any K_D greater than about 10^{-4} M is generally considered to indicate nonspecific binding. As used herein, an antibody that "binds specifically" to an antigen refers to an antibody that binds to the antigen and substantially identical antigens with high affinity, which means having a K_D of 10^{-7} M or less, 10^{-8} M or less, 1×10^{-9} M or less, 1×10^{10} M or less, or 1×10^{-11} M or less. In some aspects, the antibody specifically binds

to an antigen with a K_D between 10^{-8} M and 10^{-10} M or between 10^{-9} M and 10^{-11} M, but does not bind with high affinity to unrelated antigens.

[0110] An "antibody" according to the present disclosure includes, but is not limited to, naturally and non-naturally occurring antibodies, monoclonal antibodies, polyclonal antibodies, chimeric antibodies, humanized antibodies, human antibodies, nonhuman antibodies, bivalent antibodies, bispecific antibodies, multispecific antibodies, single chain antibodies, diabodies, and nanobodies.

[0111] An "isolated antibody," as used herein, refers to an antibody which is substantially free of other antibodies having different antigenic specificities.

[0112] The phrase "antigen binding portion" of an antibody, as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.*, human and/or cynomolgus CEACAM5). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) an Fd fragment consisting of the V_H and CH1 domains; (iv) an Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) *Nature* 341:544-546), which consists of a V_H domain; and (vi) an isolated complementarity determining region (CDR) or (vii) a combination of two or more isolated CDRs which may optionally be joined by a synthetic linker. Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*, Bird et al. (1988) *Science* 242:423-426; and Huston et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Antigen-binding portions can be produced by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact immunoglobulins.

[0113] Antibody fragments within the scope of the present invention also include F(ab')2 fragments which may be produced by enzymatic cleavage of an IgG by, for example, pepsin. Fab fragments may be produced by, for example, reduction of F(ab')2 with dithiothreitol or mercaptoethylamine. A Fab fragment is a VL-CL chain appended to a VH-CH1 chain by a

disulfide bridge. A F(ab')2 fragment is two Fab fragments which, in turn, are appended by two disulfide bridges. The Fab portion of an F(ab')2 molecule includes a portion of the Fc region between which disulfide bridges are located.

[0114] As used herein, "isotype" refers to the antibody class (*e.g.*, IgG (including IgG1, IgG2, IgG3, and IgG4), IgM, IgA (including IgA1 and IgA2), IgD, and IgE antibody) that is encoded by the heavy chain constant region genes of the antibody.

[0115] An antibody may be from any of the commonly known isotypes, including but not limited to IgA, secretory IgA, IgG and IgM. The IgG isotype is divided in subclasses in certain species: IgG1, IgG2, IgG3 and IgG4 in humans, and IgG1, IgG2a, IgG2b and IgG3 in mice. Immunoglobulins, e.g., IgG1, exist in several allotypes, which differ from each other in at most a few amino acids.

[0116] As used herein, the term "allotype" refers to naturally occurring variants within a specific isotype group, where the variants differ in a few amino acids. Anti-CEACAM5 antibodies described herein can be of any allotype. Antibodies referred to herein as "IgG1.3f" are IgG1 antibodies of the allotype "f," i.e., having 214R, 356E and 358M according to the EU index. A triple mutant (L234A, L235E, G237A) IgG1.3f variant comprises an amino acid sequence set forth in SEQ ID NO: 30. The mutation of these residues would eliminate or decrease the binding of the antibodies to Fcγ receptors and/or C1q, and thus reduce activator efficacy of the Fc domain of the IgG1 component of an antibody.

[0117] As used herein, the term "hypervariable region" (sometimes referred to as the "variable region") refers to the amino acid residues of an antibody that are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (*e.g.* residues 24-34 (CDRL1), 50-56 (CDRL2) and 89-97 (CDRL3) in the light chain variable domain and residues 31-35 (CDRH1), 50-65 (CDRH2) and 95-102 (CDRH3) in the heavy chain variable domain; Kabat et al., (1991) Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md.) and/or those residues from a "hypervariable loop" (*i.e.* residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk, (1987) *J. Mol. Biol.* 196: 901-917).

[0118] As used herein, the term "framework" or "FR" residues refers to those variable domain residues other than the hypervariable region residues defined herein as CDR residues. The residue numbering above relates to the Kabat numbering system and does not necessarily

correspond in detail to the sequence numbering in the accompanying Sequence Listing. Amino acid residues in antibodies can also be defined using other numbering systems, such as Chothia, enhanced Chothia, IMGT, Kabat/Chothia composite, Honegger (AHo), Contact, or any other conventional antibody numbering scheme.

[0119] The term "acceptor human framework" refers to a framework comprising the amino acid sequence of a V_L framework, or a V_H framework derived from a human immunoglobulin framework or a human consensus framework. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may have the same amino acid sequence as the naturally occurring human immunoglobulin framework or human consensus framework, or it may have amino acid sequence changes compared to wild-type naturally occurring human immunoglobulin framework or human consensus framework. In some aspects, the number of amino acid changes are 10, 9, 8, 7, 6, 5, 4, 3, or 2, or 1. In some aspects, the V_L acceptor human framework is identical in sequence to the V_L human immunoglobulin framework sequence or human consensus framework sequence.

[0120] An "Fc region," "Fc domain," or "Fc" refers to the C-terminal region of the heavy chain of an antibody. Thus, an Fc region comprises the constant region of an antibody excluding the first constant region immunoglobulin domain (*e.g.,* CH1 or CL).

[0121] An "effector function" refers to the interaction of an antibody Fc region with an Fc receptor or ligand, or a biochemical event that results therefrom. Exemplary "effector functions" include Clq binding, complement dependent cytotoxicity (CDC), Fc receptor binding, FcγR-mediated effector functions such as ADCC and antibody dependent cell-mediated phagocytosis (ADCP), and downregulation of a cell surface receptor (*e.g.*, the B cell receptor; BCR). Such effector functions generally require the Fc region to be combined with a binding domain (*e.g.*, an antibody variable domain).

[0122] The term "epitope" or "antigenic determinant" refers to a site on an antigen (*e.g.*, human CEACAM5) to which an immunoglobulin or antibody specifically binds. Epitopes can be formed both from contiguous amino acids (usually a linear epitope) or noncontiguous amino acids juxtaposed by tertiary folding of the protein (usually a conformational epitope). Epitopes formed from contiguous amino acids are typically, but not always, retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22 amino acids in a unique spatial conformation.

[0123] The term "monoclonal antibody," as used herein, refers to an antibody that displays a single binding specificity and affinity for a particular epitope or a composition of antibodies in which all antibodies display a single binding specificity and affinity for a particular epitope. Accordingly, the term "human monoclonal antibody" refers to an antibody or antibody composition that display(s) a single binding specificity and which has variable and optional constant regions derived from human germline immunoglobulin sequences. In one aspect, human monoclonal antibodies are produced by a hybridoma which includes a B cell obtained from a transgenic non-human animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell. Monoclonal antibodies include chimeric antibodies, human antibodies, and humanized antibodies and may occur naturally or be produced recombinantly.

[0124] The monoclonal antibodies herein also include camelized single domain antibodies. *See, e.g.,* Muyldermans *et al.* (2001) *Trends Biochem. Sci.* 26:230; Reichmann *et al.* (1999) *J. Immunol. Methods* 231:25; WO 94/04678; WO 94/25591; U.S. Pat. No. 6,005,079, which are hereby incorporated by reference in their entireties). In one aspect, provided herein are single domain antibodies comprising two V_H domains with modifications such that single domain antibodies are formed.

[0125] The term "recombinant antibody," refers to antibodies that are prepared, expressed, created or isolated by recombinant means, such as (a) antibodies isolated from an animal (*e.g.,* a mouse) that is transgenic or transchromosomal for immunoglobulin genes (*e.g.,* human immunoglobulin genes) or a hybridoma prepared therefrom, (b) antibodies isolated from a host cell transformed to express the antibody, *e.g.,* from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial antibody library (*e.g.,* containing human antibody sequences) using phage display, and (d) antibodies prepared, expressed, created or isolated by any other means that involve splicing of immunoglobulin gene sequences (*e.g.,* human immunoglobulin genes) to other DNA sequences. Such recombinant antibodies may have variable and constant regions derived from human germline immunoglobulin sequences. In certain aspects, however, such recombinant human antibodies can be subjected to in vitro mutagenesis and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

[0126] A "human" antibody refers to an antibody having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region also is derived from human germline immunoglobulin sequences. Also encompassed are antibodies derived from human germline immunoglobulin sequences that include normal somatic hypermutations which alter the germline immunoglobulin sequences relative to the wild-type germline immunoglobulin sequences.

[0127] A "humanized" antibody refers to an antibody in which some, most or all of the amino acids outside the CDR domains of a non-human antibody are replaced with corresponding amino acids derived from human immunoglobulins. In one aspect of a humanized form of an antibody, some, most or all of the amino acids outside the CDR domains have been replaced with amino acids from human immunoglobulins, whereas some, most or all amino acids within one or more CDR regions are unchanged. Any additions, deletions, insertions, substitutions or modifications of amino acids are permissible as long as they do not abrogate the ability of the antibody to bind to a particular antigen. A "humanized" antibody may retain an antigenic specificity similar to that of the original antibody.

[0128] The term “fully human antibody” refers to an antibody that comprises human immunoglobulin protein sequences only. A fully human antibody may contain murine carbohydrate chains if produced in a mouse, in a mouse cell, or in a hybridoma derived from a mouse cell. Similarly, “mouse antibody” refers to an antibody which comprises mouse immunoglobulin sequences only.

[0129] A "chimeric antibody" refers to an antibody in which the variable regions are derived from one or more species and the constant regions are derived from another species, such as an antibody in which the variable regions are derived from a mouse antibody and the constant regions are derived from a human antibody. See U.S. Pat. No. 4,816,567; and Morrison et al., (1984) *Proc. Natl. Acad. Sci. USA* 81: 6851-6855.

[0130] A "domain antibody" or “nanobody” is an immunologically functional immunoglobulin fragment containing only the variable region of a heavy chain or the variable region of a light chain. In some instances, two or more V_H regions are covalently joined with a peptide linker to create a bivalent domain antibody. The two V_H regions of a bivalent domain antibody may target the same or different antigens.

[0131] A "bivalent antibody" comprises two antigen binding sites. In some instances, the two binding sites have the same antigen specificities. However, bivalent antibodies may be bispecific.

[0132] A "bispecific" or "bifunctional antibody" is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, *e.g.*, Songsivilai & Lachmann, *Clin. Exp. Immunol.* 79:315-321 (1990); Kostelny et al., *J. Immunol.* 148, 1547-1553 (1992). Bifunctional antibodies include, for example, heterodimeric antibody conjugates (*e.g.,* two antibodies or antibody fragments joined together with each having different specificities), antibody/cell surface-binding molecule conjugates (*e.g.,* an antibody conjugated to a non-antibody molecule such as a receptor), and hybrid antibodies (*e.g.,* an antibody having binding sites for two different antigens).

[0133] A "multispecific antibody" is an antibody (*e.g.,* bispecific antibodies, tri-specific antibodies) that recognizes two or more different antigens or epitopes.

[0134] As used herein, the term "single-chain Fv" or "scFv" antibody refers to antibody fragments comprising the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker. For a review of scFvs, see Pluckthun (1994) THE PHARMACOLOGY OF MONOCLONAL ANTIBODIES, vol. 113, Rosenburg and Moore eds. Springer-Verlag, New York, pp. 269-315.

[0135] As used herein, the term "diabodies" refer to small antibody fragments with two antigen-binding sites in which the fragments comprise a heavy chain variable domain (V_H) connected to a light chain variable domain (V_L) in the same polypeptide chain (V_H-V_L or V_L-V_H). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, *e.g.*, EP 404,097; WO 93/11161; and Holliger et al. (1993) *Proc. Natl. Acad. Sci. USA* 90: 6444-6448. For a review of engineered antibody variants generally see Holliger and Hudson (2005) *Nat. Biotechnol.* 23:1126-1136.

[0136] The term "immune cell engager" or "ICE" is used herein with reference to a multifunctional molecule comprising two or more binding specificities able to redirect immune effector cells against cancer cells. Exemplary immune cell engagers include T-cell engagers (*e.g.,* bispecific T-cell engagers or BiTEs), NK-cell engagers (NKCEs), B-cell engagers, dendritic cell engagers, and macrophage cell engagers.

[0137] The terms "bispecific T cell engager" and "BiTE" are used herein interchangeably with reference to a bispecific molecule linking the targeting regions of two antibodies and/or protein binding domains, wherein one arm of the molecule is engineered to bind a protein (*e.g.*, CD3) on the surface of a cytotoxic T cell (i.e., T cell engager), and the other arm is engineered to bind to a specific protein found primarily on tumor cells, such as CEACAM5. When both targets are engaged, the BiTE molecule forms a bridge between the cytotoxic T cell and the tumor cell, enabling the T cell to recognize and kill the tumor cell. The BiTE may or may not include immunoglobulin constant regions.

[0138] The terms "bispecific NK cell engager" and "NKCE" are used herein interchangeably with reference to a bispecific molecule comprising a CEACAM5 binding domain linked by a short flexible linker region to the binding domain of cell surface protein of an NK cell (i.e., NK cell engager).

[0139] The term "binds to the same epitope" is used with reference to two or more antibodies that bind to the same segment or same segments of amino acid residues. Techniques for determining whether antibodies bind to the same epitope may be determined by epitope mapping methods described herein. Other methods involve monitoring the binding of the antibody to antigen fragments (*e.g.*, proteolytic fragments) or to mutated variations of the antigen where loss of binding due to a modification of an amino acid residue within the antigen sequence is often considered an indication of an epitope component, such as alanine scanning mutagenesis (Cunningham & Wells (1985) *Science* 244:1081), yeast display of mutant target sequence variants, or analysis of chimeras. In addition, computational combinatorial methods for epitope mapping can also be employed. These methods rely on the ability of the antibody of interest to affinity isolate specific short peptides from combinatorial phage display peptide libraries. Antibodies having the same V_H and V_L or the same CDR1, 2 and 3 sequences are expected to bind to the same epitope.

[0140] Antibodies that "compete with another antibody for binding to a target" refer to antibodies that inhibit (partially or completely) the binding of another antibody to the target. Whether two antibodies compete with each other for binding to a target, *i.e.*, whether and to what extent one antibody inhibits the binding of the other antibody to a target, may be determined using known binding competition experiments involving surface plasmon resonance (SPR) and bio-layer interferometry (BLI). In certain aspects, an antibody competes with, and inhibits binding of another antibody to a target by at least 50%, 60%, 70%, 80%, 90% or 100%. The level of inhibition

or competition may be different depending on which antibody is the "blocking antibody" (*i.e.*, the antibody that when combined with an antigen blocks another immunologic reaction with the antigen). Competition assays can be conducted as described, for example, in Ed Harlow and David Lane, Cold Spring Harb. Protoc. 2006; doi:10.1101/pdb.prot4277 or in Chapter 11 of "Using Antibodies" by Ed Harlow and David Lane, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA 1999. Competing antibodies bind to the same epitope, an overlapping epitope, or to adjacent epitopes (*e.g.,* as evidenced by steric hindrance). Two antibodies "cross-compete" if antibodies block each other both ways by at least 50%, *i.e.*, regardless of whether one or the other antibody is contacted first with the antigen in the competition experiment.

[0141] Competitive binding assays for determining whether two antibodies compete or cross-compete for binding include competition for binding to cells expressing CEACAM5, *e.g.,* by flow cytometry. Other methods include surface plasmon resonance (SPR) (*e.g.,* BIACORE®), solid phase direct or indirect radioimmunoassay (RIA), solid phase direct or indirect enzyme immunoassay (EIA), sandwich competition assay (*see* Stahli et al., *Methods in Enzymology* 9:242 (1983)); solid phase direct biotin-avidin EIA (*see* Kirkland et al., *J. Immunol.* 137:3614 (1986)); solid phase direct labeled assay, solid phase direct labeled sandwich assay (*see* Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Press (1988)); solid phase direct label RIA using 1-125 label (*see* Morel et al., *Mol. Immunol.* 25(1):7 (1988)); solid phase direct biotin-avidin EIA (Cheung et al., *Virology* 176:546 (1990)); and direct labeled RIA. (Moldenhauer et al., *Scand. J. Immunol.* 32:77 (1990)).

[0142] As used herein, the terms "specific binding," "selective binding," "selectively binds," and "specifically binds," refer to antibody binding to an epitope on a predetermined antigen. Typically, the antibody (i) binds with an equilibrium dissociation constant (K_D) of approximately less than 10^{-7} M, such as approximately less than 10^{-8} M, 10^{-9} M or 10^{-10} M or even lower when determined by, *e.g.,* surface plasmon resonance (SPR) using a predetermined antigen as the analyte and the antibody as the ligand, or Scatchard analysis of binding of the antibody to antigen positive cells, and (ii) binds to the predetermined antigen with an affinity that is at least two-fold greater than its affinity for binding to a non-specific antigen (*e.g.*, BSA, casein) other than the predetermined antigen or a closely-related antigen. Any K_D greater than about 10^{-4} M is generally considered to indicate nonspecific binding.

[0143] The term "k_{assoc}" or "k_a," as used herein, refers to the association rate of a particular antibody-antigen interaction, whereas the term "k_{dis}" or "k_d," as used herein, refers to the

dissociation rate of a particular antibody-antigen interaction. The term "K_D," as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of k_d to k_a (*i.e.* k_d/k_a) and is expressed as a molar concentration (M). K_D values for antibodies can be determined using methods well established in the art. A preferred method for determining the K_D of an antibody is by using surface plasmon resonance, preferably using a biosensor system such as a BIACORE® ® system or flow cytometry and Scatchard analysis, or bio-layer interferometry.

[0144] The term "EC50" or "IC50" in the context of an *in vitro* or *in vivo* assay using an antibody or immunoconjugate refers to the concentration of an antibody that induces a response that is 50% of the maximal response, *i.e.*, halfway between the maximal response and the baseline. In pharmacology, the potency of a compound is expressed as the half-maximal effective concentration (EC50), which refers to the concentration of a drug that induces a response halfway between the baseline and maximum. While expressing the potency of a compound by its EC50 value makes sense in a clinical context, it is counterintuitive in the context of bioactivity-guided purification, as the potency of a compound is inversely related to its EC50 value, and the most potent compound is the one with the lowest EC50. Half-maximal inhibitory concentration (IC50) is the most widely used and informative measure of a drug's efficacy. It indicates how much drug is needed to inhibit a biological process by half, thus providing a measure of potency of an antagonist drug in pharmacological research.

[0145] As used herein, the term "linked" refers to the association of two or more molecules. The linkage can be covalent or non-covalent. The linkage also can be genetic (*i.e.*, recombinantly fused). Such linkages can be achieved using a wide variety of art recognized techniques, such as chemical conjugation and recombinant protein production.

[0146] As used herein, the term "conjugate" is used with reference to an immunoconjugate or antibody drug conjugate comprising an anti-CEACAM5 antibody or antigen binding portion thereof described herein linked to a cytotoxic or therapeutic drug.

[0147] The term "linker," as used herein, refers to a chemical moiety comprising a covalent bond and/or any chain of atoms that may be used to covalently attach *e.g.,* a drug to the antibody. Linkers are known in the art and include *e.g.,* disulfide groups, thioether groups, acid labile groups, photolabile groups, peptidase labile groups and esterase labile groups. Conjugation of an antibody of the present disclosure with cytotoxic drugs or other growth inhibitory agents may be performed *e.g.* using a variety of bifunctional protein coupling agents including but not limited to N-succinimidyl pyridyldithiobutyrate (SPDB), butanoic acid 4-[(5-nitro-2-pyridinyl)dithio]-2,5-

dioxo-1 - pyrrolidinyl ester (nitro-SPDB), 4-(Pyridin-2-yldisulfanyl)-2-sulfo-butyric acid (sulfo-SPDB), N- succinimidyl (2-pyridyldithio) propionate (SPDP), succinimidyl (N-maleimidomethyl) cyclohexane-1 -carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl)-hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1 ,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al (1987). Carbon labeled 1-isothiocyanatobenzyl methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to an antibody (WO 94/11026).

[0148] In certain aspects, the linker is a "cleavable linker," which may facilitate release of the cytotoxic drug or other growth inhibitory agent inside of or in the vicinity of a cell, *e.g.,* a tumor cell. In some aspects, the linker is a linker cleavable in an endosome of a mammalian cell. For example, an acid-labile linker, a peptidase-sensitive linker, an esterase labile linker, a photolabile linker or a disulfide-containing linker (see *e.g.,* U.S. Patent No. 5,208,020) may be used.

[0149] The term “nucleic acid molecule,” as used herein, is used with reference to DNA molecules and RNA molecules. A nucleic acid molecule may be single-stranded or double-stranded, and may be a cDNA.

[0150] The term “isolated nucleic acid molecule,” as used herein in reference to nucleic acids encoding antibodies or antibody fragments (*e.g.*, V_H, V_L, CDR3), is intended to refer to a nucleic acid molecule in which the nucleotide sequences are essentially free of other genomic nucleotide sequences, *e.g.,* those encoding antibodies that bind antigens other than CEACAM5, which other sequences may naturally flank the nucleic acid in human genomic DNA.

[0151] The term “vector,” as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a “plasmid,” which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the

host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, also included are other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

[0152] Also provided are "conservative sequence modifications" of the sequences set forth herein, e.g., amino acid sequence modifications which do not abrogate the binding of the antibody encoded by the nucleotide sequence or containing the amino acid sequence, to the antigen. Such conservative sequence modifications include conservative nucleotide and amino acid substitutions, as well as nucleotide and amino acid additions and deletions. For example, modifications can be introduced into a sequence by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions include ones 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. These families include amino acids with 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, tryptophan), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in an anti-CEACAM5 antibody is preferably replaced with another amino acid residue from the same side chain family. Methods of identifying nucleotide and amino acid conservative substitutions which do not eliminate antigen binding are well-known in the art (see, *e.g.*, Brummell *et al.*, *Biochem.* 32:1180-1187 (1993); Kobayashi *et al. Protein Eng.* 12(10):879-884 (1999); and Burks *et al. Proc. Natl. Acad. Sci. USA* 94:412-417 (1997)). Alternatively, in another aspect, mutations can be introduced randomly along all or part of an anti-CEACAM5 antibody coding sequence, such as by saturation mutagenesis, and the resulting modified anti-CEACAM5 antibodies can be screened for binding activity.

[0153] For nucleic acids, the term "substantial homology" indicates that two nucleic acids, or designated sequences thereof, when optimally aligned and compared, are identical, with

appropriate nucleotide insertions or deletions, in at least about 80% of the nucleotides, usually at least about 80% to 85%, 85% to 90% or 90% to 95%, and more preferably at least about 98% to 99.5% of the nucleotides. Alternatively, substantial homology exists when the segments will hybridize under selective hybridization conditions, to the complement of the strand. For polypeptides, the term "substantial homology" indicates that two polypeptides, or designated sequences thereof, when optimally aligned and compared, are identical, with appropriate amino acid insertions or deletions, in at least about 80% of the amino acids, usually at least about 80% to 85%, 85% to 90%, 90% to 95%, and more preferably at least about 98% to 99.5% of the amino acids.

[0154] The percent identity between two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % homology = # of identical positions/total # of positions x 100), considering the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, as described in the non-limiting examples below.

[0155] The percent identity between two nucleotide sequences can be determined using the GAP program in the GCG software package (available at http://www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. The percent identity between two nucleotide or two amino acid sequences can also be determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

[0156] The nucleic acid and protein sequences described herein can further be used as a "query sequence" to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to the nucleic acid molecules described herein. BLAST protein searches

can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to the protein molecules described herein. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See www.ncbi.nlm.nih.gov.

[0157] The term "recombinant host cell" (or simply "host cell"), as used herein, is intended to refer to a cell that comprises a nucleic acid that is not naturally present in the cell and may be a cell into which a recombinant expression vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but also to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein.

[0158] The term "inhibition," as used herein, refers to any statistically significant decrease in biological activity, including partial and full blocking of the activity. For example, "inhibition" can refer to a statistically significant decrease of about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% in biological activity.

[0159] The term "immunotherapy," as used herein, refers to the treatment of a subject afflicted with, or at risk of contracting or suffering a recurrence of, a disease by a method comprising inducing, enhancing, suppressing or otherwise modifying an immune response.

[0160] The terms "immunostimulating therapy" and "immunostimulatory therapy," as used herein, refer to a therapy that results in an increase (*e.g.*, inducing or enhancing) an immune response in a subject for, *e.g.,* treating cancer.

[0161] As used herein, "immune cell" refers to the subset of blood cells known as white blood cells, which include mononuclear cells such as lymphocytes, monocytes, macrophages, and granulocytes.

[0162] As used herein, "abnormal" is used in the context of the activity or level or expression of a molecule which is outside of the normal activity or expression level (e.g., overexpressed) as compared to *e.g.,* a control sample or reference sample exhibiting a normal activity/expression profile. The term "normal" is used herein in the context of the activity or level of expression of a protein found in a population of healthy, gender- and age-matched subjects. The minimal size of this healthy population may be determined using standard statistical measures, *e.g.,*

the practitioner could consider the incidence of the disease in the general population and the level of statistical certainty desired in the results. Preferably, the normal range for activity, level or expression of a biomarker is determined from a population of subjects (*e.g.*, at least five, ten or twenty subjects), more preferably from a population of at least forty or eighty subjects, and even more preferably from more than 100 subjects.

[0163] "T effector" ("T_{eff}") cells refer to T cells (*e.g.,* CD4+ and CD8+ T cells) with cytolytic activities as well as T helper (Th) cells, which secrete inflammatory cytokines and activate and direct other immune cells but does not include regulatory T cells (Treg cells).

[0164] As used herein, "administering" refers to the physical introduction of a CEACAM5 targeting agent, such as an anti-CEACAM5 antibody, antigen binding portion thereof, bispecific molecule, multispecific molecule, conjugate (*e.g.*, antibody drug conjugate (ADC), CEACAM5 detection agent), CAR-T cell, nucleic acid, or expression vector as described herein, that binds CEACAM5) alone or in combination with another therapeutic agent to a subject, using any of the various methods and delivery systems known to those skilled in the art. Preferred routes of administration for antibodies described herein include intravenous, intraperitoneal, intramuscular, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intraperitoneal, intramuscular, intraarterial, intrathecal, intra-lymphatic, intralesional, intracapsular, intra-orbital, intracardiac, intradermal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion, as well as *in vivo* electroporation. Alternatively, an antibody described herein can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

[0165] As used herein, "cancer" refers to a broad group of diseases characterized by the uncontrolled growth of abnormal cells in the body. Unregulated cell division may result in the formation of malignant tumors or cells that invade neighboring tissues and may metastasize to distant parts of the body through the lymphatic system or bloodstream, and includes a variety of cancers, including but not limited to *e.g.,* carcinomas, melanomas, sarcomas, leukemias, lymphomas, germ cell tumors, and blastomas. Exemplary cancers for treatment include cancers of

the brain, bladder, breast, cervix, colon, head and neck, kidney, lung, non-small cell lung, mesothelioma, ovary, prostate, stomach and uterus, leukemia, and medulloblastoma.

[0166] As used herein, "cell therapy" refers to a method of treatment involving the administration of live cells (*e.g.,* CAR T cells, and NK cells).

[0167] The terms "chimeric antigen receptor" and "CAR" are used with reference to a cell-surface receptor comprising an extracellular binding domain, a transmembrane domain and at least one cytoplasmic signaling domain in a combination that is not naturally found together on a single protein. This particularly includes receptors where the extracellular domain and the cytoplasmic domain are not naturally found together on a single receptor protein. Further, the chimeric antigen receptor is different from a T cell receptor (TCR) expressed in the native T cell lymphocyte.

[0168] The term "CAR-T cells" as used herein refer to a T cell or population thereof, which has been modified through molecular biological methods to express a chimeric antigen receptor (CAR) on the surface of the T cell or population of T cells. The CAR is an engineered polypeptide having an extracellular binding domain with a pre-defined binding specificity to a desired target (*e.g.,* CEACAM5) expressed operably connected to (*e.g.,* as a fusion, or separate chains linked by one or more disulfide bonds) an intracellular part of a T cell activation domain. By bypassing MHC class I and class II restriction, CAR engineered T cells of both CD8+ and CD4+ subsets can be recruited for redirected target cell recognition.

[0169] The term "CAR-T therapy" refers to a method of inducing T cell immunity through administration of CAR-T cells.

[0170] As used herein, the term "small molecule drug" refers to a molecular entity, often organic or organometallic, that is not a polymer, that has medicinal activity, and that has a molecular weight less than about 2 kilodaltons (kDa), less than about 1 kDa, less than about 900 daltons (Da), less than about 800Da or less than about 700Da. The term encompasses most medicinal compounds termed "drugs" other than protein or nucleic acids, although a small peptide or nucleic acid analog can be considered a small molecule drug. Examples include chemotherapeutic anticancer drugs and enzymatic inhibitors. Small molecule drugs can be derived synthetically, semi-synthetically (i.e., from naturally occurring precursors), or biologically.

[0171] The terms "treat," "treating," and "treatment," as used herein, refer to any type of intervention or process performed on, or administering an active agent (*e.g.,* an anti-CEACAM5 antibody, antigen binding portion thereof, antibody drug conjugate, drug) to, the subject with the objective of preventing, reversing, alleviating, ameliorating, inhibiting, or slowing down or

preventing the progression, development, severity or recurrence of a symptom, complication, condition or biochemical indicia associated with a disease. Treatment can be of a subject having a disease or a subject who does not have a disease (*e.g.,* for prophylaxis).

[0172] As used herein, "adjunctive" or "combined" administration (co-administration) includes simultaneous administration of an anti-CEACAM5 antibody or antigen binding portion thereof and one or more additional agents and/or compounds in the same or different dosage form, or combined administration in separate dosages concurrently or sequentially. Thus, a first antibody or antigen binding portion thereof, *e.g.*, an anti-CEACAM5 antibody or antigen binding portion thereof, and second, third, or more antibodies, antigen binding portions, proteins, and/or compounds (*e.g.,* small molecules) can be simultaneously administered in a single formulation or formulated for separate administration and are administered concurrently or sequentially.

[0173] "Combination" therapy, as used herein, means administration of two or more therapeutic agents in a coordinated fashion, and includes, but is not limited to, concurrent and sequential dosing. Specifically, combination therapy encompasses both co-administration (*e.g.,* administration of a co-formulation or simultaneous administration of separate therapeutic compositions) and serial or sequential administration, provided that administration of one therapeutic agent is conditioned in some way on administration of another therapeutic agent. For example, one therapeutic agent may be administered only after a different therapeutic agent has been administered and allowed to act for a prescribed period of time. (*See, e.g.,* Kohrt *et al.* (2011) *Blood* 117:2423). For example, the anti-CEACAM5 antibody can be administered first followed by (*e.g.*, immediately followed by) the administration of a second agent (*e.g.,* an antibody or antigen binding portion thereof, and anti-cancer agent), or vice versa. In one aspect, the ADC is administered prior to administration of the second agent. In another aspect, the ADC is administered, for example, a few minutes (*e.g.,* within about 30 minutes) or at least one hour after the second agent. Such concurrent or sequential administration preferably results in both antibodies or antigen binding portions thereof being simultaneously present in treated patients.

[0174] The administration of effective amounts of the anti-CEACAM5 antibody or antigen binding portion thereof alone, or anti-CEACAM5 antibody or antigen binding portion thereof combined with another compound or agent (*e.g.,* an immune checkpoint inhibitor such as an anti-PD-1 antibody), according to any of the methods provided herein, can result in at least one therapeutic effect, including, for example, reduced tumor growth or size, reduced number of indicia of cancer (*e.g.,* metastatic lesions) appearing over time, complete remission, partial remission, or

stable disease. For example, the methods of treatment may produce a comparable clinical benefit rate (CBR = complete remission (CR)+ partial remission (PR) + stable disease (SD) lasting ≥ 6 months) better than that achieved without administration of the anti-CEACAM5 antibody or antigen binding portion thereof, or than that achieved with administration of any one of the combined antibodies, *e.g.*, the improvement of clinical benefit rate is about 20% 20%, 30%, 40%, 50%, 60%, 70%, 80% or more.

[0175] As used herein, the terms "inhibit" and "block" (*e.g.,* with regard to inhibition/blocking of CEACAM5 binding or functional activity) are used interchangeably and encompass both partial and complete inhibition/blocking by anti-CEACAM5 antibody or fragment thereof, or other inhibition/blocking of a functional activity by a therapeutic agent. The degree of inhibition may be at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% (i.e., 2-fold or 2x), 3-fold, 5-fold or 10-fold relative to a control antibody or reference antibody. Additionally, the degree of inhibition may be between 20%-95%, 20%-80%, 20%-50%, 40%-95%, 40%-80%, 40%-60%, 50%-90%, 50%-70%, 75%-95%, 75%-85%, 2-fold to 20-fold, 2-fold to 10-fold, 2-fold to 5-fold, 4-fold to 12-fold, or 4-fold to 8-fold.

[0176] The term “effective dose” or “effective dosage” is defined as an amount sufficient to achieve or at least partially achieve a desired effect. A "therapeutically effective amount" or "therapeutically effective dosage" of a drug (*e.g.,* anti-CEACAM5 antibody or antigen binding portion thereof) is any amount of the drug or therapeutic agent that, when used alone or in combination with another therapeutic agent, promotes disease regression evidenced by a decrease in severity of disease symptoms, an increase or therapeutic agent in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. A therapeutically effective amount or dosage of a drug or therapeutic agent includes a "prophylactically effective amount" or a "prophylactically effective dosage", which is any amount of the drug or therapeutic agent that, when administered alone or in combination with another therapeutic agent to a subject at risk of developing a disease or of suffering a recurrence of disease, inhibits the development or recurrence of the disease. The ability of a therapeutic agent to promote disease regression or inhibit the development or recurrence of the disease can be evaluated using a variety of methods known to the skilled practitioner, such as in human subjects during clinical trials, in animal model systems predictive of efficacy in humans, or by assaying the activity of the agent in *in vitro* assays.

[0177] By way of example, for the treatment of tumors, a therapeutically effective amount or dosage of the drug or therapeutic agent (*e.g.,* anti-CEACAM5 antibody or antigen binding portion thereof) inhibits tumor cell growth by at least about 20%, by at least about 30% by at least about 40%, by at least about 50%, by at least about 60%, by at least above 70%, by at least about 80%, or by at least about 90% relative to untreated subjects. In some aspects, a therapeutically effective amount or dosage of the drug or therapeutic agent completely inhibits cell growth or tumor growth, *i.e.*, inhibits cell growth or tumor growth by 100%. The ability of a compound or therapeutic agent, including an antibody, to inhibit tumor growth can be evaluated using the assays described herein. Alternatively, this property of a composition comprising the compound or therapeutic agent can be evaluated by examining the ability of the composition to inhibit cell growth; such inhibition can be measured *in vitro* by assays known to the skilled practitioner.

[0178] The term "patient" includes human and other mammalian subjects that receive either prophylactic or therapeutic treatment.

[0179] As used herein, the term "subject" includes any human or non-human animal. For example, the methods and compositions described herein can be used to treat a subject having cancer. The term "non-human animal" includes all vertebrates, *e.g.*, mammals and non-mammals, such as non-human primates, sheep, cats, dogs, cows, chickens, amphibians, and reptiles.

[0180] The term "sample" refers to tissue, bodily fluid, or a cell (or a fraction of any of the foregoing) taken from a patient or a subject. Normally, the tissue or cell will be removed from the patient, but *in vivo* diagnosis is also contemplated. In the case of a solid tumor, a tissue sample can be taken from a surgically removed tumor and prepared for testing. In the case of lymphomas and leukemias, lymphocytes, leukemic cells, or lymph tissues can be obtained (*e.g.*, leukemic cells from blood) and appropriately prepared. Other samples, including e.g., urine, tears, serum, plasma, cerebrospinal fluid, feces, sputum, and cell extracts can also be useful for particular cancers.

[0181] The terms "detection" or "detected", as used herein refer to qualitative and/or quantitative detection (measuring levels) with or without reference to a control.

[0182] The term "diagnosing", as used herein, means the determination of the nature of a medical condition intended to identify a pathology which affects the subject from a number of collected data.

[0183] As used herein, "comprising" is synonymous with "including," "containing," or "characterized by," and is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. As used herein, "consisting of" excludes any element, step, or ingredient

not specified in the claim element. As used herein, "consisting essentially of" does not exclude materials or steps that do not materially affect the basic and novel characteristics of the claim. In each instance herein any of the terms "comprising," "consisting essentially of," and "consisting of" may be optionally replaced with either of the other two terms, thus describing alternative aspects of the scope of the subject matter. The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein.

[0184] As used herein, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. The use of "or" or "and" means "and/or" unless stated otherwise. Furthermore, use of the term "including" as well as other forms, such as "include," "includes," and "included," is not limiting.

[0185] The term "about" as used herein when referring to a measurable value such as an amount, a temporal duration and the like, encompasses variations of up to ± 10% from the specified value. Unless otherwise indicated, all numbers expressing e.g., quantities of ingredients or properties (e.g., molecular weight, reaction conditions) described herein are to be understood as being modified by the term "about".

[0186] As used herein, “and/or” is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term “and/or” as used in a phrase such as “A and/or B” includes “A and B,” “A or B,” “A” alone, and “B” alone. Likewise, the term “and/or” as used in a phrase such as “A, B, and/or C” encompasses each of the following: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A alone; B alone; and C alone.

[0187] As used herein, recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. For example, if a concentration range is stated as 1% to 50%, it is intended that values such as 2% to 40%, 10% to 30%, or 1% to 3% are expressly enumerated in this specification. These are only examples of what is specifically intended, and all possible combinations of numerical values between and including the lowest value and the highest value enumerated are to be considered to be expressly stated in this disclosure.

[0188] As used herein, the term "stereoisomer" refers to isomers of identical constitution that differ in the arrangement of their atoms in space. Enantiomers and diastereomers are examples

of stereoisomers. Geometric isomers are also examples of stereoisomers. The term "enantiomer" refers to one of a pair of molecular species that are mirror images of each other and are not superimposable. The term "diastereomer" refers to stereoisomers that are not mirror images. The term "racemate" or "racemic mixture" refers to a composition composed of equimolar quantities of two enantiomeric species, wherein the composition is devoid of optical activity. Geometric isomers of C=C double bonds can also be present in the ADCs, and all such stable isomers are contemplated in the present invention. *Cis*- and *trans*- (or *E*- and *Z*-) geometric isomers of the ADCs of the present invention are described and may be isolated as a mixture of isomers or as separated isomeric forms.

[0189] As used herein, the term "transmembrane domain" refers to a portion of the signaling component that fuses an extracellular multimerization domain and one or more intracellular signaling domains and anchors the signaling component to the plasma membrane of the T cell. In some aspects, a “transmembrane domain” refers to a portion of the binding component that is fused to an extracellular multimerization domain and anchors the binding component to the plasma membrane of the T cell.

[0190] Various aspects described herein are described in further detail in the following subsections.

I. Anti-CEACAM5 Antibodies and Antigen Binding Portions

[0191] Provided herein are anti-CEACAM5 antibodies having desirable properties for use as therapeutic agents in treating diseases, such as cancers characterized by abnormally high levels of CEACAM5 expression.

[0192] In one aspect, provided herein is an isolated anti-CEACAM5 antibody (i.e., an antibody that binds CEACAM5) or antigen binding portion thereof defined by particular structural features.

[0193] In some aspects, the antibody or antigen binding portion described herein that binds human CEACAM5 may also bind another non-human species of CEACAM (*e.g.*, cynomolgus monkey CEACAM5). For example, the binding to another non-human species is measured by detecting a specific reactivity with purified antigen in binding assays (*e.g.*, SPR, ELISA, bio-layer interferometry) or binding to, or otherwise functionally interacting with, cells physiologically expressing CEACAM5 (*e.g.*, HCT-116 cells overexpressing CEACAM).

[0194] As used herein, the terms "Carcinoembryonic antigen-related cell adhesion molecule-5" and "CEACAM5" are used interchangeably with reference to human CEACAM5 or cynomolgus (*Macaca fascicularis*) CEACAM5, unless the context clearly dictates otherwise. The human CEACAM5 precursor polypeptide (with signal peptide) contains the amino acid sequence set forth in the SEQ ID NO: 1 (GenBank: AAH34671.1); the cDNA sequence is set forth in SEQ ID NO: 2 (GenBank: BC034671.1).

[0195] The cynomolgus CEACAM5 precursor polypeptide (with signal peptide) contains the amino acid sequence set forth in SEQ ID NO: 3 (NCBI: XP_005589491.2); the cDNA sequence is set forth in SEQ ID NO: 4 (NCBI: XM_005589434.3).

[0196] The term "CEACAM5" further includes counterparts from other species and other naturally occurring allelic, splice variants, and processed forms thereof, unless the context clearly dictates otherwise.

Antibody sequences

[0197] In some aspects, the isolated anti-CEACAM5 antibody (*e.g.*, recombinant humanized, chimeric, or human antibody) or antigen binding portion thereof described herein in **Table 16**.

[0198] Anti-CEACAM5 antibodies disclosed herein include all known forms of antibodies and other protein scaffolds with antibody-like properties. For example, the antibody can be a monoclonal antibody, a humanized antibody, a human antibody, a bispecific antibody, an immunoconjugate, a chimeric antibody, or a protein scaffold with antibody-like properties, such as fibronectin or ankyrin repeats. The antibody also can be a Fab, $F(ab')_2$, scFv, AFFIBODY, avimer, nanobody, single chain antibody, or a domain antibody. The antibody also can have any isotype or allotype, including any of the following isotypes: IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, secretory IgA (SIgA), IgD, IgE, and allotypes thereof. Full-length antibodies can be prepared from V_H and V_L sequences using standard recombinant DNA techniques and nucleic acid encoding the desired constant region sequences to be operatively linked to the variable region sequences.

[0199] In some aspects, the present disclosure comprises an isolated anti-CEACAM5 antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a heavy chain variable region (VH) comprising CDR1, CDR2, and CDR3 regions which have at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to the amino acid sequences set forth in SEQ ID NOs: 14, 15, and 16,

respectively, and a light chain variable region (VL) comprising CDR1, CDR2, and CDR3 regions which have at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to the amino acid sequences set forth in SEQ ID NOs: 19, 20, and 21, respectively. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprises: a VH comprising CDR1, CDR2, and CDR3 regions which comprises the amino acid sequences set forth in SEQ ID NOs: 14, 15, and 16, respectively, and a VL comprising CDR1, CDR2, and CDR3 regions which comprise the amino acid sequences set forth in SEQ ID NOs: 19, 20, and 21, respectively.

[0200] In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises a VH comprising CDR1, CDR2, and CDR3 regions which have at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequences set forth in SEQ ID NOs: 14, 15, and 16, respectively, and a VL comprising CDR1, CDR2, and CDR3 regions which have at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to, and wherein the VH comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 38. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises a VH comprising CDR1, CDR2, and CDR3 regions which have at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequences set forth in SEQ ID NOs: 14, 15, and 16, respectively, and a VL comprising CDR1, CDR2, and CDR3 regions which have at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to, and wherein the VH comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 17. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises a VH comprising CDR1, CDR2, and CDR3 regions which have at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequences set forth in SEQ ID NOs: 14, 15, and 16, respectively, and a VL comprising CDR1, CDR2, and CDR3 regions which have at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to, and wherein the VH comprises an amino acid sequence having at least 80%, at least 85%, at least

90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, or SEQ ID NO: 93.

[0201] In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises a VH comprising CDR1, CDR2, and CDR3 regions which have at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequences set forth in SEQ ID NOs: 14, 15, and 16, respectively, and a VL comprising CDR1, CDR2, and CDR3 regions which have at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to, and wherein the VL comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 43. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises a VH comprising CDR1, CDR2, and CDR3 regions which have at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequences set forth in SEQ ID NOs: 14, 15, and 16, respectively, and a VL comprising CDR1, CDR2, and CDR3 regions which have at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to, and wherein the VH comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 22. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises a VH comprising CDR1, CDR2, and CDR3 regions which have at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequences set forth in SEQ ID NOs: 14, 15, and 16, respectively, and a VL comprising CDR1, CDR2, and CDR3 regions which have at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to, and wherein the VH comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82,

SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 92, or SEQ ID NO: 94.

[0202] In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises a VH and a VL comprising the amino acid sequence set forth in SEQ ID NO: 38 and the amino acid sequence set forth in SEQ ID NO: 43, respectively. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises a VH and a VL comprising the amino acid sequence set forth in SEQ ID NO: 49 and the amino acid sequence sest forth in SEQ ID NO: 50; respectively. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises a VH and a VL comprising the amino acid sequence set forth in SEQ ID NO: 51 and the amino acid sequence set forth in SEQ ID NO: 52, respectively. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises a VH and a VL comprising the amino acid sequence set forth in SEQ ID NO: 67 and the amino acid sequence set forth in SEQ ID NO: 68, respectively. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises a VH and a VL comprising the amino acid sequence set forth in SEQ ID NO: 69 and the amino acid sequence set forth in SEQ ID NO: 70, respectively. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises a VH and a VL comprising the amino acid sequence set forth in SEQ ID NO: 71 and the amino acid sequence of SEQ ID NO: 72, respectively. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises a VH and a VL comprising the amino acid sequence set forth in SEQ ID NO: 73 and the amino acid sequence set forth in SEQ ID NO: 74, respectively. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises a VH and a VL comprising the amino acid sequence set forth in SEQ ID NO: 75 and the amino acid sequence set forth in SEQ ID NO: 76, respectively. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises a VH and a VL comprising the amino acid sequence set forth in SEQ ID NO: 77 and the amino acid sequence set forth in SEQ ID NO: 78, respectively. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises a VH and a VL comprising the amino acid sequence set forth in SEQ ID NO: 79 and the amino acid sequence set forth in SEQ ID NO: 80, respectively. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises a VH and a VL comprising the amino acid sequence set forth in SEQ ID NO: 81 and the amino acid sequence set forth in SEQ ID NO: 82, respectively. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises a VH and a VL comprising the amino acid sequence set forth in SEQ ID NO: 83 and the amino acid sequence

set forth in SEQ ID NO: 84, respectively. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises a VH and a VL comprising the amino acid sequence set forth in SEQ ID NO: 85 and the amino acid sequence set forth in SEQ ID NO: 86, respectively. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises a VH and a VL comprising the amino acid sequence set forth in SEQ ID NO: 87 and the amino acid sequence set forth in SEQ ID NO: 88, respectively. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises a VH and a VL comprising the amino acid sequence set forth in SEQ ID NO: 89 and the amino acid sequence set forth in SEQ ID NO: 90, respectively. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises a VH and a VL comprising the amino acid sequence set forth in SEQ ID NO: 91 and the amino acid sequence set forth in SEQ ID NO: 92, respectively. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises a VH and a VL comprising the amino acid sequence set forth in SEQ ID NO: 93 and the amino acid sequence set forth in SEQ ID NO: 94, respectively. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises a VH and a VL comprising the amino acid sequence set forth in 17 and the amino acid sequence set forth in SEQ ID NO: 22, respectively.

[0203] In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, which binds human CEACAM5 and has at least one amino acid mutation described in **Table 17**.

[0204] In some aspects, the heavy chain and the light chain comprise the amino acid sequence set forth in SEQ ID NO: 45 and the amino acid sequence set forth in SEQ ID NO: 46, respectively.

[0205] In some aspects, the anti-CEACAM5 antibody or antigen binding portion thereof is described in Table 16 and Table 17. In some aspects, the CDRs, VH, VL, heavy chain and/or light chain is described in Table 16 and Table 17.

[0206] In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, specifically binds to CEACAM5 with a K_D less than 1 X 10^{-6}M. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, specifically binds to CEACAM5 with a K_D less than 1 X 10^{-7}M. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, specifically binds to CEACAM5 with a K_D less than 1 X 10^{-8}M. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, specifically binds to CEACAM5 with a K_D less than 5 X 10^{-9}M. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, specifically binds to CEACAM5 with a K_D less than 1 X 10^{-9}M. In some aspects, the anti-

CEACAM5 antibody, or antigen binding portion thereof, specifically binds to CEACAM5 with a K_D less than 5 X 10^{-10}M.

Antibody binding

[0207] The anti-CEACAM5 antibodies or antigen binding portions thereof described herein bind to CEACAM5 (*e.g.*, human CEACAM5) in solution, to CEACAM5 attached to a solid surface, such as a microtiter plate, and/or to CEACAM5 (*e.g.*, human CEACAM5) anchored to the membrane of a cell. In some aspects, the anti-CEACAM5 antibody or antigen binding portion thereof binds to human CEACAM5, cyno CEACAM5, or both.

[0208] In some aspects, the anti-CEACAM5 antibody or antigen binding portion thereof binds to human and/or cynomolgus CEACAM5 with a K_D of 100 nM or less, such as 90 nM or less, 80 nM or less, 70 nM or less, 60 nM or less, 50 nM or less, such as 40 nM or less, 30 nM or less, 20 nM or less, 10 nM or less, 5 nM or less, 3 nM or less, 1 nM or less, 0.9 nM or less, 0.8 nM or less, 0.7 nM or less, 0.6 nM or less, 0.5 nM or less, 0.4 nM or less, 0.3 nM or less, 0.2 nM or less, 0.1 nM or less, as measured by any detection method known in the art or described herein, for example, in Example 7.

[0209] In some aspects, the anti-CEACAM5 antibody or antigen binding portion thereof binds to human and/or cynomolgus CEACAM5 with a K_D between 0.1 nM and 100 nM, between 0.1 nM and 50 nM, between 0.1 nM and 25 nM, between 0.1 nM and 10 nM, between 0.1 nM and 5 nM, between 0.1 nM and 2 nM, between 0.1 nM and 1 nM, between 0.1 nM and 0.5 nM, between 1 nM and 100 nM, between 1 nM and 50 nM, between 1 nM and 25 nM, between 1 nM and 10 nM, between 1 nM and 5 nM, between 1 nM and 2 nM, between 5 nM and 100 nM, between 5 nM and 50 nM, between 5 nM and 25 nM, between 5 nM and 10 nM, between 10 nM and 100 nM, between 10 nM and 50 nM, between 10 nM and 25 nM, between 25 nM and 100 nM, between 25 nM and 50 nM, or between 50 nM and 100 nM, as measured by any detection method known in the art or described herein.

[0210] Binding of an anti-CEACAM5 antibody or antigen binding portion thereof (and absence of binding) may be assessed qualitatively or quantitatively by any method known in the art. Exemplary binding methodologies include immunohistochemistry, flow cytometry using, *e.g.*, CEACAM5-overexpressing cells (*e.g.,* MKN-45 or HCT116-CEACAM5), surface plasmon resonance (SPR) using, *e.g.,* BIACORE® system (Cytiva), or bio-layer interferometry (BLI) using, *e.g.,* the Octet platform (ForteBio).

[0211] In some aspects, the CEACAM5 antibody or antigen binding portion thereof does not bind to or does not cross-react with other carcinoembryonic antigens (CEAs), such as CEACAM1, CEACAM3, CEACAM4, CEACAM5, CEACAM6, CEACAM7, CEACAM8, CEACAM16, CEACAM18, CEACAM19, CEACAM20, and/or CEACAM21, *e.g.*, as assessed by, *e.g.,* flow cytometry using cells that overexpress one of the foregoing CEAs, or by SPR or BLI. For example, in some aspects, the anti-CEACAM5 antibody or antigen binding portion thereof binds to one of the foregoing CEAs with a signal or affinity that is not significantly above the signal seen with a control antibody (*e.g.,* isotype control) or the signal seen in the absence of the anti-CEACAM5 antibody.

[0212] In some aspects, the anti-CEACAM5 antibody or antigen binding portion thereof binds to all or a portion of amino acids of human CEACAM5 (SEQ ID NO: 1) as described in **Example 17**. In some aspects, the anti-CEACAM5 antibody or antigen binding portion thereof binds to all or a portion of amino acids of human CEACAM5 (SEQ ID NO: 1) as described in **Example 18**.

[0213] In some aspects, the anti-CEACAM5 antibody or antigen binding portion thereof binds to (or is determined to bind to) CEACAM5-overexpressing cancer cell lines or tumor cells. In some aspects, the anti-CEACAM5 antibody or antigen binding portion thereof binds to CEACAM5 on these cells as assessed *e.g.*, by flow cytometry. For example, in some aspects, at least 5%, at least 10%, at least 20% at least 50%, at least 75%, or at least 90% of CEACAM5-expressing cells can be detected by binding of the anti-CEACAM5 antibody (*e.g.*, display a signal above that seen with an isotype control antibody) by any detection method known in the art or described herein.

[0214] In some aspects, the anti-CEACAM5 antibody or antigen binding portion thereof binds to CEACAM5 expressed on cells (*e.g.,* human and/or cynomolgus CEACAM5 expressed on, *e.g.,* MKN-45 cells) with an EC_{50} of 1000 ng/ml or less, 500 ng/ml or less, 200 ng/ml or less, 150 ng/ml or less, 100 ng/ml or less, 50 ng/ml or less, 25 ng/ml or less, 10 ng/ml or less, 5 ng/ml or less, 2 ng/ml or less, or 1 ng/ml or less, as measured by any detection method known in the art or described herein.

[0215] In some aspects, the anti-CEACAM5 antibody or antigen binding portion thereof binds to CEACAM5 expressed on cells with an EC_{50} between about 1 ng/ml and about 1000 ng/ml, between about 1 ng/ml and about 500 ng/ml, between about 1 ng/ml and about 200 ng/ml, between about 1 ng/ml and about 100 ng/ml, between about 1 ng/ml and about 50 ng/ml, between about 1

ng/ml and about 25 ng/ml, between about 1 ng/ml and about 10 ng/ml, between about 1 ng/ml and about 5 ng/ml, between about 5 ng/ml and about 500 ng/ml, between about 5 ng/ml and about 200 ng/ml, between about 5 ng/ml and about 100 ng/ml, between about 5 ng/ml and about 50 ng/ml, between about 5 ng/ml and about 25 ng/ml, between about 5 ng/ml and about 10 ng/ml, between about 10 ng/ml and about 500 ng/ml, between about 10 ng/ml and about 200 ng/ml, between about 10 ng/ml and about 100 ng/ml, between about 10 ng/ml and about 50 ng/ml, between about 10 ng/ml and about 25 ng/ml, between about 25 ng/ml and about 500 ng/ml, between about 25 ng/ml and about 200 ng/ml, between about 25 ng/ml and about 100 ng/ml, between about 25 ng/ml and about 50 ng/ml, between about 50 ng/ml and about 500 ng/ml, between about 50 ng/ml and about 200 ng/ml, between about 50 ng/ml and about 100 ng/ml, between about 100 ng/ml and about 500 ng/ml, or between about 100 ng/ml and about 200 ng/ml, as measured by any detection method known in the art or described herein.

[0216] The binding of the anti-CEACAM5 antibody or antigen binding portion thereof to CEACAM5 may also be defined using quantitative immunofluorescence by flow cytometry, which allows the number of antibody molecules bound per cell or the number of CEACAM5-expressing cells to be quantified. In some aspects, the number of CEACAM5 molecules expressed per cell or number of CEACAM5-expressing cells in a cell line or tumor sample may be quantified by quantitative immunofluorescence using an anti-CEACAM5 antibody or fragment thereof described herein.

[0217] An anti-CEACAM5 antibody or antigen binding portion thereof binds to soluble or membrane-bound human and/or cynomolgus CEACAM5 with high affinity, for example, with a K_D of 10^{-7} M or less, 10^{-8} M or less, 10^{-9} M or less, 10^{-10} M or less, 10^{-11} M or less, 10^{-12} M or less, 10^{-12} M to 10^{-7} M, 10^{-11} M to 10^{-7} M, 10^{-10} M to 10^{-7} M, or 10^{-9} M to 10^{-7} M, as measured by, *e.g.,* surface plasmon resonance or other art-recognized methods.

[0218] In some aspects, the anti-CEACAM5 antibody or antigen binding portion thereof binds to soluble or membrane-bound human and/or cynomolgus CEACAM5 with a K_D of between 10^{-7} M and 10^{-12} M, between 10^{-7} M and 10^{-11} M, between 10^{-7} M and 10^{-10} M, between 10^{-7} M and 10^{-9} M, between 10^{-7} M and 10^{-8} M, between 10^{-8} M and 10^{-12} M, between 10^{-8} M and 10^{-11} M, between 10^{-8} M and 10^{-11} M, between 10^{-8} M and 10^{-9} M, between 10^{-9} M and 10^{-12} M, between 10^{-9} M and 10^{-11} M, between 10^{-9} M and 10^{-10} M, between 10^{-10} M and 10^{-12} M, between 10^{-10} M and 10^{-11} M, or between 10^{-11} M and 10^{-12} M, as measured by, *e.g.,* surface plasmon resonance or other art-recognized methods.

Competing antibodies and antibodies that bind to the same epitope

[0219] The anti-CEACAM5 antibodies and antigen binding portions described herein are distinguished by the characteristic epitope(s) (i.e., site(s) on CEACAM5) to which they bind, e.g., Examples 16 and 17. The epitope(s) to which the antibody or fragment binds can be determined using art-recognized methods. An anti-CEACAM5 antibody or antigen binding portion thereof is considered to bind to the same epitope as a reference anti-CEACAM5 antibody (for example, MBN001) if it, *e.g.*, contacts one or more of the same residues on human CEACAM5 as the reference antibody or contacts all of the same residues at all of the same regions of human CEACAM5 as the reference antibody.

[0220] Antibodies sharing common epitope binding characteristics may considered to fall within a common "epitope bin." In some cases, a CEACAM5-binding "test antibody" may be determined to fall within a common "epitope bin" by comparison to the sequence of a given "reference" antibody (e.g., MBN001) known to fall within a particular epitope bin. In other cases, epitope binning experiments may be performed to determine whether a test antibody falls into the same "bin" as an antibody based on common binding characteristics with a reference antibody. Antibodies that reduce binding of the antibodies disclosed herein by sequence to, *e.g.,* an immobilized CEACAM5 protein or protein fragment, particularly at roughly stoichiometric concentrations, are likely to bind at the same, overlapping, or adjacent epitopes, and thus may share the desirable functional properties as one or more of the antibodies disclosed herein.

[0221] In some aspects, antibodies falling into the same epitope bin are determined by assaying for antibodies that compete for binding to CEACAM5 with particular anti-CEACAM5 antibodies described herein. Methods of determining antibody competition are known in the art.

[0222] In some aspects, BIACORE analysis can be used to assess the ability of the antibodies to compete. The ability of a test antibody to inhibit the binding of an anti-CEACAM5 antibody described herein to CEACAM5 demonstrates that the test antibody can compete with the antibody for binding to CEACAM5.

[0223] Inhibition or blocking by one antibody relative to another may be carried out by performing any suitable competitive inhibition experiment using art-recognized methods or those described herein, including but not limited to surface plasmon resonance (SPR) using *e.g.,* the BIACORE® system (Cytiva), bio-layer interferometry (BLI) using *e.g.,* the Octet platform (ForteBio), enzyme-linked immunoassay (ELISA), and flow cytometry. In some aspects, epitope binning of the anti-CEACAM5 antibodies may be performed using a recombinant CEACAM5

protein or fragment, which is biotinylated and captured onto, *e.g.,* Streptavidin biosensors which are bound by the first antibody until saturation is achieved. In some aspects, epitope binning may be carried out using a cell-based competition binding FACS assay.

[0224] Unless otherwise indicated, an antibody will be considered to compete with an anti-CEACAM5 antibody if it reduces binding of the selected antibody to human CEACAM5 (SEQ ID NO: 1), cynomolgus CEACAM5 (SEQ ID NO: 3), or fragment thereof by at least 20% when used at a roughly equal molar concentration with the selected antibody, as measured in competition ELISA experiments as outlined in the preceding two paragraphs.

[0225] In some aspects, the anti-CEACAM5 antibodies or antigen binding portions thereof comprise a linear epitope. In some aspects, the anti-CEACAM5 antibodies or antigen binding portions thereof comprise a conformational epitope.

[0226] In some aspects, the anti-CEACAM5 antibodies are screened for high affinity binding to human CEACAM5, and selected antibodies therefrom are studied, *e.g.,* using yeast display assays in which sequence variants of CEACAM5 are presented on the surface of yeast cells, MS-based protein footprinting, such as HDX-MS and Fast Photochemical Oxidation of Proteins (FPOP), and structural methods, such as X-ray crystal structure determination, molecular modeling, and nuclear magnetic resonance (NMR) spectroscopy, including NMR determination of the H-D exchange rates of labile amide hydrogens in CEACAM5 when free and when bound in a complex with an antibody of interest. Such methods can provide atomic resolution of the precise epitope bound by the antibody. In recent years, SP-cryo-EM has emerged as a complementary technique to crystallography and NMR for determining near-atomic level structures suitable for application in drug discovery (Renaud *et al. Nat Rev Drug Discov* 2018;17:471-92; Scapin *et al. Cell Chem Biol* 2018;25:1318-25; Ceska *et al. Biochemical Society Transactions* 2019: p. BST20180267).

[0227] Anti-CEACAM5 antibodies which bind to and compete for the same or similar epitopes to the antibodies disclosed herein may be raised using immunization protocols similar to those described herein, for example, in **Example 1**. In some aspects the immunization may be carried out with a construct containing the epitope bound by the anti-CEACAM5 antibodies disclosed herein. The resulting antibodies can be screened for high affinity binding to human CEACAM5 by FACS, ELISA, or SPR and/or screened for the ability to block binding of a reference antibody disclosed herein as determined by ELISA or by blocking their ability to bind to cells expressing CEACAM5 on their surface, *e.g.,* by FACS or SPR. A test antibody can be

contacted with a CEACAM5 protein, protein fragment, or CEACAM5-expressing cell prior to, at the same time as, or after the addition of the reference antibody.

[0228] Alternatively, variants of anti-CEACAM5 antibodies or antigen-binding fragments described herein can be obtained by mutagenesis of cDNA sequences encoding the heavy and light chains of the antibody.

Antibody internalization

[0229] In another aspect, provided herein is an anti-CEACAM5 antibody, or antigen binding portion thereof, which binds to human and/or cynomolgus CEACAM5 and induces internalization of the anti-CEACAM5 antibody or antigen binding portion thereof in accordance with, *e.g.,* the conditions and results described in, *e.g.,* **Example 11** or **Example 15**, or is linked to a cytotoxin for killing CEACAM5-expressing cells in accordance with the conditions and results described in, *e.g.*, **Example 12**.

[0230] The identification of internalizing anti-CEACAM5 antibodies, or antigen binding portions thereof, disclosed herein is important for development of effective antibody-drug conjugates (ADCs). An anti-CEACAM5 antibody, or antigen binding portion thereof, in accordance with the present disclosure can be evaluated for its ability to internalize into cells as determined by any well-known method in the art, including but not limited to use of the IncuCyte live-cell analysis system, Amnis IMAGESTREAM® Imaging Flow Cytometry Analysis, or laser scanning confocal microscopy.

[0231] The internalizing anti-CEACAM5 antibody, or antigen binding portion thereof, disclosed herein can be characterized or ranked in terms of their "degree of internalization" or "level of internalization," which can relate to the degree (*e.g.,* cell percentage) or level of internalization (total amount of internalized antibodies) at a given antibody concentration (*e.g.*, 100 nM) or following a given period of time (*e.g.*, 2 minutes, 5 minutes, 10 minutes or 30 minutes) relative to a control antibody (*e.g.*, MBN001), such as a non-internalizing antibody, control IgG, or other control antibody (*e.g.*, benchmark antibody).

[0232] In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, disclosed herein internalizes into at least about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% of CEACAM5-expressing cells in a cell population. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, of the present disclosure internalizes into a CEACAM5-expressing cell or population of CEACAM5-expressing cells at a level at least 2-fold,

at least 5-fold, at least 20-fold, at least 100-fold, at least 500-fold, or at least 2,000-fold greater level than a control antibody (*e.g.,* non-internalizing antibody, control IgG, other antibody, benchmark antibody).

[0233] In some aspects, the level of internalization of an anti-CEACAM5 antibody, or antigen binding portion thereof, disclosed herein into CEACAM5-expressing cells (*e.g.,* MKN45 or HCT116-hu/cyno CEACAM5) is determined by comparing area under time-course (AUC) immunofluorescence levels relative to a reference antibody, *e.g.*, as described in **Example 11**. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, disclosed herein internalizes into a CEACAM5-expressing cell or population of CEACAM5-expressing cells at antibody/cell concentrations resulting in an AUC immunofluorescence level that is at least 50%, at least 75%, at least 2-fold, at least 3-fold, at least 5-fold, at least 10-fold, at least 25-fold, at least 50-fold, or at least 100-fold greater compared to a control antibody, as described herein. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, internalizes into a CEACAM5-expressing cell *e.g.,* in accordance with the conditions and results set forth in **Example 11** and **Table 14**.

[0234] In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, disclosed herein may be characterized by its "rate of internalization" represented, *e.g.,* by its $T_{1/2}$ of internalization, which is defined as the time at which half of the maximal internalization is achieved, as measured from the time the antibody is added to the cells. In some aspects, the $T_{1/2}$ of internalization for the anti-CEACAM5 antibody, or antigen binding portion thereof, disclosed herein may be enhanced or increased by at least 10%, 30%, 50%, 75%, 2-fold, 3-fold, 5-fold or more, resulting in a reduction of the $T_{1/2}$ by at least 10%, 30%, 50%, 75%, 2-fold, 3-fold, 5-fold or more compared to a control antibody, as described herein. For example, instead of having a $T_{1/2}$ of 10 minutes, the anti-CEACAM5 antibody, or antigen binding portion thereof, may exhibit an increased rate of internalization and thereby reduce the $T_{1/2}$ to 5 minutes (*i.e.*, a two-fold increase in rate of internalization or a two-fold decrease in $T_{1/2}$). In some aspects, the $T_{1/2}$ is reduced by at least 10 minutes, 30 minutes, or 1 hour.

[0235] In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, disclosed herein may be characterized by its maximal level of internalization into a CEACAM5-expressing cell or population of CEACAM5-expressing cells, where the maximal level of internalization is represented by the level of internalization at the plateau of a graph representing the internalization plotted against antibody concentrations or times. In some aspects, the anti-

CEACAM5 antibody, or antigen binding portion thereof, disclosed herein exhibits a maximal level of internalization, which is at least 10%, 30%, 50%, 75%, 2-fold, 3-fold, 5-fold or more relative to a control antibody, as described herein.

[0236] Another way to compare internalization efficacies of the anti-CEACAM5 antibody, or antigen binding portion thereof, disclosed herein is to compare their level of internalization at a given antibody concentration (*e.g.,* 100 nM) and/or at a given time (*e.g.,* 2 minutes, 5 minutes, 10 minutes or 30 minutes).

[0237] In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, disclosed herein may be characterized by level of internalization, which ability to internalize may be determined using area under time-course (AUC) immunofluorescence analysis representing the antibody concentration at which 50% of the maximum level of internalization is obtained, as measured from the time the antibody is added to the cells, for example, as described in **Example 11**.

[0238] In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, disclosed herein exhibits an EC50 binding value less than 50 nM, less than 40 nM, less than 30 nM, less than 25 nM, less than 20 nM, less than 15 nM, less than 10 nM, less than 8 nM, less than 6 nM, less than 4 nM or less than 3 nM. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, disclosed herein exhibits an EC50 internalization value between 1 nM and 50 nM, between 4 nM and 50 nM, between 10 nM and 50nM, between 20 nM and 50 nM, between 30 nM and 50 nM, between 4 nM and 40 nM, between 4 nM and 30 nM, between 4 nM and 20 nM, between 8 nM and 40 nM, between 8 nM and 30 nM, between 8 nM and 20 nM, between 12 nM and 40 nM, between 12 nm and 30 nM, or between 12 nM and 25 nM.

[0239] In some aspects, the level of binding of the anti-CEACAM5 antibody, or antigen binding portion thereof, disclosed herein can be defined relative to that of a given control antibody, as described herein, and expressed as a percentage of the EC50 value obtained compared to the control antibody. In some aspects, the extent of binding reflected in the EC50 value can be enhanced by at least 10%, 30%, 50%, 75%, 2-fold, 3-fold, 5-fold or more, as compared to a control antibody.

[0240] In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, disclosed herein comprises a modified constant region conferring increased internalization relative to the same anti-CEACAM5 antibody, or antigen binding portion thereof, without the modified constant region, or relative to a control antibody, as described herein. Modified constant regions

for use in these aspects are described in U.S. Patent No. 10,653,791, the contents of which are herein incorporated by reference in their entirety. For example, in some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, disclosed herein comprises an IgG2 hinge or a substitution of a non-IgG2 hinge with an IgG2 hinge. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, disclosed herein comprises a hinge and/or a CH1 domain that is not an IgG2 hinge and/or IgG2 CH1 domain is replaced with an IgG2 hinge and/or IgG2 CH1 domain.

[0241] In some aspects, the anti-CEACAM5 antibody useful for the present disclosure comprises a lysine at the C-terminus of the heavy chain. In some aspects, the anti-CEACAM5 antibody useful for the present disclosure does not comprise a lysine at the C-terminus of the heavy chain. In some aspects, the anti-CEACAM5 antibody useful for the present disclosure is a compostion comprising a mixture of at least two antibodies, one without C terminus lysine, and another with C terminus lysine.

[0242] In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, disclosed herein comprising the modified constant region has a rate of internalization (as measured by $T_{1/2}$) that is increased by at least 10%, 30%, 50%, 75%, 2 fold, 3 fold, 5 fold or more, resulting in a reduction of the $T_{1/2}$ by at least 10%, 30%, 50%, 75%, 2 fold, 3 fold, 5 fold or more relative to the same anti-CEACAM5 antibody, or antigen binding portion thereof, without the modified constant region, or relative to a control antibody, as described herein.

Antibody physical properties

[0243] Each antibody or antigen binding portion thereof will have a unique isoelectric point (pI), which generally falls in the pH range between 6 and 9.5. The pI for an IgG1 antibody typically falls within the pH range of 7-9.5 and the pI for an IgG4 antibody typically falls within the pH range of 6-8. In addition, each antibody or antigen binding portion thereof will have a characteristic melting temperature, with a higher melting temperature indicating greater overall stability *in vivo* (Krishnamurthy R and Manning MC (2002) *Curr Pharm Biotechnol* 3:361-71). In general, the T_{M1} (the temperature of initial unfolding) may be greater than 60°C, greater than 65°C, or greater than 70°C. The melting point of an antibody or fragment can be measured using differential scanning calorimetry (Chen *et al* (2003) *Pharm Res* 20:1952-60; Ghirlando *et al* (1999) *Immunol Lett* 68:47-52) or circular dichroism (Murray *et al.* (2002) *J. Chromatogr Sci* 40:343-9). In a further aspect, the antibodies and antigen binding portions thereof are selected that do not degrade

rapidly. Degradation of an antibody or antigen binding portion thereof can be measured using capillary electrophoresis (CE) and MALDI-MS (Alexander AJ and Hughes DE (1995) *Anal Chem* 67:3626-32).

[0244] In some aspects, the anti-CEACAM5 antibody or antigen binding portion thereof has minimal aggregation effects, which can otherwise lead to the triggering of an unwanted immune response and/or altered or unfavorable pharmacokinetic properties. Generally, antibodies and antigen binding portions thereof are acceptable with aggregation of 25% or less, 20% or less, 15% or less, 10% or less, or 5% or less. Aggregation can be measured by several techniques, including size-exclusion column (SEC), high performance liquid chromatography (HPLC), and light scattering.

II. Bispecific or Multispecific Molecules

[0245] In some aspects, provided herein are bispecific molecules or multispecific molecules (*e.g.*, bispecific antibodies or multispecific antibodies). In some aspects, the disclosure includes a bispecific molecule comprising at least one binding region (e.g., antibody or antigen binding portion thereof) for a particular epitope on CEACAM5 (*e.g.*, human CEACAM5), as described herein, and at least one other binding region that binds another antigen. In some aspects, the disclosure comprises a multispecific molecule comprising the antibody, or antigen binding portion thereof, disclosed herein and at least two binding regions, each of which binds other antigens. Bispecific and/or multispecific molecules can be prepared as full-length antibodies or antibody binding portions (*e.g.*, $F(ab')_2$ antibodies).

[0246] Methods for making bispecific or multispecific molecules are known in the art (see, *e.g.*, PCT Publication numbers WO 05117973 and WO 06091209). For example, production of full length bispecific or multispecific molecules, e.g., antibodies, can be based on the co-expression of two paired immunoglobulin heavy chain-light chains, where the two or more chains have different specificities. Various techniques for making and isolating bispecific or multispecific molecules directly from recombinant cell culture have are also known. For example, bispecific or multispecific molecules can be produced using leucine zippers. Another strategy for making bispecific or multispecific molecules by the use of single-chain Fv (sFv) dimers has also been reported.

[0247] Examples of suitable bispecific or multispecific molecule platforms include, but are not limited to, Dual Targeting (DT)-Ig (GSK/Domantis), Two-in-one Antibody (Genentech),

Cross-linked Mabs (Karmanos Cancer Center), Fcab and mAb^2 (F-Star), CovX-body (CovX/Pfizer), Dual Variable Domain (DVD)-Ig (Abbott), IgG-like Bispecific (ImClone/Eli Lilly), Ts2Ab (Medlmmune/AZ) and BsAb (Zymogenetics), HERCULES (Biogen Idee), TvAb (Roche), ScFv/Fc Fusions, SCORPION (Emergent BioSolutions/Trubion, Zymogenetics/BMS), Dual Affinity Retargeting Technology (Fc-DART) (MacroGenics), Dual(ScFv)2-Fab (National Research Center for Antibody Medicine - China), F(ab)2 (Medarex/AMGEN), Dual-Action or Bis-Fab (Genentech), Dock-and-Lock (DNL) (ImmunoMedics), Bivalent Bispecific (Biotecnol), SEED (EMD Serono), mAb^2 (F-star), Fab-Fv (UCB-Celltech), Bispecific T Cell Engager (BiTE) (Micromet, Tandem Diabody (Tandab) (Affimed), Dual Affinity Retargeting Technology (DART) (MacroGenics), Single-chain Diabody (Academic), TCR-like Antibodies (AIT, ReceptorLogics), COMBODY (Epigen Biotech), dual targeting nanobodies (Ablynx), and Fc-engineered IgGl (Xencor).

[0248] In some aspects, the bispecific molecule comprises a first binding region (e.g., antibody or antigen binding portion thereof) which binds to CEACAM5 derivatized or linked to another functional molecule, *e.g.*, another peptide or protein (*e.g.*, another antibody or ligand for a receptor) to generate a bispecific molecule that binds to CEACAM5 and a non-CEACAM5 target molecule. In some aspects, the multispecific molecule comprises a first binding region (e.g., antibody or antigen binding portion thereof) which binds to CEACAM5 derivatized or linked to two or more functional molecules, *e.g.*, different peptides or proteins (*e.g.*, other antibodies or ligands for a receptor) to generate a multispecific molecule that binds to CEACAM5 and two or more non-CEACAM5 target molecules. An antibody may be derivatized or linked to more than one other functional molecule to generate bispecifc or multispecific molecules that bind to more than two or more different binding sites and/or target molecules. To create a bispecific or multispecific molecule, an antibody disclosed herein can be functionally linked (*e.g.*, by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other binding molecules, such as another antibody, antibody fragment, peptide, receptor, or binding mimetic, such that a multispecific molecule results.

[0249] Accordingly, bispecific molecules, for example, bispecific antibodies and bifunctional antibodies, comprising at least a first binding specificity for a particular epitope on CEACAM5 (*e.g.*, human CEACAM5) and a second binding specificity for a second target are contemplated. In some aspects, multispecific molecules, for example, multispecific antibodies and multifunctional antibodies, comprising at least a first binding specificity for a particular epitope on

CEACAM5 (*e.g.*, human CEACAM5), a second binding specificity for a second target, and a third binding specificity for a third target, wherein the second target and the third target are not the same, are contemplated. In some aspects, the second binding region and/or the third binding region specifically binds to a tumor-associated antigen. Tumor-associated antigens are known in the art. Exemplary tumor-associated antigens include, but are not limited to, AFP, ALK, BAGE proteins, β-catenin, brc-abl, BRCA1, BORIS, CA9, carbonic anhydrase IX, caspase-8, CCR5, CD19, CD20, CD30, CDK4, CEA, cyclin-B1, CYP1B1, EGFR, EGFRvIII, ErbB2/Her2, ErbB3, ErbB4, ETV6-AML, EpCAM, EphA2, Fra-1, FOLR1, GAGE proteins (*e.g.,* GAGE-1, -2), GD2, GD3, GloboH, glypican-3, GM3, gp100, Her2, HLA/B-raf, HLA/k-ras, HLA/MAGE-A3, hTERT, LMP2, MAGE proteins (*e.g.*, MAGE-1, -2, -3, -4, -6, and -12), MART-1, mesothelin, ML-IAP, Muc1, Muc2, Muc3, Muc4, Muc5, Muc16 (CA-125), MUM1, NA17, NY-BR1, NY-BR62, NY-BR85, NY-ESO1, OX40, p15, p53, PAP, PAX3, PAX5, PCTA-1, PLAC1, PRLR, PRAME, PSMA (FOLH1), RAGE proteins, Ras, RGS5, Rho, SART-1, SART-3, Steap-1, Steap-2, STn, survivin, TAG-72, TGF-β, TMPRSS2, Tn, TRP-1, TRP-2, tyrosinase, and uroplakin-3.

[0250] In some aspects, the second and/or third binding region of the bispecific or multispecific antibody specifically binds to CD3, CD4, CD8, CD11b, CD14, CD16, CD19, CD20, CD22, CD23, CD25, CD27/CD70, CD28, CD33, CD38, CD11b, CD30, CD39, CD45, CD47, CD56, CD73, CD91, CD94, CD114, CD122, CD163, CD200R, CD203, CD206, PD-1, PD-L1, PD-L2, CTLA-4, IDO, TIM-3, LAG-3, TIGIT, PVR, PVRL2, B7H3, B7H4, CSF-1R, VISTA, KIR, OX-40, GITR, 4-1BB, CEACAM5, CEACAM5L, ICOS, NKG2DA, NKG2DB, NKG2DC, NKG2DD, NKG2DF, NKG2DH, NKP46, NKP30, LILRB1, calreticulin, GARP, LRRC33, CEACAM52, CEACAM53, TGF-β1, TGF-β2, TGF-β3, FAP, cadherin 11, stanniocalcin 1, or any combination thereof. In some aspects, the second and/or third binding region has agonistic properties when binding to a target, *e.g.*, a TNF family member agonist, OX40 ligand, CD137 ligand, CD137 agonist, STING agonist, GITR agonist, ICOS agonist, CD28 agonist, or any combination thereof.

[0251] In some aspects, the antibody is a trispecific antibody comprising first, second, and third binding regions, wherein the first binding region comprises the binding specificity (*e.g.*, antigen-binding region) of an anti-CEACAM5 antibody described herein, and the second and third binding regions bind to two different targets (or different epitopes on the same target), for example, the targets described herein.

[0252] In some aspects, the antibody is a bifunctional antibody comprising an anti-CEACAM5 antibody described herein and a receptor molecule (*e.g.*, a receptor trap construct such as a TGF-β superfamily ligand receptor (*e.g.*, ActRIIB and variants thereof) or VEGFR).

[0253] In one aspect, the multispecific molecules comprise as a binding specificity at least one antibody, or an antigen binding portion thereof, including, *e.g.*, a Fab, Fab', F(ab')2, Fv, or a single chain Fv. The antibody may also be a light chain or heavy chain dimer, or any minimal fragment thereof such as a Fv or a single chain construct, as described in Ladner *et al.* U.S. Patent No. 4,946,778.

[0254] In some aspects, provided herein is a bispecific or multispecific immune cell engager (ICE) comprising a CEACAM5 binding domain linked by a short flexible linker region to at least one binding domain of a cell surface protein in an immune effector cell. Exemplary immune effector cells include T cells, NK cells, B cells, dendritic cells, and macrophage cells. Compositions and methods for preparing and using immune cell engagers are disclosed in U.S. Patent Publication No. 2017/368169, the disclosures of which are incorporated by reference herein.

[0255] In some aspects, the immune cell engager is a bispecific (BiTE) or trispecific (TriKE) T cell engager molecule comprising a CEACAM5 binding domain linked by a short flexible linker region to at least one binding domain of a T cell surface protein (i.e., T cell engager domain) in a T cell effector, such as a cytotoxic T cell. A CEACAM5-targeted BiTE or TriKE can bring $CD8^+$ CTLs into close proximity to a CEACAM5-expressing tumor cell, resulting in a high binding affinity. $CD8^+$ CTLs, like all T cells, express variable T-cell receptors (TCRs) associated with invariable CD3 subunits. In some aspects, a CEACAM5-targeted BiTE comprises a CEACAM5 binding fragment linked to a CD3ϵ binding domain engages the CD3ϵ unit of the TCR complex to form a synapse on the surface of the tumor cell, activating T cells directly and triggering cell death signaling pathways with the subsequent release of granzymes and perforins. By engaging the CD3ϵ unit, the CEACAM-based BiTE is not limited by TCR specificity and can potentially redirect the entire repertoire of T cells in a TCR–peptide–major histocompatibility complex (MHC) independent manner, which avoids the potential for immunotherapy driven downregulation of MHC-I and immune escape. Advantageously, CEACAM5-targeted BiTEs provide a means for activating exhausted T cells induced by long term exposure to CEACAM5. Exemplary T cell engager binding domains for inclusion in the BiTE or TriKE include CD3, TCRa, TCRp, TCRy, TCRC, ICOS, CD28, CD27, HVEM, LIGHT, CD40, 4-lBB, OX40, DR3, GITR, CD30, TIMl, SLAM, CD2, CD226, or a combination thereof.

[0256] In some aspects, a bispecific T cell engager molecule comprises a CEACAM5 binding domain linked by short flexible linker regions to a checkpoint inhibitor binding domain (a bispecific checkpoint inhibitory engager), *e.g.,* CTLA-4, PD-1, PD-L1, PD-L2, LAG-3, TIM-3, Galectin 9, CEACAM-1, BTLA, CD69, Galectin-1, TIGIT, CD113, CD155, GPR56, VISTA, B7-H3, B7-H4, 2B4, CD48, GARP, PD1H, LAIR1, TIM-1, or TIM-4.

[0257] In some aspects, the immune cell engager is a bispecific or trispecific natural killer (NK) cell engager (NKCE) molecule comprising a CEACAM5 binding domain linked by a short flexible linker region to at least one binding domain of an NK cell surface protein (i.e., an NK cell engager binding domain). In some aspects, the NKCE comprises an antigen binding domain, or ligand that binds to (*e.g.,* activates) CD16 (*e.g.,* CD16a, CD16b, or both), NKp46, NKp30, NKp40, NKp44, NKp46, NKG2D, DNAM1, DAP10, CD16 (*e.g.,* CD16a, CD16b, or both), CRTAM, CD27, PSGL1, CD96, CD 100 (SEMA4D), NKp80, CD244 (also known as SLAMF4 or 2B4), SLAMF6, SLAMF7, KIR2DS2, KIR2DS4, KIR3DS 1, KIR2DS3, KIR2DS5, KIR2DS 1, CD94, NKG2C, NKG2E, CD160, or a combination thereof.

[0258] The bispecific or multispecific molecules can be prepared by conjugating the constituent binding specificities, *e.g.*, the anti-FcR and anti-CEACAM5 binding specificities, using methods known in the art. For example, each binding specificity of the multispecific molecule can be generated separately and then conjugated to one another. When the binding specificities are proteins or peptides, a variety of coupling or cross-linking agents can be used for covalent conjugation. Examples of cross-linking agents include protein A, carbodiimide, N-succinimidyl-S-acetyl-thioacetate (SATA), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), o-phenylenedimaleimide (oPDM), N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), and sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohaxane-1-carboxylate (sulfo-SMCC). In some aspects, conjugating agents are SATA and sulfo-SMCC, both available from Pierce Chemical Co. (Rockford, IL).

[0259] When the binding specificities are antibodies, they can be conjugated via sulfhydryl bonding of the C-terminus hinge regions of the two heavy chains. In some aspects, the hinge region is modified to contain an odd number of sulfhydryl residues, preferably one, prior to conjugation.

[0260] Alternatively, both binding specificities can be encoded in the same vector and expressed and assembled in the same host cell. This method is particularly useful where the multispecific molecule is a mAb x mAb, mAb x Fab, Fab x F(ab')2 or ligand x Fab fusion protein. A bispecific or multispecific molecule can be a single chain molecule comprising one single chain

antibody and a binding determinant, or a single chain bispecific or multispecific molecule comprising two binding determinants. Bispecific or multispecific molecules may comprise at least two single chain molecules. Methods for preparing bispecific or multispecific molecules are described for example in U.S. Patent Number 5,260,203; U.S. Patent Number 5,455,030; U.S. Patent Number 4,881,175; U.S. Patent Number 5,132,405; U.S. Patent Number 5,091,513; U.S. Patent Number 5,476,786; U.S. Patent Number 5,013,653; U.S. Patent Number 5,258,498; and U.S. Patent Number 5,482,858.

[0261] Binding of the bispecific or multispecific molecules to their specific targets can be confirmed by, for example, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), FACS analysis, bioassay (*e.g.*, growth inhibition), or simple western blot assay. Each of these assays generally detects the presence of protein-antibody complexes of particular interest by employing a labeled reagent (*e.g.*, an antibody) specific for the complex of interest. For example, the FcR-antibody complexes can be detected using *e.g.*, an enzyme-linked antibody or antigen binding portion which recognizes and specifically binds to the antibody-FcR complexes. Alternatively, the complexes can be detected using any of a variety of other immunoassays. For example, the antibody can be radioactively labeled and used in a radioimmunoassay (RIA). The radioactive isotope can be detected by such means as the use of a α γ-β counter or a scintillation counter or by autoradiography.

III. Conjugates

[0262] The present disclosure also provides a conjugate comprising an anti-CEACAM5 antibody or antigen binding portion thereof described herein, which is linked or conjugated (*e.g.*, covalently attached) to a biologically active moiety, a binding moiety, a detectable moiety, or labeling moiety. In certain aspects, the biologically active moiety comprises a cytotoxic moiety. In some aspects, the biologically active moiety comprises a therapeutic agent, a small molecule drug, or a radioisotope.

[0263] An antibody and a drug may be directly bound to each other via their own linker groups or indirectly via a linker or other substance.

Antibody-drug conjugates (ADCs)

[0264] In some aspects, the conjugate is an antibody-drug conjugate (ADC) in which the anti-CEACAM5 antibody or antigen binding portion thereof is linked to a cytotoxic or cytostatic moiety (*i.e.*, "conjugate"). The cytotoxic moiety can be any cytotoxic or cytostatic moiety (*e.g.*,

small molecule drug) that is detrimental to and/or kills cancer cells. As used in the context of anti-cancer agents, the terms "cytotoxic moiety", "cytostatic moiety", and "therapeutic agent" may be used interchangeably with one another, unless the context explicitly suggests otherwise. A conjugate according to the present disclosure employs linkers with linkages designed to cleave, immolate and/or or otherwise breakdown specifically or non-specifically inside cells in order to release the attached payload. Parameters for ADC design considerations include conjugation site, linker length, linkage/conjugation chemistry, cleavable/non-cleavable linkage and steric hindrance.

[0265] Cytotoxic moieties, include, but are not limited to, alkylating agents and alkylating-like agents, angiogenesis inhibitors, antimetabolite agents, antimitotic agents, antitumor antibiotics, apoptosis regulators, DNA intercalating agents, DNA replication and repair inhibitors, histone deacetylase inhibitors, hormonal agents, kinase inhibitors, mitochondria inhibitors, nuclear export inhibitors, proteasome inhibitors, protein synthesis inhibitors, radionuclides, topoisomerase I inhibitors, and topoisomerase II inhibitors.

[0266] In some aspects, the cytotoxic moiety in the conjugate, *e.g.*, ADC, is an alkylating agent or alkylated-like agent. Classes of such agents include nitrogen mustards (*e.g.*, dichloroethylmethylamine, phenylbutyric acid mustard, melphalan, cyclophosphamide); ethyleneimines (*e.g.*, thiotepae); sulfonates; polyols (*e.g.*, busulfan, dibromomannitol); nitrosoureas (*e.g.*, carmustine, lomustine); platinum-based antitumor agents (*e.g.*, cisplatin, oxaliplatin, carboplatin), and the like.

[0267] Exemplary alkylating agents or alkylating-like agents for use in the conjugate, *e.g.*, ADC, of the present disclosure include, but are not limited to, altretamine; asaley (L-Leucine, N-[N-acetyl-4-[bis-(2-chloroethyl)amino]-DL-phenylalanyl]-, ethylester); AZQ (1,4-cyclohexadiene-1,4-dicarbamic acid, 2, 5-bis(1-aziridinyl)-3,6-dioxo-, diethyl ester); BCNU (N,N′-Bis(2-chloroethyl)-N-nitrosourea); busulfan (1,4-butanediol dimethanesulfonate); carboplatin; carboquone; (carboxyphthalato)platinum; carmustine (BSNU) and lomustine (CCNU); CBDCA (cis-(1,1-cyclobutanedicarboxylato)diamineplatinum(II))); CCNU (N-(2-chloroethyl)-N′-cyclohexyl-N-nitrosourea); CHIP (iproplatin; NSC 256927); chlorambucil; chlornaphazine; chlorozotocin (2-[[[(2-chloroethyl) nitrosoamino]carbonyl]amino]-2-deoxy-D-glucopyranose); cis-dichlorodiamine platinum (II) (DDP); cis-platinum (cisplatin); clomesone; cyanomorpholinodoxorubicin; cyclodisone; dacarbazine; dianhydrogalactitol (5,6-diepoxydulcitol); dibromomannitol; duocarmycin; evofosfamide; fluorodopan ((5-[(2-

chloroethyl)-(2-fluoroethyl)amino]-6-methyl-uracil); fotemustine; glufosfamide; hepsulfam; hycanthone; indolinobenzodiazepine dimer DGN462; lomustine; mannosulfan; mechlorethamine; melphalan; methyl CCNU ((1-(2-chloroethyl)-3-(trans-4-methylcyclohexane)-1-nitrosourea); mitomycin C; mitozolamide; nedaplatin; nimustine; nitrogen mustard ((bis(2-chloroethyl) methylamine hydrochloride); oxaliplatin; PCNU ((1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1-nitrosourea)); phenanthriplatin; pipobroman; piperazine alkylator ((1-(2-chloroethyl)-4-(3-chloropropyl)-piperazine dihydrochloride)); piperazinedione; pipobroman (N,N′-bis(3-bromopropionyl) piperazine); porfiromycin (N-methylmitomycin C); procarbazine, satraplatin, spirohydantoin mustard; ranimustine; semustine; streptozotocin; temozolomide; teroxirone (triglycidylisocyanurate); tetraplatin; thiotepa (N,N′,N″-tri-1,2-ethanediylthio phosphoramide); treosulfan; triaziquone; triethylenemelamine; triplatin tetranitrate; uracil nitrogen mustard (desmethyldopan); Yoshi-864 ((bis(3-mesyloxy propyl)amine hydrochloride); or any combination thereof.

[0268] In some aspects, the cytotoxic moiety in the conjugate, *e.g.*, ADC, is an angiogenesis inhibitor such as ABT-869; AEE-788; axitinib (AG-13736); AZD-2171; CP-547,632; IM-862; pegaptamib; sorafenib; BAY43-9006; pazopanib (GW-786034); vatalanib (PTK-787, ZK-222584); sunitinib; SU-11248; VEGF trap; vandetanib; ABT-165; ZD-6474; DLL4 inhibitors; or any combination thereof.

[0269] In some aspects, the cytotoxic moiety in the conjugate, *e.g.*, ADC, is an antimetabolite agent, such as a DNA/RNA antimetabolite or a folate antagonist. Exemplary antimetabolites include, but are not limited to, L-alanosine; adenosine deaminase inhibitors (*e.g.*, cladribine, fludarabine, nelarabine, pentostatin); 5-azacytidine; 5-fluorouracil; acivicin; aminopterin and aminopterin derivatives (*e.g.,* N-[2-chloro-5[[(2, 4-diamino-5-methyl-6-quinazolinyl)methyl]amino]benzoyl]L-aspartic acid (NSC 132483), N-[4-[[(2, 4-diamino-5-ethyl-6-quinazolinyl)methyl]amino]benzoyl]L-aspartic acid, N-[2-chloro-4-[[(2, 4-diamino-6-pteridinyl)methyl]amino]benzoyl]L-aspartic acid monohydrate; Baker's soluble antifol (NSC 139105); dichlorallyl lawsone ((2-(3, 3-dichloroallyl)-3-hydroxy-1,4-naphthoquinone); brequinar; folate antagonists (*e.g.*, methotrexate, PT523 ((Na-(4-amino-4-deoxypteroyl)-Nγ-hemiphthaloyl-L-ornithine), ftorafur ((pro-drug; 5-fluoro-1-(tetrahydro-2-furyl)-uracil); 5,6-dihydro-5-azacytidine; methotrexate; methotrexate derivative (N-[[4-[[(2, 4-diamino-6-pteridinyl)methyl]methylamino]-1-naphthalenyl]carbonyl]L-glutamic acid); PALA ((N-(phosphonoacetyl)-L-aspartate); pyrazofurin; purine antagonists (*e.g.*, 6-mercaptopurine, 6-

thioguanine); pyrimidine antagonists (*e.g.*, 5-fluorouracil, fluorouridine, cytarabine, capecitabine, gemcitabine), trimetrexate, and antifolate. Additional antimetabolites include3-HP; 2′-deoxy-5-fluorouridine; 5-HP; α-TGDR (α-2′-deoxy-6-thioguanosine); aphidicolin glycinate; ara C (cytosine arabinoside); 5-aza-2′-deoxycytidine; β-TGDR (β-2′-deoxy-6-thioguanosine); cyclocytidine; guanazole; hydroxyurea; inosine glycodialdehyde; macbecin IL; pyrazoloimidazole; thioguanine; thiopurine; or any combination thereof.

[0270] In some aspects, the cytotoxic moiety in the conjugate, *e.g.*, ADC, is an antimitotic agent, such as an anti-tubulin agent. Exemplary antimitotic agents include, but are not limited to, ABT-348; allocolchicine; auristatins; Aurora A-specific kinase inhibitors; Aurora B-specific kinase inhibitors and pan-Aurora kinase inhibitors; AZD-5438; AZD-1152; AZD4877; ARRY-520; BMI-1040; BMS-032; BMS-387; CVT-2584; cemadotin; colchicine and colchicine derivatives; colcimid; combretastatins; discodermolide; cryptophysins; docetaxel; dolastins; eleutherobin; estramustine; MLN-8054; flavopyridol; GPC-286199; GSK923295A; halichondrin B; maytansinoids; MCS-5A; nocodazole; paclitaxel and paclitaxel derivatives, such as 2′-N-[3-(dimethylamino)propyl]glutaramate paclitaxel; nab-paclitaxel; PD0332991; PHA-690509; rhozoxin; seliciclib; taxanes and taxane derivatives; thiocolchicine; trityl cysteine; vinca alkaloids (*e.g.*, vinblastine sulfate, vincristine, vindesine, vinblastine, and vinorelbine); VX-680; ZK-304709; or any combination thereof.

[0271] Examples of anti-tubulin agents include, but are not limited to, dolastatins (*e.g.*, auristatin E (dolastatin-10), auristatin F (MMAE), and monomethyl auristatin (MMAF)), AFP, MMAF, MMAE, AEB, and AEVB); maytansinoids, taxanes (e.g., paclitaxel, docetaxel), T67 (Tularik), baccatin derivatives, taxane analogs (*e.g.*, epothilone A and B), nocodazole, colchicine and colcimid, estramustine, cryptophysins, cemadotin, combretastatins, discodermolide, eleutherobin, or any combination thereof. Typically, the auristatin E derivative is, *e.g.*, an ester formed between auristatin E and a keto acid. For example, auristatin E can be reacted with paraacetyl benzoic acid or benzoylvaleric acid to produce AEB and AEVB, respectively.

[0272] Maytansinoids can be synthesized and/or isolated from natural sources. Maytansinoids include thiol-containing derivatives of maytansine, such as DM1 and DM4; maytansine, maytansinol, and C-3 esters of maytansinol. Thiol-containing derivatives of maytansine include compounds where the methyl group bound to the carbonyl group is replaced by a group containing a free sulfhydryl group such as the group —R—SH where R represents an alkylene group or other carbon-containing group of atoms.

[0273] In some aspects, the cytotoxic moiety in the conjugate, *e.g.*, ADC, is an antitumor antibiotic, including but not limited to, dactinomycin, anthracyclines (*e.g.*, anthramycin (AMC), daunorubicin, doxorubicin, epirubicin, and idarubicin), bleomycin, calicheamicin, duocarmycin, mithromycin, and the like.

[0274] In some aspects, the cytotoxic moiety in the conjugate, *e.g.*, ADC, is an apoptosis regulator, such as AT-101 ((−)gossypol); G3139 or oblimersen (Bcl-2-targeting antisense oligonucleotide); IPI-194; IPI-565; N-(4-(4-((4′-chloro(1,1′-biphenyl)-2-yl)methyl)piperazin-1-ylbenzoyl)-4-(((1R)-3-(dimethylamino)-1-((phenylsulfanyl)methyl)propyl)amino)-3-nitrobenzenesulfonamide); N-(4-(4-((2-(4-chlorophenyl)-5,5-dimethyl-1-cyclohex-1-en-1-yl)methyl)piperazin-1-yl)benzoyl)-4-(((1R)-3-(morpholin-4-yl)-1-((phenylsulfanyl)methyl)propyl)amino)-3-((trifluoromethyl)sulfonyl)benzenesulfonamide; GX-070 (Obatoclax®; 1H-Indole, 2-(2-((3,5-dimethyl-1H-pyrrol-2-yl)methylene)-3-methoxy-2H-pyrrol-5-yl)−)); HGS1029; GDC-0145; GDC-0152; LCL-161; LBW-242; venetoclax; agents that target TRAIL or death receptors (*e.g.*, DR4 and DR5) such as ETR2-ST01, GDC0145, HGS-1029, LBY-135, PRO-1762; drugs that target caspases, caspase-regulators, BCL-2 family members, death domain proteins, TNF family members, Toll family members, NF-kappa-B proteins, or any combination thereof.

[0275] In some aspects, the cytotoxic moiety in the conjugate, *e.g.*, ADC, is a DNA intercalating agent (*e.g.*, groove binding agents, including minor groove binders), such as anthramycin; chicamycin A; enediynes; lexitropsins; tomaymycin; DC-81; sibiromycin; pyrrolobenzodiazepine derivative; SGD-1882 ((S)-2-(4-aminophenyl)-7-methoxy-8-(3 S)-7-methoxy-2-(4-methoxyphenyl)-5-oxo-5,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5(11aH)-one); SG2000 (SJG-136; (11aS,11a′S)-8,8′-(propane-1,3-diylbis(oxy))bis(7-methoxy-2-methylene-2,3-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5(11 aH)-one)); or any combination thereof.

[0276] In some aspects, the cytotoxic moiety in the conjugate, *e.g.*, ADC, is a DNA replication and repair inhibitor selected from Altretamine; Bleomycin; Dacarbazine; Dactinomycin; Mitobronitol; Mitomycin; Pingyangmycin; Plicamycin; Procarbazine; Temozolomide; ABT-888 (veliparib); olaparib; KU-59436; AZD-2281; AG-014699; BSI-201; BGP-15; INO-1001; ONO-2231; or any combination thereof.

[0277] In some aspects, the cytotoxic moiety in the conjugate, *e.g.*, ADC, is a histone deacetylase inhibitor, such as vorinostat; romidepsin; chidamide; panobinostat; valproic acid;

belinostat; mocetinostat; abexinostat; entinostat; SB939 (pracinostat); resminostat; givinostat; quisinostat; thioureidobutyronitrile (KEVETRIN™); CUDC-10; CHR-2845 (tefinostat); CHR-3996; 4SC-202; CG200745; ACY-1215 (rocilinostat); ME-344; sulforaphane; or any combination thereof.

[0278] In some aspects, the cytotoxic moiety in the conjugate, *e.g.*, ADC, is a hormonal agent, such as anastrozole; exemestane; arzoxifene; bicalutamide; cetrorelix; degarelix; deslorelin; trilostane; dexamethasone; flutamide; raloxifene; fadrozole; toremifene; fulvestrant; letrozole; formestane; glucocorticoids; doxercalciferol; sevelamer carbonate; lasofoxifene; leuprolide acetate; megesterol; mifepristone; nilutamide; tamoxifen citrate; abarelix; prednisone; finasteride; rilostane; buserelin; luteinizing hormone releasing hormone (LHRH); histrelin; trilostane or modrastane; fosrelin; goserelin, or any combination thereof.

[0279] In some aspects, the cytotoxic moiety in the conjugate, *e.g.*, ADC, is a kinase inhibitor, such as a tyrosine kinase inhibitor or an ATP-competitive TORC1/TORC2 inhibitor. Exemplary tyrosine kinase inhibitors include, but are not limited to, afantinib, axitinib, binimetinib, bosutinib, crizotinib, dasatinib, erlotinib, fostamatinib, gefitinib, ibrutinib, imatinib, lapatinib, lestaurtinib, lenvatinib, mubritinib, nilotinib, pazopanib; pegaptanib, ruxolitinib, selemetinib, semaxanib, sildenib, sorafenib, SU6656, sunitinib, vandetanib, and vemurafenib. Exemplary TORC1/TORC2 inhibitors include PI-103, PP242, PP30, Torin 1, or any combination thereof. Additional kinases include XL019, INCB018424, ARRY-142886, ARRY-438162, PD-325901, PD-98059, AP-2357, CCI-779, everolimus, RAD-001, rapamycin, temsirolimus, LY294002, XL-147, CAL-120, ONC-21, AEZS-127, ETP-45658, PX-866, GDC-0941, BGT226, BEZ235, XL765, or any combination thereof.

[0280] In some aspects, the cytotoxic moiety in the conjugate, *e.g.*, ADC, is a mitochondria inhibitor, such as pancratistatin; phenpanstatin; rhodamine-123; edelfosine; d-alpha-tocopherol succinate; compound 11β; aspirin; ellipticine; berberine; cerulenin; GX015-070 (Obatoclax®; 1H-Indole, 2-(2-((3,5-dimethyl-1H-pyrrol-2-yl)methylene)-3-methoxy-2H-pyrrol-5-yl)−); celastrol (tripterine); metformin; Brilliant green; ME-344, or any combination thereof.

[0281] In some aspects, the cytotoxic moiety in the conjugate, *e.g.*, ADC, is a nuclear export inhibitor, such as callystatin A; delactonmycin; KPT-185 (propan-2-yl (Z)-3-[3-[3-methoxy-5-(trifluoromethyl)phenyl]-1,2,4-triazol-1-yl]prop-2-enoate); kazusamycin A; leptolstatin; leptofuranin A; leptomycin B; ratjadone; Verdinexor ((Z)-3-[3-[3,5-

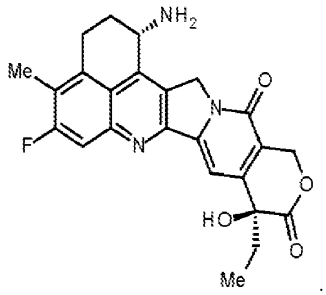
bis(trifluoromethyl)phenyl]-1,2,4-triazol-1-yl]-N-pyridin-2-ylprop-2-enehydrazide), or any combination thereof.

[0282] In some aspects, the cytotoxic moiety in the conjugate, *e.g.*, ADC, is a proteasome inhibitor, such as Bortezomib; Carfilzomib; Epoxomicin; Ixazomib; Salinosporamide A, or any combination thereof.

[0283] In some aspects, the cytotoxic moiety in the conjugate, *e.g.*, ADC, is a radionuclide agent, such as I^{131}, In^{11}, $Y^{90,}$ Lu^{177}, and the like.

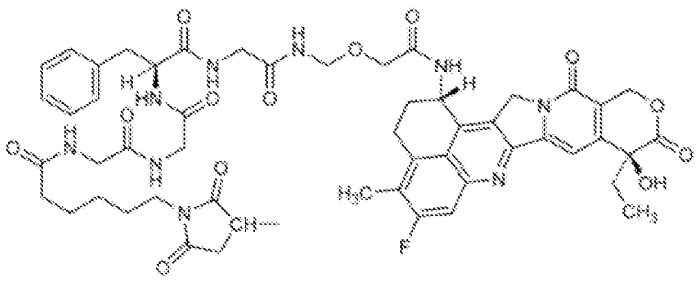
[0284] In some aspects, the cytotoxic moiety in the conjugate, *e.g.*, ADC, is a protein synthesis inhibitor, such as Streptomycin; Dihydrostreptomycin; Neomycin; Framycetin; Paromomycin; Ribostamycin; Kanamycin; Amikacin; Arbekacin; Bekanamycin; Dibekacin; Tobramycin; Spectinomycin; Hygromycin B; Paromomycin; Gentamicin; Netilmicin; Sisomicin; Isepamicin; Verdamicin; Astromicin; Tetracycline; Doxycycline; Chlortetracycline; Clomocycline; Demeclocycline; Lymecycline; Meclocycline; Metacycline; Minocycline; Oxytetracycline; Penimepicycline; Rolitetracycline; Tetracycline; Glycylcyclines; Tigecycline; Oxazolidinone; Eperezolid; Linezolid; Posizolid; Radezolid; Ranbezolid; Sutezolid; Tedizolid; Peptidyl transferase inhibitors; Chloramphenicol; Azidamfenicol; Thiamphenicol; Florfenicol; Pleuromutilins; Retapamulin; Tiamulin; Valnemulin; Azithromycin; Clarithromycin; Dirithromycin; Erythromycin; Flurithromycin; Josamycin; Midecamycin; Miocamycin; Oleandomycin; Rokitamycin; Roxithromycin; Spiramycin; Troleandomycin; Tylosin; Ketolides; Telithromycin; Cethromycin; Solithromycin; Clindamycin; Lincomycin; Pirlimycin; Streptogramins; Pristinamycin; Quinupristin/dalfopristin; Virginiamycin, or any combination thereof.

[0285] In some aspects, the cytotoxic moiety in the conjugate, *e.g.*, ADC, is a topoisomerase I inhibitor, such as camptothecin, including derivatives and analogs thereof, *e.g.*, topotecan, irinotecan, silatecan, cositecan, exatecan, lurtotecan, gimatecan, belotecan and rubitecan, NSC 100880, NSC 603071, NSC 107124, NSC 643833, NSC 629971, NSC 295500, NSC 249910, NSC 606985, NSC 74028, NSC 176323, NSC 295501, NSC 606172, NSC 606173, NSC 610458, NSC 618939, NSC 610457, NSC 610459, NSC 606499, NSC 610456, NSC 364830, and NSC 606497); morpholinisoxorubicin; SN-38, or any combination thereof. The structure of exatecan is shown below.

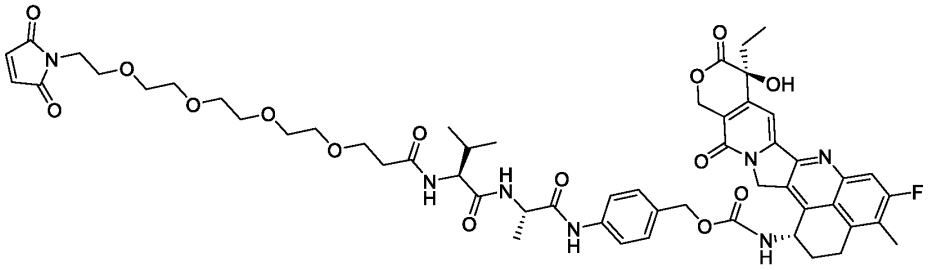


[0286] For example, the exatecan ((10S,23S)-23-amino-10-ethyl-18-fluoro-10-hydroxy-19-methyl-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracosa-1,6(11),12,14,16,18,20(24)-heptaene-5,9-dione) is a derivative of exatecan. For example, the derivative of exatecan is deruxtecan (Dxd; 6-(2,5-dioxopyrrol-1-yl)-N-[2-[[2-[[(2S)-1-[[2-[[2-[[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracosa-1,6(11),12,14,16,18,20(24)-heptaen-23-yl]amino]-2-oxoethoxy]methylamino]-2-oxoethyl]amino]-1-oxo-3-phenylpropan-2-yl]amino]-2-oxoethyl]amino]-2-oxoethyl]hexanamide), which is shown below.

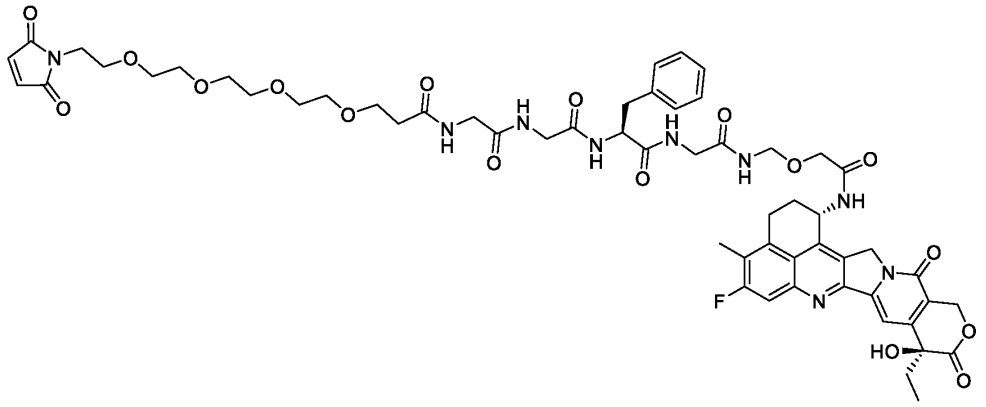
[0287] In some aspects, the linker and drug moiety (e.g., payload, e.g., cytotoxic moiety) is a structure/compound shown below:



(Compound A);



(Compound B); or



(Compound C),

or an isomer thereof (e.g., a stereoisomer thereof), a solvate thereof, or a pharmaceutically acceptably salt thereof.

[0288] In some aspects, the conjugate, *e.g.*, ADC, comprises an anti-CEACAM5 antibody, or antigen binding portion thereof, disclosed elsewhere herein (e.g., an antibody comprising a VH and a VL, which comprise the amino acid sequences set forth in SEQ ID NOs: 38 and 43, respectively) and Compound A. In some aspects, the conjugate, *e.g.*, ADC, comprises an anti-CEACAM5 antibody, or antigen binding portion thereof, disclosed elsewhere herein (e.g., an antibody comprising a VH and a VL, which comprise the amino acid sequences set forth in SEQ ID NOs: 38 and 43, respectively) and Compound B. In some aspects, the conjugate, *e.g.*, ADC, comprises an anti-CEACAM5 antibody, or antigen binding portion thereof, disclosed elsewhere herein (e.g., an antibody comprising a VH and a VL, which comprise the amino acid sequences set forth in SEQ ID NOs: 38 and 43, respectively) and Compound C.

[0289] In some aspects, the drug moiety, e.g., cytotoxic moiety, in the conjugate, *e.g.*, ADC, is a topoisomerase II inhibitor, such as amonafide (benzisoquinolinedione); m-AMSA (4′-(9-acridinylamino)-3′-methoxymethanesulfonanilide); anthrapyrazole derivative ((NSC 355644); emsacrine; etoposide (VP-16); teniposide; pyrazoloacridine ((pyrazolo[3,4,5-kl]acridine-2(6H)-propanamine, 9-methoxy-N, N-dimethyl-5-nitro-, monomethanesulfonate); bisantrene hydrochloride; daunorubicin; deoxydoxorubicin; mitoxantrone; menogaril; N,N-dibenzyl daunomycin; oxanthrazole; rubidazone; or teniposide. For example, the drug moiety, e.g., cytotoxic moiety, in the conjugate, *e.g.*, ADC, is etoposide.

[0290] In some aspects, the cytotoxic moiety is an enzymatically active toxin or fragment thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria

toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), *Momordica charantia* inhibitor, curcin, crotin, *Sapaonaria officinalis* inhibitor, gelonin, mitogellin, restrictocin, phenomycin, neomycin, and a tricothecene.

[0291] Any of these moities that include, or that may be modified to include, a site of attachment to an antibody and/or binding fragment can be included in the conjugate. It is further noted that any of the foregoing agents may be used or administered separately in a combination therapy with the anti-CEACAM5 antibodies, antigen-binding fragments thereof, or ADCs described herein.

[0292] In some aspects, an anti-CEACAM5 antibody or antigen binding portion thereof described herein is linked to a drug via a bifunctional crosslinking reagent. As used herein, a "bifunctional crosslinking reagent" refers to a reagent that possesses two reactive groups one of which is capable of reacting with an antibody, while the other one is capable of reacting with the drug to link the antibody with the drug, thereby forming a conjugate. Any suitable bifunctional crosslinking reagent can be used in connection with the disclosure, so long as the linker reagent provides for retention of the drug, *e.g.*, cytotoxicity, and targeting characteristics of the antibody. In some aspects, the linker molecule joins the drug to the antibody through chemical bonds, such that the drug and the antibody are chemically coupled (*e.g.*, covalently bonded) to each other.

[0293] Bifunctional crosslinking reagents include, but are not limited to, N-succinimidyl-3-(2-pyridyldithio)butyrate (SPDB), N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (Sulfo-SMCC), N-succinimidyl-4-(maleimidomethyl)cyclohexanecarboxylate (SMCC), N-succinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxy-(6-amidocaproate) (LC-SMCC), 4-maleimidobutyric acid N-hydroxysuccinimide ester (GMBS), 3-maleimidocaproic acid N-hydroxysuccinimide ester (EMCS), m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), N-(α-maleimidoacetoxy)-succinimide ester (AMAS), succinimidyl-6-(β-maleimidopropionamido)hexanoate (SMPH), N-succinimidyl-4-(p-maleimidophenyl)-butyrate (SMPB), N-(p-maleimidophenyl)isocyanate (PMPI), 6-maleimidocaproyl (MC), maleimidopropanoyl (MP), p-aminobenzyloxycarbonyl (PAB), N-succinimidyl-4-(2-pyridylthio)pentanoate (SPP), N-succinimidyl (4-iodoacetyl)aminobenzoate (SIAB), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-

diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody (see, *e.g.*, PCT publication number WO94/11026).

[0294] In some aspects, the bifunctional crosslinking reagent comprises a cleavable linker. In some aspects, a cleavable linker is cleavable under physiological conditions, in particular inside a cell by *e.g.*, a lysosomal or endosomal protease to release the attached payload. In some aspects, the cleavable linkers are designed to release the free payload in an unmodified form. Cleavable linkers include, *e.g.,* disulfide linkers, acid labile linkers, photolabile linkers, peptidase labile linkers, and esterase labile linkers. Typically, a peptidyl linker is at least two amino acids long or at least three amino acids long.

[0295] Disulfide containing linkers are linkers cleavable through disulfide exchange, which can occur under physiological conditions. In other aspects, the linker is cleavable under reducing conditions (*e.g.*, a disulfide linker). A variety of disulfide linkers are known in the art, including, for example, those that can be formed using SATA (N-succinimidyl-5-acetylthioacetate), SPDP (N-succinimidyl-3-(2-pyridyldithio)propionate), SPDB (N-succinimidyl-3-(2-pyridyldithio)butyrate) and SMPT (N-succinimidyl-oxycarbonyl-alpha-methyl-alpha-(2-pyridyl-dithio)toluene).

[0296] Acid labile linkers are linkers cleavable at acidic pH (pH 4-5), in *e.g.*, certain intracellular compartments, such as endosomes and lysosomes, which provide conditions suitable for cleavage. Acid labile linkers are relatively stable under neutral pH conditions, such as those in the blood, but are unstable at below pH 5.5 or 5.0. Acid-labile linkers include, *e.g.,* hydrazone, semicarbazone, thiosemicarbazone, cis-aconitic amide, orthoester, acetal, ketal, and the like.

[0297] Photolabile linkers are useful at the body surface and in many body cavities that are accessible to light. Furthermore, infrared light can penetrate tissue.

[0298] Peptidase labile linkers can be used to cleave certain peptides inside or outside cells. In one aspect, the cleavable linker is cleaved under mild conditions, i.e., conditions within a cell under which the activity of the cytotoxic agent is not affected.

[0299] In some aspects, the bifunctional crosslinking reagent comprises a non-cleavable linker. A non-cleavable linker is any chemical moiety that is capable of linking a drug to the anti-CEACAM5 antibody, or antigen-binding fragment thereof, in a stable, covalent manner.

Preferably, a non-cleavable linker is not cleavable under physiological conditions, in particular inside a cell. Thus, non-cleavable linkers are substantially resistant to acid-induced cleavage, light-induced cleavage, peptidase-induced cleavage, esterase-induced cleavage, and disulfide bond cleavage, at conditions under which the drug or the antibody remains active. Suitable crosslinking reagents that form non-cleavable linkers between a drug and an antibody are known in the art. In one aspect, the drug is linked to the antibody through a thioether bond.

[0300] Depending on the linker design, membrane permeable (lipophilic) toxins that are released inside target positive cells can pass the cell membrane and kill other cells that are in close proximity, including neighboring cancer cells that lack antigen expression (bystander effect) (Kovtun, Y. V. et al. (2006) Cancer Res. 66 (6), 3214-3221). The ability of such cytotoxic drugs to mediate local bystander killing is one selection criterium for the ADCs according to the present disclosure.

[0301] Cleaving agents can include *e.g.,* cathepsins B and D and plasmin, all of which are known to hydrolyze dipeptide drug derivatives resulting in the release of active drug inside target cells. For example, a peptidyl linker that is cleavable by the thiol-dependent protease cathepsin-B, which is highly expressed in cancerous tissue, can be used (*e.g.*, a Phe-Leu or a Gly-Phe-Leu-Gly (SEQ ID NO: 101) linker). In specific aspects, the peptidyl linker cleavable by an intracellular protease is a valine-citrulline (Val-Cit) linker or a phenylalanine-lysine (Phe-Lys) linker. One advantage of using intracellular proteolytic release of the therapeutic agent is that the agent is typically attenuated when conjugated and the serum stabilities of the conjugates are typically high.

[0302] A variety of linkers may be used in the conjugates described herein. In some aspects, the linker comprises a peptidyl linker, such as dipeptide valine (Val)-citrulline (Cit) (vc), which can be cleaved by cathepsin inside tumor cells. Additional peptidyl linkers include, but are not limited to Val-Cit, Ala-Val, Val-Ala-Val, Lys-Lys, Pro-Val-Gly-Val-Val (SEQ ID NO: 99), Ala-Asn-Val, Val-Leu-Lys, Ala-Ala-Asn, Cit-Cit, Val-Lys, Lys, Cit, Ser, or Glu. The ADCs can be prepared as described in U.S. Pat. Nos. 7,087,600; 6,989,452; and 7,129,261; PCT Publication numbers WO 02/096910; WO 07/038658; WO 07/051081; WO 07/059404; WO 08/083312; and WO 08/103693; U.S. Patent Publication numbers 20060024317; 20060004081; and 20060247295; the disclosures of which are incorporated herein by reference.

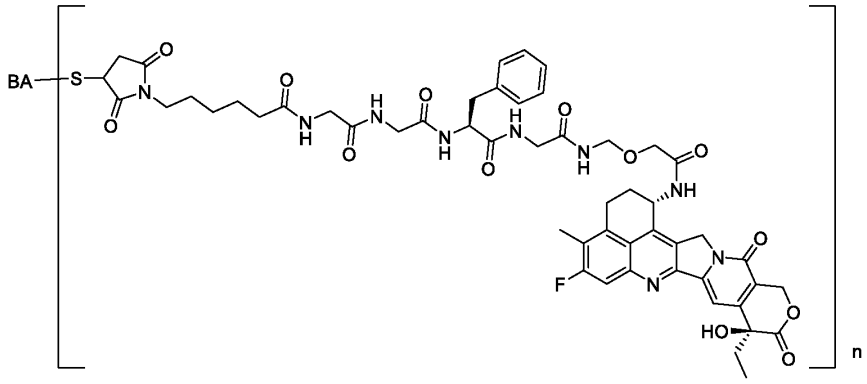
[0303] The number of cytotoxic agents linked to the antigen binding moiety of a CEACAM5- ADC (drug-to-antibody ratio: DAR) can vary and will be limited only by the number

of available attachments sites on the antigen binding moiety and the number of agents linked to a single linker.

[0304] The DAR value can vary with the nature of the antibody and the drug used along with the experimental conditions used for the conjugation (DAR, reaction time, nature of the solvents and/or cosolvents). Thus the contact between the antibody and the drug may lead to a mixture comprising several conjugates differing from one another by different drug-to-antibody ratios and may further include free antibodies and/or aggregates. The DAR that is determined is thus a mean value. DARs may be analyzed by UV spectrometry, monomer content may be analyzed by SEC-HPLC, and free drug content may be analyzed by RP-HPLC.

[0305] In some aspects, a linker will link a single cytotoxic moiety to the antigen binding moiety of an conjugate. In some aspects where the conjugate include more than one cytotoxic moiety, each agent may be the same or different. As long as the conjugate does not exhibit unacceptable levels of aggregation under the conditions of use and/or storage, conjugates with DARs of twenty, or even higher, are contemplated. In some aspects, the conjugates described herein may have a DAR in the range of about 1-10, 2-10, 1-8, 2-8, 1-6, 2-6, 1-4, or 2-4. In some specific aspects, the conjugate may have a DAR of 2, 3, 4 or 5. In some aspects, the DAR is 6. In some aspects, the DAR is 7. In some aspects, the DAR is 8. In some aspects, the DAR is 9. In some aspects, the DAR is 6 or 7. In some aspects, the DAR is 7, 7.5 or 8. In some aspects, the DAR is 7-8.

[0306] In some aspects, an ADC of the present disclosure comprises the following structure:



[ADC 101],

an isomer thereof (*e.g.*, a stereioisomer thereof,), a solvate thereof, or a pharmaceutically acceptable salt thereof, wherein n is from 1 to10, AB comprises the antibody, or antigen binding portion thereof. In some aspects, the n in the ADC is 7 or 8. In some aspects, the n in the ADC is 5 or 6. In some aspects, the n in the ADC is 9 or 10. In some aspects, the n in the ADC is 7. In some aspects, the n in the ADC is 8.

[0307] In some aspects, the disclosure provides an ADC comprising an antibody or antigen binding portion thereof and a cytotoxic moiety and having the structure of ADC 101, wherein the antibody, or antigen binding portion thereof, comprises a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 49 and the amino acid sequence set forth in SEQ ID NO: 50, respectively.

[0308] In some aspects, the disclosure provides an ADC comprising an antibody, or antigen binding portion thereof, and a cytotoxic moiety and having the structure of ADC 101, wherein wherein the antibody, or antigen binding portion thereof, comprises a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 67 and the amino acid sequence set forth in SEQ ID NO: 68, respectively.

[0309] In some aspects, the disclosure provides an ADC comprising an antibody, or antigen binding portion thereof, and a cytotoxic moiety and having the structure of ADC 101, wherein the antibody, or antigen binding portion thereof, comprises a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 69 and the amino acid sequence set forth in SEQ ID NO: 70, respectively.

[0310] In some aspects, the disclosure provides an ADC comprising an antibody, or antigen binding portion thereof, and a cytotoxic moiety and having the structure of ADC 101, wherein the antibody, or antigen binding portion thereof, comprises a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 71 and the amino acid sequence set forth in SEQ ID NO: 72; respectively.

[0311] In some aspects, the disclosure provides an ADC comprising an antibody, or antigen binding portion thereof, and a cytotoxic moiety and having the structure of ADC 101, wherein the antibody, or antigen binding portion thereof, comprises a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 73 and the amino acid sequence set forth in SEQ ID NO: 74; respectively

[0312] In some aspects, the disclosure provides an ADC comprising an antibody, or antigen binding portion thereof, and a cytotoxic moiety and having the structure of ADC 101, wherein the

antibody, or antigen binding portion thereof, comprises a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 75 and the amino acid sequence set forth in SEQ ID NO: 76; respectively.

[0313] In some aspects, the disclosure provides an ADC comprising an antibody, or antigen binding portion thereof, and a cytotoxic moiety and having the structure of ADC 101, wherein the antibody, or antigen binding portion thereof, comprises a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 77 and the amino acid sequence set forth in SEQ ID NO: 78; respectively.

[0314] In some aspects, the disclosure provides an ADC comprising an antibody, or antigen binding portion thereof, and a cytotoxic moiety and having the structure of ADC 101, wherein the antibody, or antigen binding portion thereof, comprises a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 79 and the amino acid sequence set forth in SEQ ID NO: 80; respectively.

[0315] In some aspects, the disclosure provides an ADC comprising an antibody, or antigen binding portion thereof, and a cytotoxic moiety and having the structure of ADC 101, wherein the antibody, or antigen binding portion thereof, comprises a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 81 and the amino acid sequence set forth in SEQ ID NO: 82; respectively.

[0316] In some aspects, the disclosure provides an ADC comprising an antibody, or antigen binding portion thereof, and a cytotoxic moiety and having the structure of ADC 101, wherein the antibody, or antigen binding portion thereof, comprises a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 83 and the amino acid sequence set forth in SEQ ID NO: 84; respectively.

[0317] In some aspects, the disclosure provides an ADC comprising an antibody, or antigen binding portion thereof, and a cytotoxic moiety and having the structure of ADC 101, wherein the antibody, or antigen binding portion thereof, comprises a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 85 and the amino acid sequence set forth in SEQ ID NO: 86; respectively.

[0318] In some aspects, the disclosure provies an ADC comprising an antibody, or antigen binding portion thereof, and a cytotoxic moiety and having the structure of ADC 101, wherein the antibody, or antigen binding portion thereof, comprises a VH and a VL which comprise the amino

acid sequence set forth in SEQ ID NO: 87 and the amino acid sequence set forth in SEQ ID NO: 88; respectively.

[0319] In some aspects, the disclosure provides an ADC comprising an antibody, or antigen binding portion thereof, and a cytotoxic moiety and having the structure of ADC 101, wherein the antibody, or antigen binding portion thereof, comprises a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 89 and the amino acid sequence set forth in SEQ ID NO: 90; respectively and wherein the cytotoxic moiety comprises Compound 101.

[0320] In some aspects, the disclosure provides an ADC comprising an antibody, or antigen binding portion thereof, and a cytotoxic moiety and having the structure of ADC 101, wherein the antibody, or antigen binding portion thereof, comprises a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 91 and the amino acid sequence set forth in SEQ ID NO: 92; respectively.

[0321] In some aspects, the disclosure provides an ADC comprising an antibody, or antigen binding portion thereof, and a cytotoxic moiety and having the structure of ADC 101, wherein the antibody, or antigen binding portion thereof, comprises a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 93 and the amino acid sequence set forth in SEQ ID NO: 94; respectively.

[0322] In some aspects, the disclosure provides an ADC comprising an antibody, or antigen binding portion thereof, and a cytotoxic moiety and having the structure of ADC 101, wherein the antibody, or antigen binding portion thereof, comprises a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 17 and the amino acid sequence set forth in SEQ ID NO: 22; respectively.

[0323] In some aspects, the heavy chain and the light chain comprise the amino acid sequence set forth in SEQ ID NO: 45 and the amino acid sequence set forth in SEQ ID NO: 46, respectively.

[0324] In some aspects, the anti-CEACAM5 antibody or antigen binding portion thereof is described in Table 16 and Table 17. In some aspects, the CDRs, VH, VL, heavy chain and/or light chain is described in Table 16 and Table 17.

[0325] Conjugates can also be used to modify a given biological response, where the drug moiety should not be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity (*e.g.*, lymphokines, tumor necrosis factor, IFNγ, growth factors).

[0326] Techniques for conjugating toxins or therapeutic moieties to antibodies are 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.), pp. 623-53 (Marcel Dekker, Inc. 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.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982).

[0327] In some aspects, conjugation methods result in linkages which are substantially (or nearly) non-immunogenic, *e.g.*, peptide- (i.e., amide-), sulfide-, (sterically hindered), disulfide-, hydrazone-, and ether linkages. These linkages are nearly non-immunogenic and show reasonable stability within serum (see *e.g.*, Senter, P. D., Curr. Opin. Chem. Biol. 13 (2009) 235-244; and PCT Publication numbers WO 2009/059278 and WO 95/17886).

[0328] Depending on the biochemical nature of the moiety and the antibody, different conjugation strategies can be employed. In cases where the moiety is naturally occurring or recombinant protein of between 50 to 500 amino acids, there are standard procedures in textbooks describing the chemistry for synthesis of protein conjugates (see *e.g.*, Hackenberger, C. P. R., and Schwarzer, D., Angew. Chem. Int. Ed. Engl. 47 (2008) 10030-10074). In one aspect the reaction of a maleinimido moiety with a cysteine residue within the antibody or the moiety is used. This is an especially suited coupling chemistry where *e.g.*, a Fab or Fab'-fragment of an antibody is used. Alternatively in one aspect coupling to the C-terminal end of the antibody or moiety is performed. C-terminal modification of a protein, *e.g.*, of a Fab-fragment can *e.g.*, be performed as described (Sunbul, M. and Yin, J., Org. Biomol. Chem. 7 (2009) 3361-3371).

[0329] In general, site specific reaction and covalent coupling is based on transforming a natural amino acid into an amino acid with a reactivity which is orthogonal to the reactivity of the other functional groups present. For example, a specific cysteine within a rare sequence context can be enzymatically converted in an aldehyde (see Frese, M. A., and Dierks, T., ChemBioChem. 10 (2009) 425-427). It is also possible to obtain a desired amino acid modification by utilizing the specific enzymatic reactivity of certain enzymes with a natural amino acid in a given sequence

context (see, *e.g.*, Taki, M. et al., Prot. Eng. Des. Sel. 17 (2004) 119-126; Gautier, A. *et al.* Chem. Biol. 15 (2008) 128-136; and Protease-catalyzed formation of C--N bonds is used by Bordusa, F., Highlights in Bioorganic Chemistry (2004) 389-403). Site specific reaction and covalent coupling can also be achieved by the selective reaction of terminal amino acids with appropriate modifying reagents. The reactivity of an N-terminal cysteine with benzonitriles (see Ren, H. *et al.*, Angew. Chem. Int. Ed. Engl. 48 (2009) 9658-9662) can be used to achieve a site-specific covalent coupling. Native chemical ligation can also rely on C-terminal cysteine residues (Taylor, E. Vogel; Imperiali, B, Nucleic Acids and Molecular Biology (2009), 22 (Protein Engineering), 65-96).

Immunoconjugates for CEACAM5 detection

[0330] In some aspects, the immunoconjugate comprises a detectable label for CEACAM5 detection and/or cancer diagnosis. The detectable labels may be attached to the anti-CEACAM5 antibodies, antigen binding portions thereof, or bispecific antibodies described herein according to the coupling methods described herein. The immunoconjugate detection agents of the present disclosure may be used for detecting CEACAM5 and/or diagnosing CEACAM5-associated cancers in accordance with the methods described herein.

[0331] For diagnostic purposes, detectable labels may include, for example, radioisotopes for whole body imaging, and radioisotopes, enzymes, fluorescent labels, and other suitable antibody tags for sample testing. The detectable labels can be any of the various types used currently in the field of *in vitro* diagnostics, including particulate labels including metal sols such as colloidal gold, isotopes such as I^{125} or Tc^{99} presented for instance with a peptidic chelating agent of the N_2S_2, N_3S or N_4 type, chromophores including fluorescent markers, luminescent markers, phosphorescent markers and the like, as well as enzyme labels that convert a given substrate to a detectable marker, and polynucleotide tags that are revealed following amplification such as by polymerase chain reaction. Suitable enzyme labels include horseradish peroxidase, alkaline phosphatase, and the like. For instance, the label can be the enzyme alkaline phosphatase, detected by measuring the presence or formation of chemiluminescence following conversion of 1,2 dioxetane substrates such as adamantyl methoxy phosphoryloxy phenyl dioxetane (AMPPD), disodium 3-(4-(methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)tricyclo{3.3.1.1 3,7}decan}-4-yl) phenyl phosphate (CSPD), as well as CDP and CDP-STAR® or other art-recognized luminescent substrates including, for example, chelates of suitable lanthanides, such as Terbium(III) and Europium(III). The detection means is determined by the chosen label. Appearance of the label

or its reaction products can be achieved using the naked eye, in the case where the label is particulate and accumulates at appropriate levels, or using instruments such as a spectrophotometer, a luminometer, a fluorimeter, and the like, all in accordance with standard practice.

IV. Engineered Cells

[0332] In some aspects, provided herein is an engineered cell, e.g., a chimeric antigen receptor (CAR) expressing cell, in which expreses an engineered polypeptide comprising: (1) a CEACAM5 binding domain, (2) a transmembrane domain, and (3) at least one cytoplasmic signaling domain. The CEACAM5 binding domain includes one or more CEACAM5 antigen binding portions including, but not limited to, CDRs, variable heavy domains, variable light domains, Fab regions, heavy chains, light chains, single-chain variable fragments (scFvs), CH1, CH2, and/or CH3 domains, Fc regions, fragments thereof, and combinations thereof. In certain aspects, the CEACAM5 antigen binding portion is an scFv that specifically binds to CEACAM5.

[0333] The transmembrane domain can be any transmembrane domain derived or obtained from any molecule. In some aspects, the transmembrane domain is fused to the CEACAM5 binding domain of the CAR. The transmembrane domain may be derived from either a natural or synthetic source. In some aspects, the transmembrane domain can be derived from any membrane-bound or transmembrane protein. In some aspects, the transmembrane (TM) domain is selected from a group including, but not limited to, the alpha, beta, or zeta chain of the T cell receptor, CD3-epsilon, CD3-zeta, CD4, CD5, CD8, CD9, CD16, CD22, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD134, CD137, or CD154.

[0334] The CAR also comprises at least one signaling domain, which can also be referred to as the intracellular signaling domain and/or the cytoplasmic co-stimulatory signaling domain of the CAR. The cytoplasmic signaling domain is responsible for activation of at least one of the normal effector functions of the T cell and is required for an efficient response of lymphocytes to an antigen. The term "effector function" refers to a specialized function of a cell. Effector function of a T cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines. Thus, the term "cytoplasmic costimulatory signaling domain" refers to the portion of a protein which transduces the effector function signal and directs the cell to perform a specialized function. While the entire intracellular signaling domain can be employed, in many cases it is not necessary to use the entire chain (i.e., the signaling domain can be derived from the entire protein).

To the extent that a truncated portion of the intracellular signaling domain is used, such truncated portion may be used in place of the intact chain as long as it transduces the effector function signal.

[0335] The intracellular signaling domain can be derived from and include any truncated portion of the intracellular signaling domain sufficient to transduce the effector function signal. In some aspects, the intracellular signaling domain is selected from the cytoplasmic sequences of the T cell receptor (TCR) and co-receptors that initiate signal transduction following antigen receptor engagement. In some aspects, the intracellular signaling domain comprises a domain derived from CD2, CD3-zeta, CD3-gamma, CD3-delta, CD3 -epsilon, CD5, CD7, CD22, CD27, CD28, CD30, CD40, CD66d, CD79a, CD79b, 4-1BB (CD137), 0X40, PD-l, ICOS, lymphocyte function-associated antigen-l (LFA-l), LIGHT, NKG2C, B7-H3, FcR-gamma, FcR-beta, TCR-zeta, or any combination thereof. In some aspects, the intracellular signaling domain is derived from CD3-zeta, CD28, and/or 4-1BB. In some aspects, the CAR comprises two or three cytoplasmic signaling domains, such as the cytoplasmic signaling domains are derived from CD28, CD3, and/or 4-1BB.

[0336] In some aspects, the provided herein are CAR-T cell-derived effector cells for use in patients with cancer. In some aspects, the CAR-T cell-derived effector cells are a population of activated T cells expressing a CAR engineered polypeptide comprising a CEACAM5 binding domain, a transmembrane domain, and at least one cytoplasmic signaling domain, as described herein.

[0337] T cells used in the methods disclosed herein can be isolated. Sources for the T cells include, but are not limited to, peripheral blood, umbilical cord blood, bone marrow, or other sources of hematopoietic cells. Methods for separating, enriching, and expanding the desired T cells can be employed. In some aspects, the T cells are expanded by culturing in the presence of IL-2. In other aspects, the T cells are expanded by culturing in the presence of anti-CD3 antibodies and/or anti-CD28 antibodies. In other aspects, the T cells are expanded by culturing in the presence of IL-2, and by culturing in the presence of anti-CD3 antibodies and/or anti-CD28 antibodies.

[0338] Procedures for separation of cells include, but are not limited to, density gradient centrifugation, coupling to particles that modify cell density, magnetic separation with antibody-coated magnetic beads, affinity chromatography; cytotoxic agents joined to or used in conjunction with a mAb, including, but not limited to, complement and cytotoxins, and panning with an antibody attached to a solid matrix, for example, a plate or chip, elutriation, flow cytometry, or any other convenient techniques.

[0339] The isolated T cells can be autologous or non-autologous to the subject to which they are administered in the methods of treatment disclosed herein. Autologous cells are isolated from the subject to which the population of activated T cells comprising the CAR are to be administered. In some aspects, autologous cells are isolated from the subject to which the isolated and expanded cells recombinantly expressing a CAR are to be administered. In some aspects, the cells can be obtained by leukapheresis, where leukocytes are selectively removed from withdrawn blood, made recombinant, and then re-transfused into the donor subject. Alternatively, allogeneic cells from a non-autologous donor that is not the subject can be used. In the case of a non-autologous donor, the cells are typed and matched for human leukocyte antigen (HLA) to determine an appropriate level of compatibility. For both autologous and non-autologous cells, the cells can optionally be cryopreserved until ready to be used for genetic manipulation and/or administration to a subject.

[0340] Because cytokine release is a necessary consequence of T cell activation and efficacy, for effective CAR-T cell-based therapy, it is preferred that at least a portion of the activated T cells produce one or more cytokines, such as one or more cytokines selected from the group consisting of IL-l , IL-2, TNF-α, and IFN-γ. Additionally, at least a portion of the population of activated T cells express one or more surface markers comprising CD2, CD28, CTLA4, CD40 ligand (gp39), CD18, CD25, CD69, CD16/CD56, MHC Class I, MHC Class II, CD8, CD4, CD3/TcR, CD54, LFA-l, VLA-4, or any combination thereof.

V. Antibody Engineering

[0341] The anti-CEACAM5 antibody, or antigen binding portion thereof, bispecific antibody, multispecific antibody, immunoconjugate, antibody drug conjugate (ADC), or engineered cells such as CAR as disclosed herein can be modified or engineered to improve their physical and functional properties.

Antibody engineering of the Fc region

[0342] The anti-CEACAM5 antibody, or antigen binding portion thereof or the bispecific antibody, multispecific antibody, immunoconjugate, antibody drug conjugate (ADC), or engineered cells such as CAR that comprises the anti-CEACAM5 antibody, or antigen binding portion thereof described herein may include modifications to their respective Fc regions, typically to alter one or more of their physical or functional properties, such as effector function (*e.g.*, antigen-dependent cellular cytotoxicity), Fc receptor binding, serum half-life, and complement

fixation). Furthermore, anti-CEACAM5 antibodies and antigen binding portions thereof disclosed herein can be chemically modified (*e.g.,* one or more chemical moieties can be attached to the antibody) or be modified to alter its glycosylation, again to alter one or more properties of the antibody or fragment. In the context of Fc region modifications, the numbering of residues in the Fc region is that of the EU index of Kabat.

[0343] The antibodies and antigen-binding fragments thereof disclosed herein also include antibodies and fragments with modified (or blocked) Fc regions to provide altered effector functions as described in e.g., U.S. Pat. No. 5,624,821; U.S. Patent Publication numbers US2009/280114 and US2011/142858; and PCT Publication Number WO2006/0057702. Such modifications can further include alterations to enhance or suppress various reactions of the immune system, with possible beneficial effects in diagnosis and therapy.

Altered effector functions

[0344] In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof comprises a variant Fc region that is modified (*e.g.*, by amino acid substitution, deletion and/or insertion) relative to a parent Fc sequence (*e.g.*, an unmodified Fc polypeptide that is subsequently modified to generate a variant) to increase or reduce the ability of the antibody or antigen-binding fragment to mediate one or more effector function(s) and/or to increase or decrease its binding to the Fc-gamma receptors (FcγRs), while retaining its antigen binding ability. Thus, in exemplary aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof may include one or more amino acid changes altering affinity for an effector ligand, such as an Fc receptor or the C1 component of complement. This approach is described in further detail in U.S. Patent Nos. 5,624,821 and 5,648,260.

[0345] The interaction between the constant region of an antigen binding protein (such as a anti-CEACAM5 antibody, or antigen binding portion thereof,) and various Fc receptors (FcR), including FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16), is believed to mediate the effector functions, such as ADCC and CDC, of the antigen binding protein. The Fc receptor is also important for antibody cross-linking, which can be important for anti-tumor immunity. In exemplary aspects, modifications can be made in the Fc region in order to generate an Fc variant promoting (a) increased or decreased antibody-dependent cell-mediated cytotoxicity (ADCC), (b) increased or decreased complement mediated cytotoxicity (CDC), (c) increased or decreased

affinity for C1q, (d) increased or decreased affinity for a Fc receptor relative to the parent Fc, and/or (e) increased or decreased pharmacokinetic stability.

[0346] Alterations of the Fc region may include amino acid changes, such as substitutions, deletions, insertions, glycosylation, deglycosylation, and/or addition of multiple Fc regions. Combining amino acid modifications may be particularly desirable. For example, the variant Fc region may include two, three, four, five, or more substitutions therein, *e.g.*, of the specific Fc region positions identified herein. In some aspects, the Fc region is altered by replacing at least one amino acid residue with a different amino acid residue such that the antibody has an altered affinity for an effector ligand but retains the antigen-binding ability of the parent antibody. For example, one or more amino acids selected from amino acid residues 234, 235, 236, 237, 297, 318, 320 and 322 can be replaced with a different amino acid residue. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in further detail in U.S. Patent Nos. 5,624,821 and 5,648,260, both by Winter *et al.* In some aspects, the C1q binding site may be removed from the Fc region by deleting or substituting, for example, the EKK sequence of human IgG1. In another example, one or more amino acids selected from amino acid residues 329, 331 and 322 can be replaced with a different amino acid residue such that the antibody has altered C1q binding and/or reduced or abolished complement dependent cytotoxicity (CDC). This approach is described in further detail in U.S. Patent Nos. 6,194,551 by Idusogie *et al.* In another example, one or more amino acid residues within amino acid positions 231 and 239 are altered to change the ability of the antibody to fix complement. This approach is described further in U.S. Patent No. 6,180,377.

[0347] In some aspects, provided herein are effector function-less versions of the anti-CEACAM5 antibodies or antigen binding portions thereof having *e.g.*, a mutant hIgG1f allotype (hIgG1.3f) comprising the amino acid sequence set forth in SEQ ID NO: 30. The hIgG1.3f variant is a triple mutant version of hIgG1f (L234A, L235E, G237A) which lacks FcγR binding and effector function.

[0348] In some aspects, the anti-CEACAM5 antibodies may be engineered to have different affinities and selectivities for Fc gamma receptors (FcγRs) by mutating the heavy chain constant region, including the hinge and Fc domains. Mutations can be introduced to either enhance or reduce FcγR binding. These mutations can increase or decrease FcγR-mediated cross-linking and/or signaling. For therapeutic targets, such as CEACAM5, FcγR-mediated cross-

linking of anti-CEACAM5 antibodies have the potential to provide undesirable agonist signaling and potential for toxicity absent the introduction of certain modification to obviate this problem.

[0349] Binding sites on human IgG1 for FcγR1, FcγRII, FcγRIII and FcRn have been mapped and variants with improved binding have been described (see Shields, R.L. *et al.* (2001) *J. Biol. Chem.* 276:6591-6604). Specific mutations at positions 256, 290, 298, 333, 334 and 339 were shown to improve binding to FcγRIII. Additionally, the following combination mutants were shown to improve FcγRIII binding: T256A/S298A, S298A/E333A, S298A/K224A and S298A/E333A/K334A, which has been shown to exhibit enhanced FcγRIIIa binding and ADCC activity (Shields *et al.*, 2001). Other IgG1 variants with strongly enhanced binding to FcγRIIIa have been identified, including variants with S239D/I332E and S239D/I332E/A330L mutations which showed the greatest increase in affinity for FcγRIIIa, a decrease in FcγRIIb binding, and strong cytotoxic activity in cynomolgus monkeys (Lazar *et al.*, 2006). Introduction of the triple mutations into antibodies such as alemtuzumab (CD52-specific), trastuzumab (HER2/neu-specific), rituximab (CD20-specific), and cetuximab (EGFR-specific) translated into greatly enhanced ADCC activity *in vitro*, and the S239D/I332E variant showed an enhanced capacity to deplete B cells in monkeys (Lazar *et al.*, 2006).

[0350] In some aspects, the anti-CEACAM5 antibodies may be engineered for reduced FcγR binding and potential for cross-linking and/or signaling, specifically, reduced engagement of the "low affinity" FcγRs hCD32a/FcγRIIa, hCD32b/FcγRIIb, hCD16a/FcγRIIIa, and hCD16b/FcγRIIIb. Engagement of the "high affinity" receptor CD64/FcγRI is generally believed to be of lower concern due to saturation of this receptor with serum IgG. Therefore, in some aspects, the anti-CEACAM5 antibodies may comprise an IgG1.3 Fc region, which is essentially devoid of binding to CD16, CD32a, CD32b and CD64 and lacks ADCC, ADCP and CDC functions (see U.S. Patent No. 10,077,306 and U.S. Patent Publication No. US2022/0106400).

[0351] In some aspects, the Fc region may be engineered for increased antibody dependent cellular cytotoxicity (ADCC) and/or increased FcγR binding by modifying one or more amino acids at the following positions: 234, 235, 236, 238, 239, 240, 241, 243, 244, 245, 247, 248, 249, 252, 254, 255, 256, 258, 262, 263, 264, 265, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 298, 299, 301, 303, 305, 307, 309, 312, 313, 315, 320, 322, 324, 325, 326, 327, 329, 330, 331, 332, 333, 334, 335, 337, 338, 340, 360, 373, 376, 378, 382, 388, 389, 398, 414, 416, 419, 430, 433, 434, 435, 436, 437, 438 or 439 (as described *e.g.*, in U.S. Patent No. 6,737,056) wherein the numbering of the residues in the Fc region is that of the EU index as in

Kabat. Exemplary substitutions include 236A, 239D, 239E, 268D, 267E, 268E, 268F, 324T, 332D, and 332E. Exemplary variants include 239D/332E, 236A/332E, 236A/239D/332E, 268F/324T, 267E/268F, 267E/324T, and 267E/268F/324T. Other modifications for enhancing FcyR and complement interactions include but are not limited to substitutions 298A, 333A, 334A, 326A, 247I, 339D, 339Q, 280H, 290S, 298D, 298V, 243L, 292P, 300L, 396L, 305I, and 396L. These and other modifications are reviewed in Strohl, 2009, Current Opinion in Biotechnology 20:685-691.

[0352] In some aspects, the Fc region is modified to decrease the ability of the anti-CEACAM5 antibody or antigen binding portion thereof described herein to mediate effector function and/or to increase anti-inflammatory properties by modifying residues 243 and 264. In one aspect, the Fc region of the anti-CEACAM5 antibody or antigen binding portion thereof is modified by changing the residues at positions 243 and 264 to alanine. In another aspect, the Fc region is modified to decrease the ability of the anti-CEACAM5 antibody or antigen binding portion thereof to mediate effector function and/or to increase anti-inflammatory properties by modifying residues 243, 264, 267 and 328.

[0353] Other Fc modifications to the Fc region include those for reducing or ablating binding to FcγRs and/or complement proteins, thereby reducing or ablating Fc-mediated effector functions, such as ADCC, ADCP, and CDC. Modifications for altering binding to FcyRIIb include one or more substitutions, insertions, and deletions at positions 234, 235, 236, 237, 239, 266, 267, 268, 269, 325, 326, 327, 328, and 332, wherein numbering is according to the EU index. In one aspect, the Fc variants provide selectively enhanced affinity to FcyRIIb relative to one or more activating receptors. Exemplary substitutions include but are not limited to 234G, 235G, 236R, 237K, 267R, 269R, 325L, and 328R. Other Fc variants for enhancing binding to FcyRIIb include 235Y/267E, 236D/267E, 236R/328R, 239D/268D, 239D/267E, 267E/268D, 267E/268E, and 267E/328F. Other modifications for reducing FcyR and complement interactions include substitutions 297A, 234A, 235A, 237A, 318A, 228P, 236E, 268Q, 309L, 330S, 331 S, 220S, 226S, 229S, 238S, 233P, and 234V, as well as removal of the glycosylation at position 297 by mutational or enzymatic means or by production in organisms such as bacteria that do not glycosylate proteins. These and other modifications are reviewed in Strohl, 2009, Current Opinion in Biotechnology 20:685-691.

[0354] In certain aspects, the Fc region may be modified to remove an ADCC site. ADCC sites can be found, for example, in Molec. Immunol. 29 (5): 633-9 (1992) with regard to ADCC

sites in IgG1. In addition, IgG1 mutants containing L235V, F243L, R292P, Y300L and P396L mutations were found to exhibit enhanced binding to FcγRIIIa and concomitantly enhanced ADCC activity in transgenic mice expressing human FcγRIIIa in models of B cell malignancies and breast cancer (Stavenhagen *et al.*, 2007; Nordstrom *et al.*, 2011). Other Fc mutants that may be used include: S298A/E333A/L334A, S239D/I332E, S239D/I332E/A330L, L235V/F243L/R292P/Y300L/ P396L, and M428L/N434S. Specific examples of variant Fc domains are disclosed for example, in U.S. Patent No. 6,096,871 and PCT Publication number WO 97/34631.

[0355] Optionally, the Fc region may comprise a non-naturally occurring amino acid residue at additional and/or alternative positions (see, *e.g.,* U.S. Pat. Nos. 5,624,821; 6,277,375; 6,737,056; 6,194,551; 7,317,091; 8,101,720; PCT Patent Publication numbers WO 00/42072; WO 01/58957; WO 02/06919; WO 04/016750; WO 04/029207; WO 04/035752; WO 04/074455; WO 04/099249; WO 04/063351; WO 05/070963; WO 05/040217, WO 05/092925 and WO 06/020114).

[0356] In one aspect, the hinge region of Fc is modified such that the number of cysteine residues in the hinge region is altered, *e.g.*, increased or decreased. For example, in one aspect, the number of cysteine residues in the hinge region of CH1 is increased to provide increased the stability of the antibody or decreased to provide enhanced assembly of the light and heavy chains or as described in U.S. Patent No. 5,677,425.

[0357] In some aspects, the, changes to the Fc region may be made to increase the biological half-life of the anti-CEACAM5-directed therapeutic antibodies and immunoconjugates so as to facilitate less frequent dosing, with the concomitant increase convenience and decreases use of material (Presta (2005) *J. Allergy Clin. Immunol.* 116:731 at 734-35). Various approaches may be employed. For example, in certain aspects, this may be achieved by increasing the binding affinity of the Fc region for the neonatal Fc receptor (FcRn). For example, one or more of more of following residues can be mutated: 252, 254, 256, 433, 435, 436, as described in U.S. Pat. No. 6,277,375. Specific exemplary substitutions include one or more of the following: T252L, T254S, and/or T256F. Alternatively, to increase the biological half-life, the antibody can be altered within the CH1 or CL region to contain a salvage receptor binding epitope taken from two loops of a CH2 domain of an Fc region of an IgG, as described in U.S. Patent Nos. 5,869,046 and 6,121,022 by Presta *et al.*

[0358] Other Fc variants for increased binding to FcRn and/or improved pharmacokinetic properties include substitutions at positions 259, 308, 428, and 434, including for example 259I, 308F, 428L, 428M, 434S, 434H, 434F, 434Y, and 434M. Other variants that increase Fc binding to FcRn include: 250E, 250Q, 428L, 428F, 250Q/428L (Hinton *et al.*, 2004, J. Biol. Chem. 279(8): 6213-6216, Hinton et al. 2006 Journal of Immunology 176:346-356), 256A, 272A, 286A, 305A, 307A, 307Q, 31 1A, 312A, 376A, 378Q, 380A, 382A, 434A (Shields et al, Journal of Biological Chemistry, 2001, 276(9):6591-6604), 252F, 252T, 252Y, 252W, 254T, 256S, 256R, 256Q, 256E, 256D, 256T, 309P, 31 1 S, 433R, 433S, 433I, 433P, 433Q, 434H, 434F, 434Y, 252Y/254T/256E, 433K/434F/436H, 308T/309P/311S (Dall'Acqua *et al.* Journal of Immunology, 2002, 169:5171-5180, Dall'Acqua *et al.*, 2006, Journal of Biological Chemistry 281:23514-23524). Other modifications for modulating FcRn binding are described in Yeung *et al.*, 2010, J Immunol, 182:7663-7671.

[0359] In another aspect, the Fc hinge region may be mutated to decrease the biological half-life of the antibody or fragment. For example, one or more amino acid mutations may be introduced into the CH2-CH3 domain interface region of the Fc hinge fragment such that the antibody or fragment has impaired Staphylococcal protein A (SpA) binding relative to native Fc-hinge domain SpA binding as described in U.S. Patent No. 6,165,745.

[0360] In certain aspects, hybrid IgG isotypes with particular biological characteristics may be used. For example, in certain aspects, one or more regions and/or mutations from an IgG2 or IgG4. In one aspect, the anti-CEACAM5 antibody or antigen binding portion thereof described herein is an IgG4 isotype antibody or fragment comprising a serine to proline mutation at a position corresponding to position 228 (S228P; EU index) in the hinge region of the heavy chain constant region. This mutation has been reported to abolish the heterogeneity of inter-heavy chain disulfide bridges in the hinge region (Angal *et al. supra*; position 241 is based on the Kabat numbering system). When using an IgG4 constant domain, it is usually preferable to include the substitution S228P, which mimics the hinge sequence in IgG1 and thereby stabilizes IgG4 molecules.

[0361] In another aspect, an IgG1/IgG3 hybrid variant may be constructed by substituting IgG1 positions in the CH2 and/or CH3 region with the amino acids from IgG3 at positions where the two isotypes differ. Thus, a hybrid variant IgG antibody may be constructed that comprises one or more substitutions, *e.g.*, 274Q, 276K, 300F, 339T, 356E, 358M, 384S, 392N, 397M, 4221, 435R, and 436F. In other aspects described herein, an IgG1/IgG2 hybrid variant may be constructed by substituting IgG2 positions in the CH2 and/or CH3 region with amino acids from

IgG1 at positions where the two isotypes differ. Thus, a hybrid variant IgG antibody may be constructed that comprises one or more substitutions, *e.g.*, one or more of the following amino acid substitutions: 233E, 234L, 235L, 236G (referring to an insertion of a glycine at position 236), and 327A.

[0362] In some aspects, the variant Fc region may also comprise a sequence alteration wherein amino acids involved in disulfide bond formation are removed or replaced with other amino acids. Such removal may avoid reaction with other cysteine-containing proteins present in the host cell used to produce the antibodies described herein. Even when cysteine residues are removed, single chain Fc domains can still form a dimeric Fc domain that is held together non-covalently. In other aspects, the Fc region may be modified to make it more compatible with a selected host cell. For example, one may remove the PA sequence near the N-terminus of a typical native Fc region, which may be recognized by a digestive enzyme in *E. coli* such as proline iminopeptidase.

[0363] The anti-CEACAM5 antibody or antigen binding portion thereof disclosed herein may contain one or more glycosylation sites. Such glycosylation sites may result in increased immunogenicity of the antibody or fragment or an alteration of the pK of the antibody due to altered antigen-binding (Marshall *et al.* (1972) *Annu Rev Biochem* 41:673-702; Gala and Morrison (2004) *J Immunol* 172:5489-94; Wallick *et al* (1988) *J Exp Med* 168:1099-109; Spiro (2002) *Glycobiology* 12:43R-56R; Parekh *et al* (1985) *Nature* 316:452-7; Mimura *et al.* (2000) *Mol Immunol* 37:697-706). Glycosylation has been known to occur at motifs containing an N-X-S/T sequence.

[0364] Therefore, in some aspects, the glycosylation properties of the anti-CEACAM5 antibody or antigen binding portion thereof described herein may be modified. For example, one or more glycosylation sites within the Fc domain may be modified or removed. Residues that are typically glycosylated (*e.g.*, asparagine) may confer a cytolytic response. Such residues may be deleted or substituted with unglycosylated residues (*e.g.*, alanine) to produce an aglycosylated antibody. In certain aspects, glycosylation can be altered to, for example, increase the affinity of the antibody for antigen. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region framework glycosylation sites to thereby eliminate glycosylation at that site. The resulting aglycosylation may increase the affinity of the antibody for antigen. Such an approach is described in further detail in U.S. Patent Nos. 5,714,350 and 6,350,861 by Co *et al.* Glycosylation of the constant region on N297 may be prevented by

mutating the N297 residue to another residue, *e.g.,* N297A, and/or by mutating an adjacent amino acid, *e.g.,* 298 to thereby reduce glycosylation on N297.

[0365] Additionally, or alternatively, an anti-CEACAM5 antibody or antigen binding portion thereof described herein can be engineered with an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNac structures. Non-fucosylated antibodies harbor a tri-mannosyl core structure of complex-type N-glycans of Fc without fucose residue. These glycoengineered antibodies that lack core fucose residue from the Fc N-glycans may exhibit stronger ADCC than fucosylated equivalents due to enhancement of FcγRIIIa binding capacity. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery.

[0366] Cells with altered glycosylation machinery can be used as host cells in which to express recombinant antibodies described herein to thereby produce an antibody with altered glycosylation. For example, EP 1,176,195 by Hanai *et al.* describes a cell line with a functionally disrupted FUT8 gene, which encodes a fucosyl transferase (i.e., alpha-1,6-fucosyltransferase), such that antibodies expressed in such a cell line exhibit hypofucosylation. Recombinant host cells which have been genetically modified to inactivate the FUT8 gene encoding an alpha-1,6-fucosyltransferase are available. See, *e.g.*, the POTELLIGENT™ technology system available from BioWa, Inc. (Princeton, N.J.) in which CHOK1SV cells lacking a functional copy of the FUT8 gene produce monoclonal antibodies having enhanced antibody dependent cell mediated cytotoxicity (ADCC) activity that is increased relative to an identical monoclonal antibody produced in a cell with a functional FUT8 gene. Aspects of the POTELLIGENT™ technology system are described in U.S. Patent Numbers 7,214,775 and 6,946,292, and PCT Publication numbers WO00/61739 and WO02/31240.

[0367] PCT Publication number WO 03/035835 by Presta describes a variant CHO cell line, Lec13 cells, with reduced ability to attach fucose to Asn(297)-linked carbohydrates, also resulting in hypofucosylation of antibodies expressed in that host cell (see also Shields, R.L. *et al.* (2002) J. Biol. Chem. 277:26733-26740). PCT Publication number WO 99/54342 by Umana *et al.* describes cell lines engineered to express glycoprotein-modifying glycosyl transferases (*e.g.*, beta(1,4)-N-acetylglucosaminyltransferase III (GnTIII)) such that antibodies expressed in the engineered cell lines exhibit increased bisecting GlcNac structures which results in increased ADCC activity of the antibodies (see also Umana *et al.* (1999) Nat. Biotech. 17:176-180).

[0368] Another modification of the antibodies described herein is pegylation. In some aspects, the anti-CEACAM5 antibody or antigen-binding fragment described herein is pegylated to, for example, increase the biological (*e.g.*, serum) half-life of the antibody. To pegylate an antibody, the antibody, or fragment thereof, typically is reacted with polyethylene glycol (PEG), such as a reactive ester or aldehyde derivative of PEG, under conditions in which one or more PEG groups become attached to the antibody or antibody fragment. Preferably, the pegylation is carried out via an acylation reaction or an alkylation reaction with a reactive PEG molecule (or an analogous reactive water-soluble polymer). As used herein, the term "polyethylene glycol" is intended to encompass any of the forms of PEG that have been used to derivatize other proteins, such as mono (C1-C10) alkoxy- or aryloxy-polyethylene glycol or polyethylene glycol-maleimide. In certain aspects, the antibody to be pegylated is an aglycosylated antibody. Methods for pegylating proteins can be applied to the antibodies described herein. See for example, European patent number EP 0 154 316 by Nishimura *et al.* and European patent number EP 0 401 384 by Ishikawa *et al.*

[0369] Effector functions can be measured in a number of ways including for example via binding of the FcγRIII to Natural Killer cells or via FcγRI to monocytes/macrophages to measure for ADCC effector function. For example, an antigen binding protein of the present invention can be assessed for ADCC effector function in a Natural Killer cell assay. Examples of such assays can be found in Shields *et al.*, 2001 *J. Biol. Chem.*, Vol. 276, p 6591-6604; Chappel *et al.*, 1993 J. Biol. Chem., Vol 268, p 25124-25131; Lazar *et al.*, 2006 PNAS, 103; 4005-4010.

[0370] The affinities and binding properties of an Fc region for its ligand may be determined by a variety of *in vitro* assay methods (biochemical or immunological based assays) including, but not limited to, equilibrium methods (*e.g.*, enzyme-linked immunosorbent assay (ELISA), or radioimmunoassay (RIA)), or kinetics (*e.g.*, BIACORE analysis), and other methods such as indirect binding assays, competitive inhibition assays, fluorescence resonance energy transfer (FRET), gel electrophoresis, and chromatography (*e.g.*, gel filtration). These and other methods may utilize a label on one or more of the components being examined and/or employ a variety of detection methods including but not limited to chromogenic, fluorescent, luminescent, or isotopic labels. A detailed description of binding affinities and kinetics can be found in Paul, W. E., ed., Fundamental Immunology, 4th Ed., Lippincott-Raven, Philadelphia (1999), which focuses on antibody-immunogen interactions.

[0371] With regard to the above-described modifications for increasing or decreasing one or more of the functional properties described herein (*e.g.*, biochemical, immunochemical, cellular, physiological or other biological activities, as determined using methods known to the art and described herein), the resulting increase in a given parameter may represent a statistically significant increase of at least 10% of the measured parameter, more preferably at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% (i.e., 2-fold), 3-fold, 5-fold or 10-fold. Conversely, the resulting decrease in a measured parameter may represent a statistically significant decrease of at least 10% of the measured parameter, e.g., at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100%, 3-fold, 5-fold or 10-fold.

[0372] Any of the above-described modifications may be employed alone or in combination with any of the above-described modifications or those described in the next section in order to further enhance or decrease effector functions or other desirable properties (e.g., stability, expression).

Antibody engineering of variable regions

[0373] In some aspects, the anti-CEACAM5 antibodies or antigen binding portions thereof are engineered with modifications to framework residues within the variable domains of the parental antibody, *e.g.*, to improve the properties of the antibody or antigen binding portion thereof. Typically, such framework modifications are made to decrease the immunogenicity of the anti-CEACAM5 antibodies or antigen binding portions thereof. This is usually accomplished by replacing non-CDR residues in the variable domains (*i.e.*, framework residues) in a parental (*e.g.*, rodent) antibody with analogous residues from the immune repertoire of the species in which the antibody is to be used, *e.g.*, human residues in the case of human therapeutics. Such an antibody is referred to as a "humanized" antibody . In some cases, it is desirable to increase the affinity, or alter the specificity of an engineered (*e.g.*, humanized) antibody. One approach is to "back-mutate" one or more framework residues to the corresponding germline sequence. More specifically, an antibody that has undergone somatic mutation can contain framework residues that differ from the germline sequence from which the antibody is derived. Such residues can be identified by comparing the antibody framework sequences to the germline sequences from which the antibody is derived. Another approach is to revert to the original parental (*e.g.*, rodent) residue at one or more positions of the engineered (*e.g.*, humanized) antibody, *e.g.*, to restore binding affinity that

may have been lost in the process of replacing the framework residues. (See, *e.g.*, U.S. Patent No. 5,693,762, U.S. Patent No. 5,585,089 and U.S. Patent No. 5,530,101.)

[0374] In certain aspects, the anti-CEACAM5 antibodies and antigen binding portions thereof are engineered (*e.g.,* humanized) to include modifications in the framework and/or CDRs to improve their properties. Such engineered changes can be based on molecular modeling. A molecular model for the variable region for the parental (non-human) antibody sequence can be constructed to understand the structural features of the antibody and used to identify potential regions on the antibody that can interact with the antigen. Conventional CDRs are based on alignment of immunoglobulin sequences and identifying variable regions. Kabat *et al.*, (1991) Sequences of Proteins of Immunological Interest, Kabat, *et al.*; National Institutes of Health, Bethesda, Md. ; 5th ed.; NIH Publ. No. 91-3242; Kabat (1978) *Adv. Prot. Chem.* 32:1-75; Kabat, *et al.*, (1977) *J. Biol. Chem.* 252:6609-6616. Chothia and coworkers carefully examined conformations of the loops in crystal structures of antibodies and proposed hypervariable loops. Chothia, *et al.*, (1987) *J Mol. Biol.* 196:901-917 or Chothia, *et al.*, (1989) *Nature* 342:878-883. There are variations between regions classified as "CDRs" and "hypervariable loops". Later studies (Raghunathan *et al.*, (2012) *J. Mol Recog.* 25, 3, 103-113) analyzed several antibody–antigen crystal complexes and observed that the antigen binding regions in antibodies do not necessarily conform strictly to the "CDR" residues or "hypervariable" loops. The molecular model for the variable region of the non-human antibody can be used to guide the selection of regions that can potentially bind to the antigen. In practice, the potential antigen binding regions based on model differ from the conventional "CDR"s or "hyper variable" loops. Commercial scientific software such as MOE (Chemical Computing Group) can be used for molecular modeling. Human frameworks can be selected based on best matches with the non-human sequence both in the frameworks and in the CDRs. For FR4 (framework 4) in VH, VJ regions for the human germlines are compared with the corresponding non-human region. In the case of FR4 (framework 4) in VL, J-kappa and J-Lambda regions of human germline sequences are compared with the corresponding non-human region. Once suitable human frameworks are identified, the CDRs are grafted into the selected human frameworks. In some cases, certain residues in the VL-VH interface can be retained as in the non-human (parental) sequence. Molecular models can also be used for identifying residues that can potentially alter the CDR conformations and hence binding to antigen. In some cases, these residues are retained as in the non-human (parental) sequence. Molecular models can also be used to identify solvent exposed amino acids that can result in unwanted effects

such as glycosylation, deamidation and oxidation. Developability filters can be introduced early on in the design stage to eliminate/minimize these potential problems.

[0375] Another type of framework modification involves mutating one or more residues within the framework region, or even within one or more CDR regions, to remove T cell epitopes to thereby reduce the potential immunogenicity of the antibody. This approach is also referred to as "deimmunization" and is described in further detail in U.S. Patent No. 7,125,689. In certain aspects, one or more glycosylation sites in either the light or heavy chain immunoglobulin variable regions, such as the framework regions, may be modified or removed to reduce immunogenicity. In particular aspects, it will be desirable to change certain amino acids containing exposed side-chains to another amino acid residue in order to provide for greater chemical stability of the final antibody, so as to avoid deamidation or isomerization. The deamidation of asparagine may occur on NG, DG, NG, NS, NA, NT, QG or QS sequences and result in the creation of an isoaspartic acid residue that introduces a kink into the polypeptide chain and decreases its stability (isoaspartic acid effect). Isomerization can occur at DG, DS, DA or DT sequences. In certain aspects, the antibodies provided herein do not contain deamidation or asparagine isomerism sites. For example, an asparagine (Asn) residue may be changed to Gln or Ala to reduce the potential for formation of isoaspartate at any Asn-Gly sequences, particularly within a CDR.

[0376] A similar problem may occur at an Asp-Gly sequence. Reissner and Aswad (2003) *Cell. Mol. Life Sci.* 60:1281. Isoaspartate formation may debilitate or completely abrogate binding of an antibody to its target antigen. *See*, Presta (2005) *J. Allergy Clin. Immunol.* 116:731 at 734.

[0377] In various aspect, the asparagine is changed to glutamine (Gln). It may also be desirable to alter an amino acid adjacent to an asparagine (Asn) or glutamine (Gln) residue to reduce the likelihood of deamidation, which occurs at greater rates when small amino acids occur adjacent to asparagine or glutamine. *See*, Bischoff & Kolbe (1994) *J. Chromatog.* 662:261. In addition, any methionine residues (typically solvent exposed Met) in CDRs may be changed to Lys, Leu, Ala, or Phe or other amino acids in order to reduce the possibility that the methionine sulfur would oxidize, which could reduce antigen-binding affinity and also contribute to molecular heterogeneity in the final antibody preparation. *Id.* Additionally, in order to prevent or minimize potential scissile Asn-Pro peptide bonds, it may be desirable to alter any Asn-Pro combinations found in a CDR to Gln-Pro, Ala-Pro, or Asn-Ala. Antibodies with such substitutions are subsequently screened to ensure that the substitutions do not decrease the affinity or specificity of

the antibody for CEACAM5, or other desired biological activity to unacceptable levels. See **Table 1** for exemplary stabilizing CDR variants.

Table 1. Exemplary stabilizing CDR variants

CDR Residue	Stabilizing Variant Sequence
Asn-Gly (N-G)	Gln-Gly, Ala-Gly, or Asn-Ala (Q-G), (A-G), or (N-A)
Asp-Gly (D-G)	Glu-Gly, Ala-Gly or Asp-Ala (E-G), (A-G), or (D-A)
Met (M)	Lys, Leu, Ala, or Phe (K), (L), (A), or (F)
Asn (N)	Gln or Ala (Q) or (A)
Asn-Pro (N-P)	Gln-Pro, Ala-Pro, or Asn-Ala (Q-P), (A-P), or (N-A)

VI. Nucleic Acid Molecules

[0378] Also provided herein are nucleic acids, or a set of nucleic acids, that encode the anti-CEACAM5 antibodies or antigen binding portions thereof. The nucleic acids include nucleotide sequences encoding heavy and/or light chain variable regions, antibody heavy or light chain sequences, antigen binding portions thereof, bispecific antibodies, or multispecific antibodies including sequence fragments thereof.

[0379] The nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. A nucleic acid described herein can be, for example, DNA or RNA and may or may not contain intronic sequences. In certain aspects, the nucleic acid is a cDNA molecule. The nucleic acids described herein can be obtained using standard molecular biology techniques. For antibodies expressed by hybridomas (*e.g.*, hybridomas prepared from transgenic mice carrying human immunoglobulin genes as described further below), cDNAs encoding the light and heavy chains of the antibody made by the hybridoma can be obtained by standard PCR amplification or cDNA cloning techniques.

[0380] In some aspects, provided herein are nucleic acid molecules that encode the VH and/or VL sequences, or heavy and/or light chain sequences, of any of the anti-CEACAM5 antibodies or antigen binding portions thereof. Host cells comprising the nucleotide sequences (*e.g.,* nucleic acid molecules) described herein are encompassed herein. Once DNA fragments

encoding VH and VL segments are obtained, these DNA fragments can be further manipulated by standard recombinant DNA techniques, for example to convert the variable region genes to full-length antibody chain genes, to Fab fragment genes or to a scFv gene. In these manipulations, a VL- or VH-encoding DNA fragment is operatively linked to another DNA fragment, such as an antibody constant region or a flexible linker, whereby the two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments remain in-frame.

[0381] An isolated DNA encoding the VH region can be converted to a full-length heavy chain gene by operatively linking the VH-encoding DNA to another DNA molecule encoding heavy chain constant regions (hinge, CH1, CH2 and/or CH3). Any sequences of human heavy chain constant region genes can be used for the purpose of the present disclosure (see *e.g.*, Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification.

[0382] An isolated DNA encoding the VL region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operatively linking the VL-encoding DNA to another DNA molecule encoding the light chain constant region, CL. Any sequences of human light chain constant region genes can be used for the present disclosure (see *e.g.*, Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The light chain constant region can be a kappa or lambda constant region.

[0383] In some aspects, the nucleic acid molecules with conservative substitutions do not alter the resulting amino acid sequence upon translation of the nucleic acid molecule. In other aspects, the CEACAM5 coding regions in the nucleic acid molecules are codon-optimized for improved expression.

[0384] In another aspect, provided herein are host cells transformed with the nucleic acids or expression vectors encoding the anti-CEACAM5 antibodies or antigen binding portions of the present disclosure. The host cells can be any eukaryotic or prokaryotic cell capable of expressing the CEACAM5 antibodies or antigen binding portions of the present disclosure, including immunoglobulin heavy and light chains thereof. The host cells may be used in methods for producing the CEACAM5 antibodies described herein as further described herein.

VII. Methods of production

[0385] The anti-CEACAM5 antibodies or antigen binding portion thereof provided herein can be prepared using a wide variety of techniques including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof.

[0386] Various methods for making monoclonal antibodies described herein are available in the art. For example, the monoclonal antibodies can be made using the hybridoma method first described by Kohler *et al.* , Nature, 256:495 (1975), or any later developments thereof, or by recombinant DNA methods (U.S. Pat. No. 4,816,567). For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al , Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed., 1988); Hammer-ling, *et al.* , in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981). Methods for producing and screening for specific antibodies using hybridoma technology are known in the art. In another example, antibodies useful in the methods and compositions described herein can also be generated using various art-recognized phage display methods, such as isolation from antibody phage libraries generated using the techniques described in McCafferty *et al.*, Nature, 348:552-554 (1990). Clackson *et al.* , Nature, 352:624-628 (1991) and Marks *et al.*, J. Mol. Biol, 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (*e.g.,* nM range) human antibodies by chain shuffling (Marks *et al.*, Bio/Technology, 10:779-783 (1992)), as well as combinatorial infection and in vivo recombination as a strategy for constructing very large phage libraries (Waterhouse *et al.*, Nuc. Acids. Res., 21 :2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

[0387] Human antibodies can be made by a variety of methods known in the art, including phage display methods using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Pat. Nos. 4,444,887 and 4,716,111; and PCT publication numbers WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741, the contents of which are herein incorporated by reference in their entireties. Human antibodies can also be produced using transgenic mice which express human immunoglobulin genes, and upon immunization are capable of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For an overview of this technology for producing human antibodies, see, Lonberg and Huszar, 1995, Int. Rev. Immunol. 13:65-93. Phage display

technology (McCafferty *et al.*, Nature 348:552-553 (1990)) also can be used to produce human antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. Human antibodies can also be generated by in vitro activated B cells (see U.S. Pat. Nos. 5,567,610 and 5,229,275, the contents of which are herein incorporated by reference in their entireties). Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, *e.g.*, a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope (Jespers *et al.*, 1994, Bio/technology 12:899-903).

[0388] Chimeric antibodies can be prepared based on the sequence of a murine monoclonal antibody. DNA encoding the heavy and light chain immunoglobulins can be obtained from the murine hybridoma of interest and engineered to contain non-murine (*e.g.*, human) immunoglobulin sequences using standard molecular biology techniques. For example, to create a chimeric antibody, the murine variable regions can be linked to human constant regions using methods known in the art (see *e.g.*, U.S. Patent No. 4,816,567 to Cabilly *et al.*).

[0389] Humanized forms of anti-CEACAM5 antibodies (*e.g.,* humanized forms of mouse anti-CEACAM5 antibodies) are chimeric antibodies which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies are typically human immunoglobulins (recipient antibody) in which residues from a CDR or hypervariable region of the recipient are replaced by residues from a CDR or hypervariable region of a non-human species (donor antibody), such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies can comprise residues which are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. 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 hypervariable loops 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 optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see 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).

[0390] The framework and CDR regions of a humanized antibody need not correspond precisely to the parental sequences, *e.g.*, the donor antibody CDR or the consensus framework can be mutagenized by substitution, insertion and/or deletion of at least one amino acid residue so that the CDR or framework residue at that site does not correspond exactly to either the donor antibody or the consensus framework. As used herein, the term "consensus framework" refers to the framework region in the consensus immunoglobulin sequence. As used herein, the term "consensus immunoglobulin sequence" refers to the sequence formed from the most frequently occurring amino acids (or nucleotides) in a family of related immunoglobulin sequences (see *e.g.*, Winnaker, From Genes to Clones (Veriagsgesellschaft, Weinheim, Germany 1987). In a family of immunoglobulins, each position in the consensus sequence is occupied by the amino acid occurring most frequently at that position in the family. Where two amino acids occur equally frequently, either can be included in the consensus sequence. As used herein, "Vernier zone" refers to a subset of framework residues that may adjust CDR structure and fine-tune the fit to antigen as described by Foote and Winter (1992, J. Mol. Biol. 224:487-499, which is incorporated herein by reference). Vernier zone residues form a layer underlying the CDRs and can impact on the structure of CDRs and the affinity of the antibody. Human immunoglobulin (Ig) sequences that can be used as a recipient are known in the art.

[0391] Framework residues in the human framework regions can be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods known in the art, *e.g.,* by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, *e.g.*, Queen *et al.*, U.S. Pat. No. 5,585,089; Riechmann *et al.*, Nature 332:323 (1988), which are incorporated herein by reference in their entireties.) Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most

substantially involved in influencing antigen binding. Antibodies can be humanized using a variety of techniques known in the art, including, but not limited to, those described in Jones *et al.*, Nature 321 :522 (1986); Verhoeyen *et al.*, Science 239: 1534 (1988), Sims et al., J. Immunol. 151 : 2296 (1993); Chothia and Lesk, J. Mol. Biol. 196:901 (1987), Carter *et al.*, Proc. Natl. Acad. Sci. U.S.A. 89:4285 (1992); Presta *et al.*, J. Immunol. 151 :2623 (1993), Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka *et al.*, Protein Engineering 7(6):805-814 (1994); Roguska. *et al.*, PNAS 91 :969-973 (1994); PCT publication number WO 91/09967, PCT/: US98/16280, US96/18978, US91/09630, US91/05939, US94/01234, GB89/01334, GB91/01134, GB92/01755; WO90/14443, WO90/14424, WO90/14430, EP 229246, EP 592,106; EP 519,596, EP 239,400, U.S. Pat. Nos. 5,565,332, 5,723,323, 5,976,862, 5,824,514, 5,817,483, 5,814,476, 5,763,192, 5,723,323, 5,766,886, 5,714,352, 6,204,023, 6, 180,370, 5,693,762, 5,530, 101, 5,585,089, 5,225,539; 4,816,567, each incorporated by reference herein.

[0392] In one aspect, the antibodies described herein are human monoclonal antibodies. Such human monoclonal antibodies directed against CEACAM5 can be generated using transgenic or transchromosomic mice carrying parts of the human immune system rather than the mouse system. These transgenic and transchromosomic mice include mice referred to herein as HuMAb mice and KM mice, respectively, and are collectively referred to herein as "human Ig mice."

[0393] The HuMAb mouse® (Medarex, Inc.) contains human immunoglobulin gene miniloci that encode unrearranged human heavy (μ and γ) and κ light chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous μ and κ chain loci (see *e.g.*, Lonberg, *et al.* (1994) Nature 368(6474): 856-859). Accordingly, the mice exhibit reduced expression of mouse IgM or κ, and in response to immunization, the introduced human heavy and light chain transgenes undergo class switching and somatic mutation to generate high affinity human IgGκ monoclonal (Lonberg, N. *et al.* (1994), *supra*; reviewed in Lonberg, N. (1994) *Handbook of Experimental Pharmacology* 113:49-101; Lonberg, N. and Huszar, D. (1995) *Intern. Rev. Immunol.* 13: 65-93, and Harding, F. and Lonberg, N. (1995) *Ann. N.Y. Acad. Sci.* 764:536-546). The preparation and use of HuMab mice, and the genomic modifications carried by such mice, is further described in Taylor, L. *et al.* (1992) *Nucleic Acids Research* 20:6287-6295; Chen, J. *et al.* (1993) *International Immunology* 5: 647-656; Tuaillon *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:3720-3724; Choi *et al.* (1993) *Nature Genetics* 4:117-123; Chen, J. *et al.* (1993) *EMBO J.* 12: 821-830; Tuaillon *et al.* (1994) *J. Immunol.* 152:2912-2920; Taylor, L. *et al.* (1994) *International Immunology* 6: 579-591; and Fishwild, D. *et al.* (1996) *Nature Biotechnology* 14:

845-851, the contents of all of which are hereby specifically incorporated by reference in their entirety. See further, U.S. Patent Nos. 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,789,650; 5,877,397; 5,661,016; 5,814,318; 5,874,299; and 5,770,429; all to Lonberg and Kay; U.S. Patent No. 5,545,807 to Surani *et al.*; PCT Publication Nos. WO 92/03918, WO 93/12227, WO 94/25585, WO 97/13852, WO 98/24884 and WO 99/45962, all to Lonberg and Kay; and PCT Publication No. WO 01/14424 to Korman *et al.*

[0394] In certain aspects, antibodies described herein are raised using a mouse that carries human immunoglobulin sequences on transgenes and transchomosomes, such as a mouse that carries a human heavy chain transgene and a human light chain transchromosome. Such mice, referred to herein as "KM mice", are described in detail in PCT Publication WO 02/43478 to Ishida *et al.*

[0395] Still further, alternative transgenic animal systems expressing human immunoglobulin genes are available in the art and can be used to raise anti-CEACAM5 antibodies described herein. For example, an alternative transgenic system referred to as the Xenomouse (Abgenix, Inc.) can be used; such mice are described in, for example, U.S. Patent Nos. 5,939,598; 6,075,181; 6,114,598; 6, 150,584 and 6,162,963 to Kucherlapati *et al.*

[0396] Moreover, alternative transchromosomic animal systems expressing human immunoglobulin genes are available in the art and can be used to raise anti-CEACAM5 antibodies described herein. For example, mice carrying both a human heavy chain transchromosome and a human light chain tranchromosome, referred to as "TC mice" can be used; such mice are described in Tomizuka *et al.* (2000) *Proc. Natl. Acad. Sci. USA* 97:722-727. Furthermore, cows carrying human heavy and light chain transchromosomes have been described in the art (Kuroiwa *et al.* (2002) *Nature Biotechnology* 20:889-894) and can be used to raise anti-CEACAM5 antibodies described herein.

[0397] Additional mouse systems described in the art for raising human antibodies, e.g., human anti-CEACAM5 antibodies, include (i) the VelocImmune® mouse (Regeneron Pharmaceuticals, Inc.), in which the endogenous mouse heavy and light chain variable regions have been replaced, via homologous recombination, with human heavy and light chain variable regions, operatively linked to the endogenous mouse constant regions, such that chimeric antibodies (human V/mouse C) are raised in the mice, and then subsequently converted to fully human antibodies using standard recombinant DNA techniques; and (ii) the MeMo® mouse (Merus Biopharmaceuticals, Inc.), in which the mouse contains unrearranged human heavy chain

variable regions but a single rearranged human common light chain variable region. Such mice, and methods for raising antibodies, are described in, for example, WO 2009/15777, US 2010/0069614, WO 2011/072204, WO 2011/097603, WO 2011/163311, WO 2011/163314, WO 2012/148873, US 2012/0070861 and US 2012/0073004.

[0398] In some aspects, the anti-CEACAM5 antibodies or antigen binding portions thereof, bispecific molecules (e.g., bispecific antibodies), and/or multispecific molecules (e.g., multispecific antibodies) are produced by culturing suitable host cells transformed with one or more nucleic acids or expression vectors encoding the CEACAM5 antibodies or antigen binding portions thereof, bispecific molecules (e.g., bispecific antibodies), and/or multispecific molecules (e.g., multispecific antibodies) described in the present disclosure under conditions allowing for small-scale or large-scale production and purification of the antibodies or antigen binding portions thereof, bispecific molecules, and/or multispecific molecules.

[0399] In some aspects, a method for producing antibodies comprises culturing a cell transiently or stably expressing one or more constructs encoding one or more polypeptide chains in the antibody; and purifying the antibodies produced from the cultured cells. Any cell capable of producing a functional antibody may be used.

[0400] In some aspects, cells are stably transformed with DNAs encoding partial or full-length light and heavy chains obtained by standard molecular biology techniques (*e.g.,* PCR amplification or cDNA cloning using a hybridoma that expresses the antibody of interest) inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences to facilitate their intended function of transcribing and translating the antibody gene(s) or antigen binding portions thereof. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain and heavy chain genes or fragments thereof can be inserted into the same or separate expression vectors by standard methods. In some aspects, the light and heavy chain variable regions of the antibodies or antigen binding portions thereof described herein can be used to create full-length antibody genes of any antibody isotype by inserting them into expression vectors already encoding heavy chain constant and light chain constant regions of the desired isotype such that the VH segment is operatively linked to the CH segment(s) within the vector and the VL segment is operatively linked to the CL segment within the vector. Additionally, the expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell, whereby the antibody chain genes or antigen binding portions thereof are cloned into the vector

such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene or antigen binding portion thereof. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (i.e., a signal peptide from a non-immunoglobulin protein).

[0401] Mammalian host cells for expressing the recombinant antibodies or antigen binding portions described herein include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin, (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, *e.g.*, as described in R. J. Kaufman and P. A. Sharp (1982) *Mol. Biol. 159*:601-621), NSO myeloma cells, COS cells and SP2 cells. In particular, for use with NSO myeloma cells, another preferred expression system is the GS gene expression system disclosed in WO 87/04462, WO 89/01036 and EP 338,841. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods. In other aspects, the cell is a yeast cell, an insect cell or a bacterial cell programmed to express the antibodies or antigen binding portions.

[0402] The antibodies in the present disclosure may be isolated from antibody expressing cells following culture and maintenance in any appropriate culture medium, such as RPMI, DMEM, and AIM V®. The antibodies can be purified using protein purification methodologies (*e.g.*, affinity purification, chromatography), including the use of Protein-A or Protein-G immunoaffinity purification. Typically, the antibodies are engineered for secretion into culture supernatants for isolation therefrom.

[0403] A anti-CEACAM5 antibody, antigen binding portion thereof, bispecific molecule, multispecific molecule, immunoconjugate, or antibody drug conjugate (ADC), as disclosed herein can be tested for desired properties, including particular binding specificities, binding affinities, targeted cell populations, described in the Examples, for example, protein-protein binding assays, biochemical screening assays, immunoassays, and/or cell-based assays. An aspect of the disclosure provides molecules that may be used to screen for antibodies or antigen binding portions thereof that bind CEACAM5. Exemplary assays include, but are not limited to, fluorescense-activated cell sorting (FACS), enzyme-linked immunoabsorbent assay (ELISA), surface plasmon resonance (SPR) analysis, bio-layer interferometry (*e.g.*, ForteBio assay), and Scatchard analysis.

[0404] In some aspects, the antibodies or antigen binding portions thereof are tested for specific binding to CEACAM5 (*e.g.,* human CEACAM5). Methods for analyzing binding affinity, cross-reactivity, and binding kinetics of various anti-CEACAM5 antibodies or antigen binding portions thereof include standard assays known in the art, for example, BIACORE™ surface plasmon resonance (SPR) analysis using a BIACORE™ 2000 SPR instrument (Biacore AB, Uppsala, Sweden) or bio-layer interferometry (*e.g.*, ForteBio assay), as described in the Examples.

[0405] In some aspects, functional assays may be employed. Antibodies or antigen binding portions thereof can also be tested for their ability to inhibit the proliferation or viability of cells (either *in vivo* or *in vitro*), such as tumor cells, using various methods (*e.g.*, ^{3}H-thymidine incorporation, immunohistochemistry with proliferation markers, animal cancer models). For example, the antibodies or antigen binding portions thereof can be tested for their anti-tumor activity *in vivo* (*e.g.*, as monotherapy or combination therapy), using in tumor xenograft models.

[0406] In some aspects, the functional activity of the CEACAM5 antibodies or antigen binding portions thereof are assayed for their ability to reduce CEACAM5 activation or function. In some aspects, the anti-CEACAM5 antibody, or antigen binding portion thereof, inhibits (or is determined to inhibit) CEACAM5 activation or function by, for example, 10% or more, for example, 20% or more, 30% or more, 40% or more, 50% or more, 60% or more, 70% or more, 80% or more, 90% or more, or 95% or more, relative to a control (e.g., a control antibody), as measured by ELISA.

[0407] Exemplary criteria for determining whether an anti-CEACAM5 antibody or antigen binding portion thereof exhibits certain properties (*e.g.*, binding, inhibition of activation, activation) are shown in **Table 2**.

Table 2.

Antibody Property	Positive
Binding to CEACAM5, as assessed by ELISA	≥2 SD above the mean of a negative control
Binding to CEACAM5, as assessed by flow cytometry	≥2 SD above the mean (median fluorescent intensity, MFI, on a homogeneous cell line or cell population) of a negative control
Binding to CEACAM5, as assessed by surface plasmon resonance, bio-layer interferometry)	≥100-fold difference in affinity relative to a negative control

VIII. Pharmaceutical Compositions

[0408] Also provided herein are pharmaceutical compositions comprising an anti-CEACAM5 antibody, antigen binding portion thereof, bispecific antibody, multispecific antibody, immunoconjugate, antibody drug conjugate (ADC), nucleic acids, expression vector, or engineered cells ("a CEACAM5 targeting agent") as disclosed herein and a carrier (*e.g.*, pharmaceutically acceptable carrier). Such compositions are useful for various therapeutic applications, such as cancer treatment.

[0409] In some aspects, the pharmaceutical compositions may further include other compounds, drugs, and/or agents for various therapeutic applications. Such compounds, drugs, and/or agents can include, for example, an anti-cancer agent, a chemotherapeutic agent, an immunosuppressive agent, an immunostimulatory agent, an immune checkpoint inhibitor, and/or an anti-inflammatory agent. Exemplary compounds, drugs, and agents that can be formulated together or separately with the CEACAM5 targeting agent described in the next section (*i.e.*, Section IX; Uses and Methods).

[0410] As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. In some aspects, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (*e.g.*, by injection or infusion). Depending on the route of administration, the active compound, *i.e.*, antibody, immunoconjugate, or bispecific molecule, may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

[0411] The pharmaceutical compounds described herein may include one or more pharmaceutically acceptable salts. A "pharmaceutically acceptable salt" refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired toxicological effects (see *e.g.*, Berge, S.M., *et al.* (1977) *J. Pharm. Sci.* 66:1-19). Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylenediamine, N-

methylglucamine, chloroprocaine, choline, diethanolamine, ethylenediamine, procaine and the like.

[0412] A pharmaceutical composition described herein may also include a pharmaceutically acceptable antioxidant. Examples of pharmaceutically acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

[0413] Examples of suitable aqueous and nonaqueous carriers that may be employed in the pharmaceutical compositions described herein include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures, *supra*, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

[0414] Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. Except insofar as any media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions described herein is contemplated. A pharmaceutical composition may comprise a preservative or may be devoid of a preservative. Supplementary active compounds can be incorporated into the compositions.

[0415] A composition described herein can be administered via one or more routes of administration using one or more of a variety of methods. The route and/or mode of administration can vary depending upon the desired results. Routes of administration for the CEACAM5 targeting agents described herein include *e.g.*, intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration

other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

[0416] Alternatively, a CEACAM5 targeting agent described herein can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically.

[0417] The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. *See, e.g., Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

IX. Uses and Methods

[0418] The CEACAM5 targeting agent described herein have numerous *in vitro* and *in vivo* utilities as described herein.

Cancer treatment

[0419] In one aspect, provided herein is a method of treating cancer comprising administering to a subject in need thereof a CEACAM5 targeting agent in an effective amount so that the growth of a cancerous tumor is inhibited or reduced and/or that regression and/or that prolonged survival is achieved. In some aspects, the CEACAM5 targeting agent is an anti-CEACAM5 antibody or antigen binding portion thereof. In some aspects, the CEACAM5 targeting agent is a bispecific antibody, multispecific antibody, antibody drug conjugate (ADC), chimeric antigen receptor (CAR), or CAR-T cell-derived effector cell, comprising as described herein an anti-CEACAM5 antibody or antigen binding portion thereof. In some aspects, the CEACAM5 targeting agent is an immune cell engager (ICE), such as a T cell engager molecule, or natural killer (NK) cell engager (NKCE) molecule, comprising an anti-CEACAM5 antibody or antigen binding portion thereof as described herein.

[0420] In some aspects, the CEACAM5 targeting agent described herein (e.g., an anti-CEACAM5 antibody or antigen binding portion thereof) may be administered in combination with additional cytotoxic or therapeutic agent(s), for example as described herein.

[0421] Cancers that express CEACAM5 whose growth may be inhibited using the anti-CEACAM5 antibodies described herein include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include, but are not limited to, basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and CNS cancer; breast cancer (*e.g.* estrogen-receptor positive breast cancer HER2-positive breast cancer; triple negative breast cancer); cancer of the peritoneum; cervical cancer; cholangiocarcinoma; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; liver cancer (*e.g.*, hepatocellular carcinoma; hepatoma); intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; lung cancer (*e.g.*, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); lymphoma including Hodgkin's and non-Hodgkin's lymphoma; melanoma; myeloma; neuroblastoma; oral cavity cancer (*e.g.*, lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; teratocarcinoma; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; as well as other carcinomas and sarcomas; as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia); chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblasts leukemia; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), tumors of primitive origins and Meigs' syndrome.

[0422] Additional cancers which express CEACAM5 and can be treated using the CEACAM5 targeting agent described herein include metastatic pancreatic cancer, metastatic adenocarcinoma of the pancreas, stomach cancer, fibrotic cancer, glioma, malignant glioma, diffuse intrinsic pontine glioma, recurrent childhood brain neoplasm renal cell carcinoma, clear-

cell metastatic renal cell carcinoma, metastatic castration resistant prostate cancer, stage IV prostate cancer, metastatic melanoma, malignant melanoma, recurrent melanoma of the skin, melanoma brain metastases, malignant melanoma of head and neck, squamous cell non-small cell lung cancer, metastatic breast cancer, follicular lymphoma, advanced B-cell NHL, HL including diffuse large B-cell lymphoma (DLBCL), multiple myeloma, chronic myeloid leukemia, adult acute myeloid leukemia in remission, adult acute myeloid leukemia with Inv(16)(p13.1q22), CBFB-MYH11, adult acute myeloid leukemia with t(16:16) (p13.1:q22), CBFB-MYH11, adult acute myeloid leukemia with t(8:21)(d22:q22), RUNX1-RUNX1T1, adult acute myeloid leukemia with t(9:11)(p22:q23), MLLT3-MLL, adult acute promyelocytic leukemia with tO15:17)(q22:q12), PML-RARA, alkylating agent-related acute myeloid leukemia, Richter's syndrome, adult glioblastoma, adult gliosarcoma, recurrent glioblastoma, recurrent childhood rhabdomyosarcoma, recurrent Ewing sarcoma/peripheral primitive neuroectodermal tumor, recurrent neuroblastoma, recurrent osteosarcoma, colorectal cancer, MSI positive colorectal cancer, MSI negative colorectal cancer, nasopharyngeal nonkeratinizing carcinoma, recurrent nasopharyngeal undifferentiated carcinoma, cervical adenocarcinoma, cervical adenosquamous carcinoma; cervical squamous cell carcinoma, recurrent cervical carcinoma, anal canal squamous cell carcinoma, metastatic anal canal carcinoma, recurrent anal canal carcinoma, recurrent head and neck cancer, squamous cell of head and neck, head and neck squamous cell carcinoma (HNSCC), ovarian carcinoma, colon cancer, advanced GI cancer, gastric adenocarcinoma, gastroesophageal junction adenocarcinoma, bone neoplasms, soft tissue sarcoma, bone sarcoma, thymic carcinoma, urothelial carcinoma, Merkel cell carcinoma, recurrent Merkel cell carcinoma, mycosis fungoides, Sezary syndrome, neuroendocrine cancer, nasopharyngeal cancer, basal cell skin cancer, squamous cell skin cancer, dermatofibrosarcoma trotuberans, glioma, mesothelioma, myelodysplastic syndromes (MDS), myelofibrosis (MF), myeloproliferative neoplasms, and acute myeloid leukemia (AML).

[0423] Cancers may be, *e.g.*, metastatic or primary cancers; desmoplastic or non-desmoplastic cancers; or recurrent cancers.

[0424] In some aspects, the cancer is associated with fibrosis. In some aspects, the cancer is associated with infiltration of CD4+ regulatory T cells. In some aspects, the cancer is associated with infiltration of CD8+ regulatory T cells. In some aspects, the cancer is associate with infiltration of regulatory B cells. In some aspects, the cancer is associated with infiltration of myeloid-derived suppressor cells. In some aspects, the cancer is associated with infiltration of

tumor-associated macrophages. In some aspects, the cancer is associated with infiltration of innate lymphoid cells. In some aspects, the cancer is associated with infiltration of cancer-associated fibroblasts. In some aspects, the cancer is associated with a radiation-related increase in the above cell types.

[0425] In some aspects, the anti-CEACAM5 antibodies, antigen-binding fragments, or ADCs described herein are used to treat myelodysplastic syndromes (MDSs). MDSs are a diverse group of malignant disorders marked by bone marrow failure due to defective hematopoiesis and production of dysplastic cells. TGF-β is a primary driver in MDS (Geyh *et al.*, Haematologica 2018;103:1462-71) and agents that inhibit the function of TGF-β have been proposed as therapeutics (Mies *et al.*, Curr Hematol Malig Rep 2016;11:416-24). Furthermore, MDSCs are known to be dysregulated in MDS (Chen *et al.*, JCI 2013;123:4595-611) and agents that reduce MDSC levels in the bone marrow are potential therapeutics.

[0426] In some aspects, the cancer is resistant to checkpoint inhibitor(s). In some aspects, the cancer is intrinsically refractory or resistant (*e.g.*, resistant to a PD-1 pathway inhibitor, PD-1 pathway inhibitor, or CTLA-4 pathway inhibitor). In some aspects, the resistance or refractory state of the cancer is acquired. In some aspects, the CEACAM5 targeting agent described herein can be used in combination with checkpoint inhibitors to overcome resistance of the cancer to the checkpoint inhibitors. In some aspects, the CEACAM5 targeting agent described herein can be used to treat tumors with a mesenchymal and/or EMT signature together with checkpoint inhibitors in combination or sequentially with agents that induce a mesenchymal phenotype, such as MAPK pathway inhibitors.

[0427] In some aspects, the CEACAM5 targeting agent described herein are used to enhance the viability of immune cells *ex vivo*, *e.g.*, in adoptive NK cell transfer. Accordingly, in some aspects, CEACAM5 targeting agents are used in combination with adoptively transferred NK cells to treat cancer. In some aspects, the CEACAM5 targeting agent described herein are used to treat tumors with MHC loss or MHC down-regulation, as monotherapy or in combination with NK activating or enhancing treatment.

Combination therapy

[0428] The CEACAM5 targeting agents described herein can be used in combination with various treatments or agents (or in the context of a multispecific antibody or bifunctional partner) known in the art for the treatment of disease or condition, as described herein.

[0429] In some aspects, a method of treating cancer comprises administering to a subject in need thereof an effective amount of a CEACAM5 targeting agent described herein in combination with another therapeutic agent, such as a second antibody, a therapeutic protein or a small molecule drug. In some aspects, the therapeutic protein is a checkpoint inhibitor. In some aspects, the small molecule drug is a chemotherapeutic agent as described herein. In some aspects, the another therapeutic agent comprises an anti-cancer agent.

[0430] Suitable anti-cancer agents for use in combination therapy with the CEACAM5 targeting agent described herein include, but are not limited to, surgery, chemotherapeutic agents, growth inhibitory agents, cytotoxic agents, radiotherapy and agents used in radiation therapy, anti-angiogenesis agents, apoptotic agents, anti-tubulin agents, and other agents to treat cancer, such as anti-HER-2 antibodies (*e.g.*, HERCEPTIN®), anti-CD20 antibodies, an epidermal growth factor receptor (EGFR) antagonist (*e.g.*, a tyrosine kinase inhibitor), HER1/EGFR inhibitor (*e.g.,* erlotinib (TARCEVA®)), platelet derived growth factor inhibitors (*e.g.,* GLEEVEC (Imatinib Mesylate)), COX-2 inhibitors (*e.g.*, celecoxib), interferons, and cytokines; antagonists (*e.g.*, neutralizing antibodies) that bind to and/or neutralize the activity of one or more of the following targets: PD-l, PD-L1, PD-L2 (*e.g.,* pembrolizumab; nivolumab; MK-3475; AMP-224; MPDL3280A; MEDI0680; MSB0010718C; and/or MEDI4736); CTLA4 (*e.g.*, tremelimumab (PFIZER) and ipilimumab); LAG3 (*e.g.,* BMS- 986016); CD 103; TIM-3 and/or other TIM family members; anti-VEGF antibodies (*e.g.*, Bevacizumab); CEACAM1, CEACAM6, and/or other CEACAM family members; ErbB2, ErbB3, ErbB4, PDGFR-beta, BlyS, APRIL, BCMA or VEGF receptor(s), TRAIL/Apo2, PARP inhibitors (*e.g.,* AZD-2281, Lynparza OCEACAM5arib, Rubraca Rucaparib; (Zejula) niraparib), DNA damage repair inhibitors (*e.g.*, ATMi, ATRi, DNAPKi), and other bioactive and organic chemical agents, including those described in section VII. Combinations thereof are also specifically contemplated for the methods described herein.

[0431] In some aspects, the anti-CEACAM5 antibody or ADC is administered with an anti-cancer agent, such as an EGFR inhibitor; a HER2 inhibitor; a histone deacetylase inhibitor; a hormone; a mitotic inhibitor; a phosphatidylinositol-3-kinase (PI3K) inhibitor; an Akt inhibitor; a mammalian target of rapamycin (mTOR) inhibitor; a proteasomal inhibitor; a poly(ADP-ribose) polymerase (PARP) inhibitor; a Ras/MAPK pathway inhibitor; a centrosome declustering agent; a multi-kinase inhibitor; a serine/threonine kinase inhibitor; a tyrosine kinase inhibitor; a VEGF/VEGFR inhibitor; a microtubule targeting drug; a topoisomerase poison drug; or a combination thereof.

[0432] In some aspects, the anti-CEACAM5 ADC is administered along with an immune checkpoint inhibitor. Exemplary immune checkpoint inhibitors include, but are not limited to, agents (*e.g.,* antibodies) that bind to PD-1, PD-L1, PD-L2, LAG-3, CTLA4, TIGIT, ICOS, OX40, PVR, PVRIG, VISTA, TIM3, SIRPα, ILT2, ILT3, ILT4, or ILT5.

[0433] Any anti-PD-1 antibody can be used in combination with the CEACAM5 targeting agent in the presently described methods. Various human monoclonal antibodies that bind specifically to PD-1 with high affinity have been disclosed in U.S. Patent No. 8,008,449.

[0434] In some aspects, the anti-PD-1 antibody is pembrolizumab, nivolumab, cemiplimab, spartalizumab, camrelizumab, sintilimab, tislelizumab, toripalimab, dostarlimab, retifanlimab, pimivalimab, dostarlimab, serplulimab, zimberelimab, acrixolimab, MEDI-0680, AM-0001, STI-1110, AGEN2034, BCD-100, sasanlimab, BI 754091, or SSI-361.

[0435] In some aspects, the anti-PD-1 antibody used in combination with the CEACAM5 targeting agent comprises the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, CDRL3, the VH and the VL, and/or the heavy and light chains of any of pembrolizumab, nivolumab, cemiplimab, spartalizumab, camrelizumab, sintilimab, tislelizumab, toripalimab, dostarlimab, retifanlimab, pimivalimab, MEDI-0680, GLS-010, AM-0001, STI-1110, AGEN2034, BCD-100, sasanlimab, BI 754091, or SSI-361.

[0436] In some aspects, the anti-PD-1 antibody used in combination with the CEACAM5 targeting agent is selected from the group consisting of nivolumab (OPDIVO®; formerly designated 5C4, BMS-936558, MDX-1106, or ONO-4538), pembrolizumab (KEYTRUDA®; formerly designated lambrolizumab and MK-3475; *see* WO 2008/156712A1), PDR001 (*see* WO 2015/112900), MEDI-0680 (formerly designated AMP-514; *see* WO 2012/145493), REGN-2810 *see* WO 2015/112800), JS001 (*see* Liu and Wu, 2017), BGB-A317 (*see* WO 2015/035606 and US 2015/0079109), INCSHR1210 (SHR-1210; *see* WO 2015/085847; Liu and Wu, 2017), TSR-042 (ANB011; *see* WO 2014/179664), GLS-010 (WBP3055; *see* Liu and Wu, 2017), AM-0001 (*see* WO 2017/123557), STI-1110 (*see* WO 2014/194302), AGEN2034 (*see* WO 2017/040790), and MGD013 (*see* WO 2017/106061).

[0437] In some aspects, the anti-PD-1 antibody used in combination with the CEACAM5 targeting agent is pembrolizumab (Merck; also known as KEYTRUDA®, lambrolizumab, and MK-3475; *see*, for example, WO 2008/156712). Pembrolizumab is a humanized monoclonal IgG4 (S228P) antibody directed against human cell surface receptor PD-1 (programmed death-1 or

programmed cell death-1). Pembrolizumab is described, for example, in U.S. Patent Nos. 8,354,509 and 8,900,587.

[0438] In some aspects, the anti-PD-1 antibody used in combination with the CEACAM5 targeting agent comprises nivolumab (also known as OPDIVO®, 5C4, BMS-936558, MDX-1106, and ONO-4538). Nivolumab is a fully human IgG4 (S228P) PD-1 immune checkpoint inhibitor antibody that selectively prevents interaction with PD-1 ligands (PD-L1 and PD-L2), thereby blocking the down-regulation of antitumor T-cell functions (*see*, for example, U.S. Patent No. 8,008,449; Wang et al., 2014 Cancer Immunol Res. 2(9):846-56).

[0439] In some aspects, the anti-PD-1 antibody used in combination with the CEACAM5 targeting agent is cemiplimab (Regeneron; also known as LIBTAYO or REGN-2810; *see*, for example, WO 2015/112800 and U.S. Patent No. 9,987,500).

[0440] In some aspects, the anti-PD-1 antibody used in combination with the CEACAM5 targeting agent is spartalizumab (Novartis; also known as PDR001; *see*, for example, WO 2015/112900 and U.S. Patent No. 9,683,048).

[0441] In some aspects, the anti-PD-1 antibody used in combination with the CEACAM5 targeting agent is camrelizumab (Jiangsu Hengrui Medicine; also known as SHR-1210 or INCSHR1210; *see*, for example, WO 2015/085847; Si-Yang Liu et al., J. Hematol. Oncol. 10:136 (2017)).

[0442] In some aspects, the anti-PD-1 antibody used in combination with the CEACAM5 targeting agent is MEDI-0680 (AstraZeneca; also known as AMP-514; *see*, for example, WO 2012/145493). In some aspects, the anti-PD-1 antibody is pimivalimab (also known as JTX-4014; *see*, for example, Papadopoulos, et al., 2022, IOTECH, Vol. 16, Supplement 1, 100284). In some aspects, the anti-PD-1 antibody is toripalimab (TAIZHOU JUNSHI PHARMA; also known as JS001; *see*, for example, Si-Yang Liu et al., J. Hematol. Oncol. 10:136 (2017)). In some aspects, the anti-PD-1 antibody is tislelizumab (Beigene; also known as BGB-A317; *see*, for example, WO 2015/35606 and US 2015/0079109). In some aspects, the anti-PD-1 antibody is dostarlimab (Tesaro Biopharmaceutical; also known as ANB011 or TSR-042; *see*, for example, WO2014/179664). In some aspects, the anti-PD-1 antibody is GLS-010 (Wuxi/Harbin Gloria Pharmaceuticals; also known as WBP3055; *see*, for example, Si-Yang Liu et al., J. Hematol. Oncol. 10:136 (2017)). In some aspects, the anti-PD-1 antibody is AM-0001 (Armo BioSciences).

[0443] In some aspects, the anti-PD-1 antibody is STI-1110 (Sorrento Therapeutics; *see*, for example, WO 2014/194302). In some aspects, the anti-PD-1 antibody is AGEN2034 (Agenus;

see, for example, WO 2017/040790). In some aspects, the anti-PD-1 antibody is retifanlimab (Macrogenics, also known as MGA012, AEX-1188, and INCMGA-00012; *see*, for example, WO 2017/19846). In some aspects, the anti-PD-1 antibody is BCD-100 (Biocad; *see*, for example, Kaplon et al., mAbs 10(2):183-203 (2018). In some aspects, the anti-PD-1 antibody is sintilimab (Innovent; also known as IBI308; *see*, for example, WO 2017/024465, WO 2017/025016, WO 2017/132825, and WO 2017/133540). In some aspects, the anti-PD-1 antibody is sasanlimab (Pfizer; also known as PF-06801591; *see*, for example, US 2016/0159905). In some aspects, the anti-PD-1 antibody is BI 754091 (Boehringer Ingelheim; *see*, for example, Zettl M et al., Cancer. Res. (2018);78(13 Suppl):Abstract 4558). In some aspects, the anti-PD-1 antibody is SSI-361 (Lyvgen Biopharma Holdings Limited, *see*, for example, US 2018/0346569).

[0444] Other anti-PD-1 monoclonal antibodies suitable for the methods of the present disclosure have been described in, for example, U.S. Patent Nos. 6,808,710, 7,488,802, 8,168,757, 8,354,509, and 9,205,148, US Publication No. 2016/0272708, and PCT Publication Nos. WO 2012/145493, WO 2008/156712, WO 2015/112900, WO 2012/145493, WO 2015/112800, WO 2014/206107, WO 2015/35606, WO 2015/085847, WO 2014/179664, WO 2017/020291, WO 2017/020858, WO 2016/197367, WO 2017/024515, WO 2017/025051, WO 2017/123557, WO 2016/106159, WO 2014/194302, WO 2017/040790, WO 2017/133540, WO 2017/132827, WO 2017/024465, WO 2017/025016, WO 2017/106061, WO 2017/19846, WO 2017/024465, WO 2017/025016, WO 2017/132825, and WO 2017/133540 each of which is incorporated by reference in its entirety.

[0445] Examples of anti-PD-L1 antibodies useful in combination with the CEACAM5 targeting agent according to the methods of the present disclosure include the antibodies disclosed in US Patent No. 9,580,507. In some aspects, the anti-PD-L1 antibody is atezolizumab, durvalumab, avelumab, envafolimab, cosibelimab, BMS-936559, STI-1014, CX-072, LY3300054, FAZ053, CS-1001, SHR-1316, CBT-502, KN035, or BGB-A333.

[0446] In some aspects, the anti-PD-L1 antibody is BMS-936559 (also known as 12A4, MDX-1105; *see*, *e.g.,* U.S. Patent No. 7,943,743 and WO 2013/173223).

[0447] In some aspects, the anti-PD-L1 antibody is STI-1014 (Sorrento; *see*, for example, WO 2013/181634). STI-104 is designated H6 in U.S. Patent No. 9,175,082. In some aspects, the anti-PD-L1 antibody is CX-072 (Cytomx; *see*, for example, WO 2016/149201). In some aspects, the anti-PD-L1 antibody is LY3300054 (Eli Lilly Co.; *see*, *e.g.*, WO 2017/034916). In some aspects, the anti-PD-L1 antibody is FAZ053 (Novartis). In some aspects, the anti-PD-L1 antibody

is CK-301 (Checkpoint Therapeutics; *see*, for example, Gorelik et al., AACR:Abstract 4606 (Apr 2016)). CK-301 is also referred to as cosibelimab. In some aspects, the anti-PD-L1 antibody is CS-1001. *See*, for example, Zhou et al., Journal of Clinical Oncology, Meeting Abstract, 2020 ASCO Annual Meeting I, Lung Cancer – Non-Small Cell Metastatic, e21687, and Zhang et al., Cancer Research, 2020, 80 (16_Supplement): 3260. In some aspects, the anti-PD-L1 antibody is SHR-1316. *See*, for example, Mu et al., Thorac Cancer, 2021 May;12(9):1373-1381, and Wu et al., Anals of Oncology, Abstract, Vol. 33, Supplement 2, S72, April 2022. In some aspects, the anti-PD-L1 antibody is CBT-502 (also known as TQB2450; *see*, for example, Wei et al., Mol Cancer Ther (2018) 17 (1_Supplement): A200). In some aspects, the anti-PD-L1 antibody is KN035 (3D Med/Alphamab; also referred to as envafolimab; *see*, for example, Zhang et al., Cell Discov. *7*:3 (March 2017) and Shimizu et al., Invest New Drugs, 2022 Oct;40(5):1021-1031).

[0448] In some aspects, the anti-PD-L1 antibody is BGB-A333 (BeiGene; *see*, for example, Desai et al., JCO 36 (15suppl):TPS3113 (2018) and Desai et al., 2023, British Journal of Cancer 128, 1418–1428). In certain aspects, the PD-L1 antibody is atezolizumab. Atezolizumab is a fully humanized IgG1 monoclonal anti-PD-L1 antibody. Atezolizumab (Roche) is also known as TECENTRIQ®; MPDL3280A, RG7446. *See*, for example, US 8,217,149 and Herbst et al. (2013) *J.* Clin. Oncol. 31 (suppl):3000). Atezolizumab is designated YW243.55S70 in U.S. Patent No. 8,217,149. In certain aspects, the PD-L1 antibody is durvalumab. Durvalumab is a human IgG1 kappa monoclonal anti-PD-L1 antibody. Durvalumab (AstraZeneca) is also known as IMFINZI® or MEDI-4736. Durvalumab is designated 2.14H9OPT in U.S. Patent No. 8,779,108. *See*, for example, WO 2011/066389. In certain aspects, the PD-L1 antibody is avelumab. Avelumab is a human IgG1 lambda monoclonal anti-PD-L1 antibody. Avelumab (Pfizer) is also known as BAVENCIO® or MSB-0010718C. Avelumab is designated A09-246-2 in U.S. Patent No. 9,624,298. *See*, for example, WO 2013/079174.

[0449] In some aspects, the anti-CTLA-4 antibody useful in combination with the CEACAM5 targeting agent is tremelimumab, ipilimumab, botensilimab, BMS-986218, BMS-986288, BMS-986249, IBI310, MK-1308 (quavonlimab), AGEN-1884 (zalifrelimab), ONC-392, ADG116, or CS1002.

[0450] In some aspects, the anti-CTLA-4 antibody useful in combination with the CEACAM5 targeting agent is MK-1308. MK-1308 is also known as quavonlimab. *See*, for example, Perets et al. 2021, Ann Oncol 32(3):395-403.

[0451] In some aspects, the anti-CTLA-4 antibody useful in combination with the CEACAM5 targeting agent is AGEN-1884. AGEN-1884 is also known as zalifrelimab. *See*, for example, WO 2016/196237.

[0452] In some aspects, the anti-CTLA-4 antibody useful in combination with the CEACAM5 targeting agent is tremelimumab. Tremelimumab, sold under the brand name IMJUDO®, is a fully human monoclonal antibody used for the treatment of hepatocellular carcinoma and non-small cell lung cancer. Tremelimumab (AstraZeneca) is also known as ticilimumab, CP-675,206; *see* WO 2000/037504 and Ribas, *Update Cancer Ther. 2(3)*: 133-39 (2007)).

[0453] In some aspects, the anti-CTLA-4 antibody useful in combination with the CEACAM5 targeting agent is ipilimumab. Ipilimumab (sold under the brand name YERVOY®, which was first approved for the treatment of metastatic melanoma, has since been approved for use in other cancers. Hoos *et al.* (2010) *Semin. Oncol.* 37:533; Hodi *et al.* (2010) *N. Engl. J. Med.* 363:711; Pardoll (2012) *Nat. Immunol.* 13(12):1129. In 2011, ipilimumab is a human antibody, which has an IgG1 constant region, was approved in the US and EU for the treatment of unresectable or metastatic melanoma based on an improvement in overall survival in a phase III trial of previously treated patients with advanced melanoma. Hodi *et al.* (2010) *N. Engl. J. Med.* 363:711. Tumor regressions and disease stabilization were frequently observed. Ipilimumab is also known as MDX-010 and 10D1. *See* U.S. Patent No. 6,984,720.

[0454] In some aspects, the anti-CTLA-4 antibody is an activatable anti-CTLA-4 antibody, such as an activatable anti-CTLA-4 antibody wherein the light chains of the antibody comprise a cleavable moiety and a masking moiety at the amino termini. The masking moiety interferes with binding to CTLA-4, but is preferentially released in the tumor microenvironment after cleavage of the cleavable moiety by proteases that are more prevalent and/or active in tumors than in peripheral tissues (*see*, in particular, WO 2018/085555). Such preferential cleavage in the tumor microenvironment enables full CTLA-4 blocking, promoting anti-tumor immune response, while minimizing CTLA-4 blockade in normal tissue, thereby reducing the risk of potential systemic toxicity of an anti-CTLA-4 antibody. In some aspects, the activatable anti-CTLA-4 antibody is an activatable form of ipilimumab, such as an antibody comprising light chains modified to comprise a masking moiety and a cleavable moiety, as disclosed, for example, in WO 2018/085555. An example of an activatable anti-CTLA-4 antibody that has entered human clinical trials is BMS-

986249 (NCT03369223: "A Study of BMS-986249 Alone and in Combination with Nivolumab in Advanced Solid Tumors"). In some aspects, the anti-CTLA-4 antibody is BMS-986249.

[0455] In some aspects, the anti-CTLA-4 antibody shows an enhanced Fcγ receptor (CD16) binding. Whether an anti-CTLA-4 antibody shows an enhanced Fcγ receptor binding is assessed by comparison with the Fcγ receptor binding of ipilimumab. Anti-CTLA-4 antibodies with enhanced Fcγ receptor (CD16) binding have been proposed as therapeutic agents for treatment of cancer through depletion of Treg cells. *See*, in particular, WO 2014/089113. In some aspects, the anti-CTLA-4 antibody shows an Fcγ receptor (CD16) binding that is at least a two-fold enhanced when compared to the Fcγ receptor binding of ipilimumab.

[0456] Examples of anti-CTLA-4 antibodies that show enhanced Fcγ receptor (*i.e.*, FcγRIIIA or CD16) binding are nonfucosylated anti-CTLA-4 antibodies. In some aspects, the anti-CTLA-4 antibody is a nonfucosylated anti-CTLA-4 antibody. Non-fucosylated anti-CTLA-4 antibodies lack fucose residues in its N-linked glycans. In some aspects, the non-fucosylated anti-CTLA-4 antibody is produced by expressing the chains of the antibody in a mammalian cell under conditions that prevent fucosylation, including but not limited to use of mammalian cells with genetic modifications preventing fucosylation, or growth of the cells expressing the antibody in medium containing one or more chemical compounds that inhibit fucosylation. In some aspects, the genetic modification that prevents fucosylation is inactivation, *e.g.* knock-out, of the FUT8 gene. In some aspects, the anti-CTLA-4 antibody is a hypofucosylated anti-CTLA-4 antibody.

[0457] An exemplary nonfucosylated anti-CTLA-4 antibody that has entered human clinical trials is BMS-986218 (*e.g.*, NCT03110107: "First-In-Human Study of Monoclonal Antibody BMS-986218 by Itself and in Combination with Nivolumab in Participants with Advanced Solid Tumors"). BMS-986218 is a nonfucosylated antibody developed to increase the effects of CTLA-4 blockade by enhancing binding to Fcγ receptor, thus promoting APC-mediated T cell priming. In some aspects, the anti-CTLA-4 antibody is BMS-986218. *See*, for example, PCT/US18/19868.

[0458] In some aspects, the Fc region of the anti-CTLA-4 antibody contains amino acid substitutions in the antibody constant region to enhance binding to activating Fcγ receptors. Exemplary substitutions are G236A, S239D, A330L and I332E (all residue numbering per the EU numbering system). In some aspects, the anti-CTLA-4 antibody comprises a human IgG1 constant domain with S239D, A330L and I332E mutations.

[0459] In some aspects, the anti-CTLA-4 antibody is an activatable and nonfucosylated anti-CTLA-4 antibody.

[0460] Human monoclonal antibodies that bind specifically to CTLA-4 with high affinity that are suitable for the methods of the present disclosure have been disclosed in U.S. Patent Nos. 6,984,720. Other anti-CTLA-4 monoclonal antibodies have been described in, for example, U.S. Patent Nos. 5,977,318, 6,051,227, 6,682,736, and 7,034,121 and International Publication Nos. WO 2012/122444, WO 2007/113648, WO 2016/196237, and WO 2000/037504, each of which is incorporated by reference herein in its entirety.

[0461] In some aspects, the anti-LAG-3 antibody useful in combination with the CEACAM5 targeting agent according to the methods of the present disclosu is relatlimab (BMS-986016), IMP731 (H5L7BW), MK4280 (28G-10, favezelimab), REGN3767 (fianlimab), GSK2831781, humanized BAP050, IMP-701 (LAG525, ieramilimab), aLAG-3(0414), aLAG-3(0416), Sym022, TSR-033, TSR-075, XmAb841 (XmAb22841), MGD013 (tebotelimab), BI754111, FS118, P 13B02-30, AVA-017, 25F7, AGEN1746, RO7247669, INCAGN02385, IBI-110, EMB-02, IBI-323, LBL-007, ABL501

[0462] In some aspects, the anti-LAG-3 antibody useful in combination with the CEACAM5 targeting agent comprises the the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, CDRL3, the VH and the VL, and/or the heavy and light chains of any of relatlimab (BMS-986016), IMP731 (H5L7BW), MK4280 (28G-10, favezelimab), REGN3767 (fianlimab), GSK2831781, humanized BAP050, IMP-701 (LAG525, ieramilimab), aLAG-3(0414), aLAG-3(0416), Sym022, TSR-033, TSR-075, XmAb841 (XmAb22841), MGD013 (tebotelimab), BI754111, FS118, P 13B02-30, AVA-017, 25F7, AGEN1746, RO7247669, INCAGN02385, IBI-110, EMB-02, IBI-323, LBL-007, or ABL501.

[0463] In some aspects, the anti-LAG-3 antibody useful in combination with the CEACAM5 targeting agent comprises relatlimab (BMS-986016). In some aspects, the anti-LAG-3 antibody comprises IMP731 (H5L7BW). In some aspects, the anti-LAG-3 antibody comprises MK4280 (28G-10, favezelimab). MK-4280 (28G-10, favezelimab) described in WO2016028672 and U.S. Publication No. 2020/0055938. In some aspects, the anti-LAG-3 antibody comprises REGN3767 (fianlimab). REGN3767 (fianlimab) is described, for example, in Burova E, *et al.*, *J. Immunother. Cancer* (2016); 4(Supp. 1):P195 and U.S. Patent No. 10,358,495. In some aspects, the anti-LAG-3 antibody comprises GSK2831781. In some aspects, the anti-LAG-3 antibody comprises humanized BAP050. Humanized BAP050 is described, for example, in

WO2017/019894. In some aspects, the anti-LAG-3 antibody comprises IMP-701 (LAG525, ieramilimab) IMP-701 (LAG525; ieramilimab) is described, for example, in U.S. Patent No. 10,711,060 and U.S. Publ. No. 2020/0172617. In some aspects, the anti-LAG-3 antibody comprises aLAG-3(0414). In some aspects, the anti-LAG-3 antibody comprises aLAG-3(0416). In some aspects, the anti-LAG-3 antibody comprises Sym022. In some aspects, the anti-LAG-3 antibody comprises TSR-033. In some aspects, the anti-LAG-3 antibody comprises TSR-075. In some aspects, the anti-LAG-3 antibody comprises XmAb841 (XmAb22841). In some aspects, the anti-LAG-3 antibody comprises MGD013 (tebotelimab). In some aspects, the anti-LAG-3 antibody comprises BI754111. In some aspects, the anti-LAG-3 antibody comprises FS118. In some aspects, the anti-LAG-3 antibody comprises P 13B02-30. In some aspects, the anti-LAG-3 antibody comprises AVA-017. In some aspects, the anti-LAG-3 antibody comprises 25F7. 25F7 is described, for example, in U.S. Publ. No. 2011/0150892. In some aspects, the anti-LAG-3 antibody comprises AGEN1746. In some aspects, the anti-LAG-3 antibody comprises RO7247669. In some aspects, the anti-LAG-3 antibody comprises INCAGN02385. In some aspects, the anti-LAG-3 antibody comprises IBI-110. In some aspects, the anti-LAG-3 antibody comprises EMB-02. In some aspects, the anti-LAG-3 comprises IBI-323. In some aspects, the anti-LAG-3 antibody comprises LBL-007. In some aspects, the anti-LAG-3 antibody comprises ABL501.

[0464] In general, any anti-LAG-3 antibody useful in combination with the CEACAM5 targeting agent can be used. Antibodies that bind to LAG-3 have been disclosed in Int'l Publ. No. WO/2015/042246 and U.S. Publ. Nos. 2014/0093511 and 2011/0150892, each of which is incorporated by reference herein in its entirety. Disclosure relating to the anti-LAG-3 antibodies described herein and other anti-LAG-3 antibodies useful in the methods of the present disclosure can be found in, for example: US 10,188,730, WO 2016/028672, WO 2017/106129, WO2017/062888, WO2009/044273, WO2018/069500, WO2016/126858, WO2014/179664, WO2016/200782, WO2015/200119, WO2017/019846, WO2017/198741, WO2017/220555, WO2017/220569, WO2018/071500, WO2017/015560, WO2017/025498, WO2017/087589, WO2017/087901, WO2018/083087, WO2017/149143, WO2017/219995, US2017/0260271, WO2017/086367, WO2017/086419, WO2018/034227, WO2018/185046, WO2018/185043, WO2018/217940, WO19/011306, WO2018/208868, WO2014/140180, WO2018/201096, WO2018/204374, and WO2019/018730. The contents of each of these references are incorporated by reference in their entirety.

[0465] Several experimental treatment protocols involve *ex vivo* activation and expansion of antigen specific T cells and adoptive transfer of these cells into recipients in order to generate antigen-specific T cells against tumors. *Ex vivo* activation in the presence of the anti-CEACAM5 antibodies described herein with or without an additional immunostimulating therapy (*e.g.*, an immune checkpoint inhibitor) can be expected to increase the frequency and activity of the adoptively transferred T cells.

[0466] In some aspects, the anti-CEACAM5 targeting agent described herein may also be administered with a standard of care treatment, or another treatment, such as radiation, surgery, or chemotherapy. The anti-CEACAM5 targeting agent may be combined with a vaccination protocol. Many experimental strategies for vaccination against tumors have been devised (see Rosenberg, S., 2000, Development of Cancer Vaccines, ASCO Educational Book Spring: 60-62; Logothetis, C., 2000, ASCO Educational Book Spring: 300-302; Khayat, D. 2000, ASCO Educational Book Spring: 414-428; Foon, K. 2000, ASCO Educational Book Spring: 730-738; see also Restifo, N. and Sznol, M., Cancer Vaccines, Ch. 61, pp. 3023-3043 in DeVita *et al.* (eds.), 1997, Cancer: Principles and Practice of Oncology, Fifth Edition). In one of these strategies, a vaccine is prepared using autologous or allogeneic tumor cells. These cellular vaccines have been shown to be most effective when the tumor cells are transduced to express GM-CSF. GM-CSF has been shown to be a potent activator of antigen presentation for tumor vaccination (Dranoff et al. (1993) *Proc. Natl. Acad. Sci U.S.A.* 90: 3539-43).

Diagnostics

[0467] In another aspect, the anti-CEACAM5 targeting agent described herein may be used for diagnostic purposes, including sample testing and *in vivo* imaging, and for this purpose the antibody (or binding fragment thereof) can be conjugated to an appropriate detectable agent, to form an immunoconjugate. For diagnostic purposes, appropriate agents are detectable labels that include radioisotopes, for whole body imaging, and radioisotopes, enzymes, fluorescent labels, and other suitable antibody tags for sample testing. For diagnostic purposes, appropriate agents are detectable labels that include radioisotopes, for whole body imaging, and radioisotopes, enzymes, fluorescent labels, and other suitable antibody tags for sample testing.

[0468] The detectable labels can be any of the various types used currently in the field of *in vitro* diagnostics, including particulate labels, isotopes, chromophores, fluorescent markers, luminescent markers, metal labels (*e.g.*, for CyTOF, imaging mass cytometry), phosphorescent

markers and the like, as well as enzyme labels that convert a given substrate to a detectable marker, and polynucleotide tags that are revealed following amplification such as by polymerase chain reaction. Suitable enzyme labels include horseradish peroxidase, alkaline phosphatase and the like. For instance, the label can be the enzyme alkaline phosphatase, detected by measuring the presence or formation of chemiluminescence following conversion of 1,2 dioxetane substrates such as adamantyl methoxy phosphoryloxy phenyl dioxetane (AMPPD), disodium 3-(4-(methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)tricyclo{3.3.1.1 3,7}decan}-4-yl) phenyl phosphate (CSPD), as well as CDP and CDP-STAR® or other art-recognized luminescent substrates including, for example, chelates of suitable lanthanides, such as Terbium(III) and Europium(III). The detection means is determined by the chosen label. Appearance of the label or its reaction products can be achieved using the naked eye, in the case where the label is particulate and accumulates at appropriate levels, or using instruments such as a spectrophotometer, a luminometer, a fluorimeter, and the like, all in accordance with standard practice.

[0469] In one aspect, a method of detecting the presence of CEACAM5 in a sample (*e.g.*, tissue culture cells, cell lysates, cells from a subject) comprises contacting the sample with an CEACAM5 detection agent (*e.g.*, anti-CEACAM5 antibody, antigen binding portion thereof, as described herein) under conditions that allow for specific binding between the CEACAM5 detection agent and CEACAM5 to form a complex; and detecting the formation of a complex. In certain aspects, a detectable label is attached to the CEACAM5 detection agent and the labeled CEACAM5 detection agent is contacted with the sample, for a time sufficient for specific binding to occur and facilitate detection of CEACAM5 in the sample. In other aspects, the CEACAM5 detection agent is contacted with the sample, for a time sufficient for specific binding to occur, and then a reagent, *e.g.*, a labeled antibody that specifically binds to a portion of the CEACAM5 detection agent (*e.g.*, Fc region), is added to facilitate detection of CEACAM5 bound to the CEACAM5 detection agent in the sample. In certain aspects, the CEACAM5 detection agent is a humanized antibody, fully human antibody, or a chimeric antibody having human variable regions and murine constant regions or portions thereof. The CEACAM5 detection agent may be used in any detection method known in the art (e.g., ELISA assay, immunofluorescence assay, flow cytometry) or described herein.

[0470] In some aspects, a method for diagnosing a cancer in a subject comprises contacting a biological test sample (*e.g.*, from a tumor biopsy) and a control sample (*e.g.,* corresponding to healthy tissue) with a CEACAM5 detection agent (*e.g.*, anti-CEACAM5 antibody, antigen binding

portion thereof, as described herein) under conditions allowing for formation of a complex between the CEACAM5 detection agent and CEACAM5, wherein an increased level of complex formation in the test sample compared to the control sample is indicative of the subject having a cancer associated with CEACAM5 expression.

[0471] In one aspect, a method of diagnosing a cancer in a subject comprises: (a) contacting a sample from the subject with a CEACAM5 detection agent such that an antibody-antigen complex is formed; (b) measuring the amount of the complex formed; and (c) comparing the amount of the complex in the sample with the amount in a control wherein an elevated level of the complex in the sample relative to the control indicates the subject has cancer. In some aspects, the sample is a tissue sample or blood sample.

[0472] The CEACAM5 detection agents described herein can be useful for diagnosing any CEACAM5 expressing cancer, including but not limited to colorectal, breast, lung, pancreatic, ovarian, uterine, prostate, stomach, cervical, esophageal, thyroid, bladder, endometrial, liver (*e.g.,* cholangiocarcinoma), and skin cancers.

[0473] In some aspects, the same CEACAM5 detection agent is used both as component of the diagnostic assay and as a therapeutic agent for treating a disease, such as cancer. In some aspects, the CEACAM5 detection agent is used as component of an assay in the context of a therapy targeting a CEACAM5 expressing tumor in order to diagnose the presence of a cancer in a subject; to determine susceptibility of a cancer patient to a CEACAM5 targeting agent described herein; to monitor the effectiveness of cancer treatment using a CEACAM5 targeting agent described herein; or to detect recurrence of the cancer after treatment. The assays include one or more steps for detecting expression of the surface protein CEACAM5 on tumor cells according to methods known in the art and described herein.

[0474] In another aspect, provided herein is a method of selecting a cancer patient for treatment with a CEACAM5 targeting agent described herein (*e.g.*, comprises contacting a biological test sample from the patient and a control sample with CEACAM5 detecting agent described herein under conditions allowing for formation of a complex between the CEACAM5 detecting agent and CEACAM5, wherein an increased level of complex formation in the test sample compared to the control sample is indicative of the cancer being amenable to treatment with the antibody or ADC.

[0475] In another aspect, a method of determining the response of a patient afflicted with cancer to treatment with a CEACAM5 targeting agent described herein comprises contacting a

biological test sample from the patient and a control sample with a CEACAM5 detecting agent described herein under conditions allowing for formation of a complex between the CEACAM5 detecting agent and CEACAM5, wherein an increased level of complex formation in the test sample compared to the control sample is indicative of the cancer being unresponsive to treatment. The biological test sample may be derived from the same cell type as the biological sample submitted for analysis, but which was obtained from the subject previously in time, upon or after completion of the anti-CEACAM5 cancer therapy.

[0476] In another aspect, a method of determining whether a cancer in a patient has relapsed or metastasized comprises (a) identifying a patient having a cancer, (b) administering a labeled (*e.g.*, radiolabeled) anti-CEACAM5 antibody or antigen binding portion thereof described herein to the patient and determining the biodistribution of the labeled anti-CEACAM5 antibody, and (c) periodically repeating step (b) to determine whether the biodistribution of the labeled anti-CEACAM5 antibody has changed, wherein a change in the biodistribution of the labeled anti-CEACAM5 antibody is indicative of the cancer being metastasized.

[0477] In some aspects, the CEACAM5 detection agent described herein may be used for staging of cancers (*e.g.*, in radio-imaging). As such, they may be used alone or in combination with other cancer markers.

[0478] In some aspects, the method of detecting the presence of a cancer in a subject *in vivo*, comprising the steps of administering a detectably-labelled antibody to a patient; and detecting localization of the detectably labelled antibody in the patient by imaging.

[0479] In the above methods, the control sample can be a normal, non-cancerous, biological sample of the same type, or a reference value determined as representative of the antibody binding level in normal biological sample of the same type.

[0480] The biological test sample for diagnostic purposes may encompass a variety of sample types obtained from a subject and can be used in a diagnostic or monitoring assay. Biological samples include but are not limited to blood and other liquid samples of biological origin, solid tissue samples such as a biopsy specimen or tissue cultures or cells derived therefrom, and the progeny thereof. Therefore, biological samples encompass clinical samples, cells in culture, cell supernatants, cell lysates, serum, plasma, biological fluid, and tissue samples, in particular tumor sample. In some aspects, the biological sample may be a formalin-fixed and paraffin embedded (FFPE) tissue sample.

XI. Kits

[0481] Also provided are kits comprising a CEACAM5 targeting agent described herein (*e.g.*, anti-CEACAM5 antibody, antigen binding portion thereof, bispecific antibody, immunoconjugate, or antibody drug conjugate (ADC)), and instructions for use.

[0482] Also provided are kits comprising the CEACAM5 detection agents described herein (*e.g.,* anti-CEACAM5 antibodies, antigen binding portions thereof, bispecific antibodies, and immunoconjugates) for detection and quantification of CEACAM5 *in vitro* according to the detection methods described herein. The kit may additionally include one or more detection reagents, including *e.g.*, fluorescently labeled secondary antibody detection reagents and the like. In some aspects, the kit includes a CEACAM5 polypeptide or antibody coupled to a solid support, *e.g.,* a tissue culture plate or beads (*e.g.,* Sepharose beads). The kits may include a label indicating the intended use of the contents of the kit, including any writing, marketing materials or recorded material supplied on or with the kit, or which otherwise accompanies the kit.

[0483] In some aspects, the kits comprise the CEACAM5 targeting agent in unit dosage form, such as in a single dose vial or a single dose pre-loaded syringe, optionally contained in a single vial or container, along with *e.g.*, instructions for use in treating a cancer using the targeting agent as described herein.

[0484] The present disclosure is further illustrated by the following examples, which should not be construed as further limiting. The contents of all figures and all references, Genbank sequences, issued patents, and published patent applications cited throughout this disclosure are expressly incorporated herein by reference.

EXAMPLES

[0485] Commercially available reagents referred to in the Examples below were used according to manufacturer's instructions unless otherwise indicated. Unless otherwise noted, the present disclosure uses standard procedures of recombinant DNA technology, such as those described hereinabove and in the following textbooks: Sambrook *et al.*, supra; Ausubel *et al.*, *Current Protocols in Molecular Biology* (Green Publishing Associates and Wiley Interscience, N.Y., 1989); Innis *et al.*, *PCR Protocols: A Guide to Methods and Applications* (Academic Press, Inc.: N.Y., 1990); Harlow *et al.*, *Antibodies: A Laboratory Manual* (Cold Spring Harbor Press:

Cold Spring Harbor, 1988); Gait, *Oligonucleotide Synthesis* (IRL Press: Oxford, 1984); Freshney, *Animal Cell Culture,* 1987; Coligan *et al.*, *Current Protocols in Immunology,* 1991.

[0486] The following Examples describe the isolation and characterization of anti-CEACAM5 monoclonal antibodies. The CDR sequences, variable region sequences, and full-length heavy and light chain sequences of anti-CEACAM5 antibodies are provided below, for example in **Table 16.**

Example 1: Immunization of humanized mice with human CEACAM5 and cynomolgus CEACAM5 and generation of antibodies

[0487] Human anti-CEACAM5 monoclonal antibodies (mAbs) were generated by immunizing BMS proprietary chimeric mouse strains and BMS chimeric mouse strains with a fixed human light chain. Murine subjects were immunized following either a short 3-week protocol or a 4-week (+) protocol for up to 8 times using HOCK, intraperitoneal, and/or subcutaneous injections. Seven cohorts of mice were immunized with one or more of the following immunogens listed in **Table 3**. Spleen and lymph node cells from the mice were harvested and antibodies specific to CEACAM5 were identified by hybridoma, single B cell cloning and immune library selections.

Table 3. Immunization protocol for generating human anti-CEACAM5 mAbs.

Materials	Description	Source
1	Human CEACAM5 extracellular domain (ECD) protein His tag	R&D Systems (4128-CM)
2	Human CEACAM5 extracellular domain (ECD) protein Fc fusion	R&D Systems (10449-CM)
3	Cynomolgus CEACAM5 extracellular domain (ECD) protein	Sino Biological (90891-C08H)
4	Cells expressing full-length human CEACAM5 protein	Internal material
5	Cells expressing full-length cynomolgus CEACAM5 protein	Internal material
6	DNA encoding full-length human CEACAM5 protein	Internal material
7	DNA encoding full-length cynomolgus CEACAM5 protein	Internal material
8	Cells expressing human CEACAM5 A3-B3 domain	Internal material
9	Cells expressing cynomolgus CEACAM5 A3-B3 domain	Internal material
10	DNA encoding human CEACAM5 A3-B3 domain	Internal material
11	DNA encoding cynomolgus CEACAM5 A3-B3 domain	Internal material

Example 2: CEACAM5 hybridoma methods, screening of hybridoma antibodies and recombinant production.

[0488] Hybridomas were generated from the harvested lymph node and spleen cells by electric field-based electrofusion. Fused cells were plated into multi-well plates in selective hypoxanthine-aminopterin-thymidine medium (HAT) medium for seven days, and the hybridoma supernatants were subsequently screened by ELISA for binding to biotin-human CEACAM5, cynomolgus CEACAM5, biotin-human CEACAM5 A3-B3, or biotin-cynomolgus CEACAM5 A3-B3 using a standard ELISA protocol. The supernatants were also counter-screened versus biotin-human CEACAM1 and biotin-human CEACAM6. Briefly, CEACAM5 biotinylated reagents were added at 1-2 µg/mL to streptavidin-coated and blocked high-binding Nunc MaxiSorp ELISA plates (ThermoFisher), whereas CEACAM5 unbiotinylated targets were directly coated on MAXISORP™ blocked ELISA plates at 1-2µg/mL. Hybridoma clones were then detected with peroxidase AFFINIPURE™ $F(ab')_2$ fragment goat anti-mouse IgG, Fcγ fragment specific material (Jackson Immunoresearch). Reagents for hybridoma supernatant clone screening by ELISA are shown in **Table 4**.

Table 4. Reagents used for screening CEACAM5 hybridoma supernatants by ELISA

Biotin-Human CEACAM5/CD66e Fc, AVITAG™ Protein, CF (R&D Systems)
Biotin-Human CEACAM1 / CD66a Protein, AVITAG™,His Tag (Acro Biosystems)
Biotin-Human CEACAM6 / CD66c Protein, His, AVITAG™ (Acro Biosystems)
Biotin-Human CEACAM5 A3-B3 domain (internal material)
Biotin-Cynomolgus CEACAM5 A3-B3 domain internal material
Cynomolgus CEACAM5/CD66e Protein (His Tag) (Sino Biological)

[0489] Positive human and cynomolgus CEACAM5 cross-reactive binders (not recognizing either human CEACAM1 protein or human CEACAM6 protein) were selected and the respective VH region and VL region were sequenced by Next Generation Sequencing (NGS) using a MiSeq sequencing system (Illumina). Individual mAb clones were recombinantly generated with a human IgG1.3 Fc (IgG1 inactive variant) following plasmid transient transfections into the human Expi293F cell line (ThermoFisher). The mAbs were purified via protein-A purification using standard protocols and subsequently characterized as described below.

Example 3: Single B cell cloning and screening of clones by ELISA

[0490] A single B cell cloning (SBC) approach was additionally utilized to isolate CEACAM5 mAbs from the mice immunized as described above. The lymph nodes and/or spleen cells from the immunized mice were incubated with two or more of the soluble antigens described in **Table 5**, along with antibodies that target B cell markers and mouse Ig isotypes. The subsequent mix of cells and antigens were passed through a flow cytometer, and B cells that bound both human and cynomologous CEACAM5 (extracellular domain and/or A3-B3 domain) were selected via Fluorescence Activated Cell Sorting (FACS) to identify cross reactive antibodies. In some instances, cells were also incubated with human CEACAM1 and CEACAM6, and a negative selection was performed to facilitate isolation of CEACAM5-specific antibodies that do not recognize CEACAM1 and CEACAM6.

Table 5. Antigens for single B cell cloning (SBC).

Antigen	Selection type/purpose
Human CEACAM5 ECD HA_Biotin	Positive
Cynomolgus CEACAM5 ECD AF700	Positive
Human CEACAM5 A3-B3-HA_Biotin	Positive
Cynomolgus CEACAM5 A3-B3-AF700	Positive
Human CEACAM1-PE-Cy7	Negative
Human CEACAM6-PE-Cy7	Negative

[0491] Antigen specific B cells were individually sorted into 96 well plates containing lysis buffer, thereby lysing the cells and releasing the RNA. The lysed cells were subjected to multiple rounds of PCR to isolate the VH and VL regions of the captured BCR (B cell receptor) and to append promoter/signal peptide and constant region blocks to the requisite ends of the variable region. The final PCR reactions generated Transcriptionally Active PCR (TAP) (Liang *et al.*, J Biol Chem. 2002: 277 (5): 3593-8) products that were transfected via high-throughput methods into Expi293 cells to generate small-scale amounts of antibodies as IgG1.3 mAbs for screening.

[0492] Following small-scale TAP production and purification, the TAP mAbs were screened by a standard ELISA. Briefly, plates were coated with the same CEACAM5, CEACAM1 or CEACAM6 reagents shown in **Table 5** on streptavidin-coated plates (if biotinylated) or directly

coated on MAXISORP™ high-binding ELISA plates (if non-biotinylated). Antibody binding was detected using an Fcγ fragment specific Peroxidase AFFINIPURE™ F(ab')$_2$ Fragment Goat Anti-Human IgG (Jackson Immunoresearch).

Example 4. Screening of CEACAM5 SBC mAbs by flow cytometry using CHO-s cell lines overexpressing CEACAM family members.

[0493] CHO-S (ThermoFisher) cell lines expressing various forms of CEACAM5 and CEACAM1/6 were generated for use in SBC antibody screening and counterscreening, respectively, and for use as immunization tools. Briefly, coding regions for CEACAM5 proteins shown in **Table 6** were inserted into an expression plasmid containing an osteonectin signal sequence. The CHO-S cells were transfected with these plasmids using a Lonza Nucleofector II device (Lonza Bioscience). At 72 hours post transfection, cells were placed under 600 mg/ml hygromycin antibiotic selection. After antibiotic selection, cells were labeled with human CEACAM-5/CD66e Alexa fluor 647-conjugated antibody (R&D Systems). The CEACAM5 positive cells were sorted via FACS. Sorted cells were grown in selective media and used for assays.

Table 6. Cell lines for screening CEACAM5 mAbs by flow cytometry.

Name	Description
CHO-hCEACAM5	CHO-s cell line overexpressing full-length human CEACAM5
CHO-cyCEACAM5	CHO-s cell line overexpressing full-length cynomolgus CEACAM5
CHO-hCEACAM1	CHO-s cell line overexpressing full-length human CEACAM1
CHO-hCEACAM6	CHO-s cell line overexpressing full-length human CEACAM6
CHO-hCEACAM5-A3-B3	CHO-s cell line overexpressing C-terminal A3-B3 domains of human CEACAM5
CHO-cyCEACAM5-A3-B3	CHO-s cell line overexpressing C-terminal A3-B3 domains of cynomolgus CEACAM5
CHO-S	Parental cell line

[0494] Antibodies resulting from SBC were screened by standard flow cytometry methods using the recombinant CHO-S cells described above. The CEACAM5-overexpressing cells were resuspended in FACS buffer (DPBS, no Ca/Mg, 2-5%, filtered FBS, 0.09% sodium azide). Parental CHO-S cells were stained at a temperature of 37°C for 15 minutes using the CFSE CellTrace kit (Invitrogen). The cells were washed three times, and then resuspended in FACS buffer. A mixture containing equal numbers (10^6 cells/well) of parental CHO-S and CEACAM5-overexpressing cells

was plated on 384-well plates. Equal concentrations of primary TAP antibodies were added to the cells, and the plates were incubated at 4°C for one hour.

[0495] Sequence-unique TAP clones cross-reacting with CHO-S cells expressing full-length human and cynomolgus CEACAM5, but not full-length human CEACAM1 and CEACAM6, were selected for further characterization. Expression plasmids encoding the selected CEACAM5-specific clones were transiently transfected into mammalian cells for protein A purification of the corresponding mAbs, and subsequent FACS kinetics and binning analyses.

Example 5. Immune library selections and screening of CEACAM5 antibodies

[0496] Using spleen and lymph node cells from the mice described in Example 1, immune libraries were generated and selections were performed to identify additional CEACAM5 binding antibodies. The VH mRNA and VL mRNA were isolated from the antibody-producing B cells of the immunized mice. An immune library was constructed in a manner that maintained the original VH-VL pairing that was present in the mouse B cell. This library was expressed by yeast surface display and sorted against either human CEACAM5 protein, cynomolgus CEACAM5 protein, or human CEACAM5 A3-B3 protein using FACS. Single yeast cells that displayed CEACAM5 positive antibodies were sorted into plates, and TAP DNA products were generated from the antibody genes.

[0497] The TAP DNA was transiently transfected into HEK cells for expression as IgGs. In parallel, deep sequencing of the immune libraries was also performed. Libraries were sorted against human CEACAM5, cynomolgus CEACAM5, human CEACAM5 A3-B3, human CEACAM1, and human CEACAM6. The selected antibodies were sequenced via NGS, and antibodies demonstrating the desired binding profile (human/cynomolgus CEACAM5 cross reactive and selective over CEACAM1 and CEACAM6) were identified. These antibodies were gene synthesized in IgG expression vectors and transiently transfected into HEK cells for expression. Expressed IgGs resulting from immune library TAP and deep sequencing were subjected to ELISA screening against human CEACAM5, cynomolgus CEACAM5, human CEACAM1, and human CEACAM6.

Example 6. Sequencing of CEACAM5(+) IgG-expressing antibody clones

[0498] The VH and VL regions from the positive human CEACAM5 mAbs described in examples 2, 3, and 5 were sequenced by NGS. Approximately 380 sequence-unique clones binding either human or cynomolgus CEACAM5 were identified, comprising 173 sequence families (as

defined by 80% sequence homology in HCDR3). Of these CEACAM5 positive sequences, 188 antibodies (57 sequence families) were shown to bind both human and cynomolgus CEACAM5 expressed on HCT116 cells (as described below), and 75 clones (31 sequences families) demonstrated specificity over CEACAM1 and CEACAM6. Further characterization (as described herein) led to the isolation of multiple antibodies including MBN001.The amino acid sequences of the VH and VL CDRs are provided in **Table 16**.

Example 7: Epitope binning

[0499] High throughput SPR-based epitope binning sandwich assays were performed using the Carterra LSA Platform to group the anti-CEACAM5 mAbs into bins sharing common binding epitopes. Pairwise competition experiments were performed by high-throughput Carterra SPR microfluidics under a classic sandwich format using a microarray-based 96 x 96 microfluidic system array. Binning results were analyzed using Carterra microfluidics' binning software for heat map generation and network plotting. The competing antibody relationships allow for the anti-CEACAM5 antibodies to be clustered into bins, where a bin represents a family of anti-CEACAM5 antibodies sharing an identical blocking profile when tested against the other anti-CEACAM5 antibodies. **See FIG. 1A**. Based on the assay results, the newly generated antibodies binned to 7 distinct epitope groups. MBN001 mAb was identified as being a Bin 1 binder of CEACAM5. Cross-reactivity to other CEACAM5 family members, including CEACAM1 and CEACAM6 were determined by SPR as described above, and epitope bins 4, 6, 7 and 8 were determined to cross-react with CEACAM1 and CEACAM6. See **FIG. 1B**.

Example 8: Binding specificity of anti-CEACAM5 antibodies in cell lines expressing CEACAM5

[0500] This Example analyzed the cell binding specificity of the anti-human CEACAM5 mAbs (e.g., MBN001, MBN002, and MBN003) described in Example 6. **FIG. 2A**, **FIG. 2B**, **FIG. 2C** and **FIG. 2D** are a set of graphs showing cell-based binding of antibodies against cell lines expressing different levels of human CEACAM5: medium BXPC-3 (**FIG. 2A**), low Ls174T cells (**FIGs. 2B**), high MKN-45 (**FIGs. 2C**), and negative HCT-116 (**FIGs. 2D**). Each of the tested antibodies (mAb MBN001, mAb MBN002, and mAb MBN003) bound the cells expressing human CEACAM5 comparable to mAb Control 3. Each of the mAbs was further evaluated for binding specificity using human colorectal carcinoma cell lines (HCT116) and CHOS cell lines overexpressing human CEACAM5, cynomolgus CEACAM5, human CEACAM1 and human

CEACAM6. As shown in **Table 7**, the antibody MBN001exhibited specific binding to human CEACAM5 and cynomolgus CEACAM5, but not to human CEACAM1 or CEACAM6.

Table 7. Specificity of binding of anti-CEACAM5 mAbs to human and cynomolgus CEACM5- and/or CEACAM6-expressing cell lines.

mAb	HCT116-parental single-point MFI (Mean)	HCT116-huCEACAM5 single-point MFI (Mean)	HCT116-cyCEACAM5 single-point MFI (Mean)	HCT116-huCEACAM6 single-point MFI (Mean)	CHO-S-huCEACAM1 single-point MFI (Mean)
MBN001	32.8	12758	8348	29.5	41.9

[0501] CEACAM5 mAbs such as MBN002, MBP018, MBP003, MBP001 and MBP002 were examined for binding to human CEACAM7 (R&D Systems catalog # 9010-CM-050) by SPR using CEACAM7 mAb (R&D Systems catalog # MAB44782) as a positive control. No binding was observed for the CEACAM5 mAbs (data not shown). The CEACAM5 mAbs were similarly examined for binding to human CEACAM8 (R&D Systems catalog # 9639-CM-050). Commercial CEACAM8 mAb (R&D Systems catalog # MAB4246) showed binding to CEACAM8 but no binding was observed for the CEACAM5 mAbs (data not shown).

Example 9: Binding kinetics of anti-CEACAM5 antibodies

[0502] Analytical binding studies were carried out by surface plasmon resonance (SPR) to characterize the binding kinetics of selected anti-CEACAM5 antibody MBN001 to full-length human CEACAM5 and cynomolgus CEACAM5 and to the A3-B3 regions of human CEACAM5 and cynomolgus CEACAM5 (all internally generated proteins).

[0503] SPR analyses were conducted at 37° C using a Biacore 8K instrument primed with 1xHBSP+ running water and docked with a CM5 sensor chip equilibrated to room temperature, followed by re-priming of the instrument. The chip surface was activated by injecting an ethyl(dimethylaminopropyl) carbodiimide (EDC)/N-hydroxysuccinimide (NHS) mixture for 7 minutes at 10 µL/min. The activated chip surface was then immobilized with an anti-human Fc capture reagent (at 25 µg/mL in acetate pH 5 buffer) injected on the chip surface for 7 minutes at 10 µL/min to yield an immobilization level of about 9000 RU. The remaining chip surface was blocked by injecting ethanolamine for 7 minutes at 10 µL/min.

[0504] To analyze the kinetics of antibody binding, anti-CEACAM5 antibody (10 nm in HBSP+ buffer) was first captured onto the chip for 20 seconds at 10 µL/min for binding to the Fc capture reagent, followed by CEACAM5 analyte binding in which each of a series of recombinant

full-length CEACAM5 or A3-B3 CEACAM5 proteins diluted 3x from 500 nM to 0.23 nM in HBSP+ buffer was flowed across the chip The analyte association time was 3 minutes at 30 µL/min; the antigen dissociation time was 10 minutes at 30µl/min; and regeneration involved two injections of 3M magnesium chloride ($MgCl_2$) for 30 seconds at 30 µL/min.

[0505] The kinetic data for anti-CEACAM5 mAb:CEACAM5 binding were fit to a 1:1 Langmuir binding with Rmax to provide estimates of the kinetic and affinity values for the corresponding interactions. Estimates of the binding kinetics and affinities for the selected antibody to full length CEACAM5 (hu/cy) and A3-B3 regions of CEACAM5 (hu/cy) are shown in **Tables 8-11**.

Table 8. Kinetics of anti-CEACAM5 mAb binding to full-length huCEACAM5.

mAb	Full-length huCEACAM5 ka (1/Ms)	Full-length huCEACAM5 kd (1/s)	Full-length huCEACAM5 K_D (M)	Full-length huCEACAM5 % Rmax
MBN001	6.86E+04	6.15E-03	8.96E-08	98

Table 9. Kinetics of anti-CEACAM5 mAb binding to full-length cyCEACAM5.

mAb	Full-length cyCEACAM5 ka (1/Ms)	Full-length cyCEACAM5 kd (1/s)	Full-length cyCEACAM5 KD (M)	Full-length cyCEACAM5 % Rmax
MBN001	1.91E+04	1.19E-02	6.21E-07	23

Table 10. Kinetics of anti-CEACAM5 mAb binding to A3-B3 huCEACAM5.

mAb	A3-B3 huCEACAM5 ka (1/Ms)	A3B3 huCEACAM5 kd (1/s)	A3-B3 huCEACAM5 KD (M)	A3-B3 huCEACAM5 % Rmax
MBN001	6.31E+04	8.12E-03	1.29E-07	84

Table 11. Kinetics of anti-CEACAM5 mAb antibody binding to A3-B3 cyCEACAM5.

mAb	A3-B3 cyCEACAM5 ka (1/Ms)	A3-B3 cyCEACAM5 kd (1/s)	A3-B3 cyCEACAM5 KD (M)	A3-B3 cyCEACAM5 % Rmax
MBN001	3.43E+04	2.49E-02	7.26E-07	24

Example 10: In vitro binding of anti-CEACAM5 antibodies in CEACAM5-expressing cell lines

[0506] Selected anti-CEACAM5 antibody MBN001 was evaluated for binding to the CEACAM5-expressing cell lines MKN45, HCT116-huCEACAM5, and HCT116-cyCEACAM5, along with the HCT-116 parent control. Bin 1 (Control #1) and Bin 2 (Control #2) antibodies were used as controls. The results of these experiments are shown in **Table 12** (EC50 values) and **Table 13** (Amax values).

Table 12. EC50 values for selected anti-CEACAM5 mAb

mAb	EC50 MKN45 (nM)	EC50 HCT-116 huCEACAM5 (nM)	EC50 HCT-116 cyCECAM5 (nM)	EC50 HCT-116 (nM)
MBN001	33.8	3.33	82.2	Weak
Control #1	63.73	ND	82.02	Weak
Control #2	5.74	5.47	14.51	Weak

ND= Not Determined

Table 13: Amax values for selected anti-CEACAM5 mAb

mAb	Amax MKN45 (MFI)	Amax HCT-116 huCEACAM5 (MFI)	Amax HCT-116 cyCECAM5 (MFI)	Amax HCT-116 (MFI)
MBN001	4159	6793	25518	43.9

[0507] Data show that the MBN001 bound both huCEACAM5 expressing lines and cynoCEACAM-5 expressing lines. Furthermore, MBN001 had improved cellular binding compared to control antibodies.

Example 11: Screening of anti-CEACAM5 mAbs capable of internalizing into cells

[0508] Anti-CEACAM5 mAbs were screened for their ability to internalize into huCEACAM5-expressing cells. The internalization assay utilized the MKN-45 cell line, the HCT116-huCEACAM5 cell line, the parental HCT116 control cell line and the LS174T cell line. The internalization assay was performed in 96-well plates using the IncuCyte S3 Live-Cell Analysis System. Plates were scanned for phase contrast and red fluorescence and images were automatically analyzed using the integrated INCUCYTE® software for phase confluence (measure

of cell area) and red fluorescence object area. As the labeled antibodies internalize into the acidic environment of endosomes and lysosomes, the intensity of red fluorescence inside the cells increases. The internalization signals are represented as red fluorescence object areas normalized to the total cell areas (phase confluence).

[0509] Briefly, a number ($1x10^4$) of viable cells from the MKN-45, HCT116-huCEACAM5, LS174T and HCT116 parental cell lines were added to 96-well plates and incubated at 37° C/5% CO_2 for about four hours prior to adding the antibody treatments. Antibody treatments were prepared by combining test antibodies or isotype control antibodies with PHRODO™ red secondary Fab reagent (ThermoFisher Scientific) to provide final concentrations of test antibodies (25 nM) to pHrodo reagent (75 nM) following addition to the cells. Following an incubation at 37° C for 30 minutes, 50 µl of each antibody treatment was added to 50 µl of cells in 96-well plates. The plates were then inserted in the INCUCYTE® system and further incubated at 37° C for 30 minutes prior to the reading by the IncuCyte detection machine. The IncuCyte settings were set to a 24-hour duration with reads every 30 minutes, a 10x objective with 3 images/well, and with both phase and red fluorescent channels.

[0510] **Table 14** shows a quantitative assessment of the internalization of the antibodies MBN001, MBN002 and MBN003 as described herein and as measured by an area under time-course (AUC) analysis. Internalization data were collected for selected antibodies in the MKN45, LS174T and HCT 116-CEACAM5 cell lines. Bin 1 (Control #1) and Bin 2 (Control #2) antibodies were used as controls. The data show the tested antibodies were internalized into the test cell lines and that in many cases the antibodies had improved, or comparable internalization compared to the control antibodies.

Table 14. Internalization of antibodies into HCT116-huCEACAM5 and MKN-45 cell lines.

Antibody	HCT 116 CEACAM5 (AUC)	MKN-45 (AUC)
Control-1	2.232	4.668
Control-2	0.8051	1.952
Unstained	0.00174	0.04971
hIgG1.3f isotype	0.004163	0.01135
Secondary-only	0.005398	0.01089

Example 12: ADC conjugation and screening of anti-CEACAM5 mAbs and ADCs in cytotoxicity assays

[0511] Multiple assays (e.g., direct ands indirect cytotoxicity assay) were performed to investigate the potential utility of the anti-CEACAM5 antibodies to serve as cytotoxic immunoconjugates for cancer therapy. A general time course and protocol for both the direct and indirect cytotoxicity assays are shown below. These data were used in part along with the binding and internalization data to select antibodies for *in vivo* efficacy testing.

Methods and materials utilized in the examples are listed below:

Bioconjugation of anti-CEACAM5 mAbs and DXd linker payload and characterization

[0512] Antibodies were treated with 30 equivalent of tris(2-carboxyethyl)phosphine (TCEP) in PBS, pH 7.4, 2 mM EDTA at 37 °C for 1 hour followed by purification using a ZEBA™ spin column to remove excessive TCEP. The reduced antibodies were eluted with PBS, pH 6.8, 2 mM EDTA and reacted with 10-12 equivalent of maleimide-functionalized payload linker in the same buffer at room temperature for 1 hour. The final product was purified using a HiTrap SP cation exchange chromatography column. The linear salt concentration gradient elution was carried out by changing sodium chloride (NaCl) concentrations from 0 to 1 molar (M). Buffer A was 50 mM sodium acetate pH5, and buffer B 50 mM sodium acetate pH5, 1 M NaCl. The samples were analyzed by LC-MS using an Agilent 1290 Infinity ultra-performance liquid chromatograph (UPLC) system coupled to a 6530 Accurate-Mass Q-TOF mass spectrometer (Agilent, Santa Clara, CA). The analytical column used was a Waters BEH C4 column, 1.7 μm, 2.1 mm × 50 mm, held at 60 °C. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The system was operated at a flow rate of 200 μL/min. The gradient condition was as follows: 0−2 min, held at 27% B; 2−9 min, slow ramp from 27% to 37% B; 9−9.5 min, linear ramp from 37% to 90% B; 9.5−12.3 min, held at 90% B. The MS settings were as follows: polarity = positive, capillary voltage = 4.2 kV, sample cone = 40 V, source offset = 15 V, source temperature = 140 °C, desolvation temperature = 325 °C. The data acquisition range was 900−3200 m/z. Deconvolution was performed using an Agilent MassHunter Walkup software. Monomer percentage was analyzed by size exclusion chromatography (SEC) using an Agilent 1200 Infinity HPLC. The analytical column used was Zorbax 4 um, GF-250, 9.4 x 250 mm. The mobile phase consisted of 0.1 M sodium carbonate, 0.2 M methylammonium chloride (MaCl), pH7.2, 5 mM glycine, 15% acetonitrile (ACN).

Hydrophobic interaction chromatography (HIC) analysis

[0513] The HIC analysis was performed using a Tosoh Bioscience 4.6 um ID x 3.5 cm, 2.5 um butyl-nonporous resin column. The mobile phase A buffer was a solution of 25 mM Tris-HCL, 1.5 M ammonium sulfate, pH8.0. The mobile phase B buffer was a solution of 25 mm Tris-HCL pH8.0, 5% isopropanol. The linear gradient elution (0% B to 100% B) was carried out.

Evaluation of ADC Stability in SCID Mouse Serum.

[0514] The ADCs were diluted to a target concentration of 50 μg/mL in 500 μL of SCID mouse serum and incubated at 37 °C. From the incubating sample plate, a volume (80 μL) of serum samples was collected and immediately frozen on days 0, 1, 2, and 5. All the ADCs were extracted from serum using immuno-capture methods and analyzed by LC-HRMS as previously reported. In brief, the ADCs were captured from serum using a generic anti-human F(ab′)2 reagent. The immobilized ADC was digested "on-bead" with IdeS enzyme, followed by elution of F(ab′)2 and interchain disulfide reduction to separate LC and Fd′. The MS data was analyzed following deconvolution using the Intact Mass software (v3.6, Protein Metrics, Inc.).

Direct cytotoxicity assay

[0515] Briefly, the following low, medium, and high CEACAM5-expressing cell lines were plated into 384-well plates in 20 μl cell suspensions: 2,500 Ls174T cells (low); 1,100 BxPC-3 cells (medium); 250 HCT-CEA (stably transformed cells; high); and 2,000 MKN-45 (high). The cells were plated in complete RPMI media supplemented with 10% HI-FBS and 1% Pen/Strep and incubated for 20-24 hours at 37° C. Amounts of 2x solutions of anti-CEACAM-5 mAb dilutions (each diluted 4-fold relative to the preceding sample) were prepared in complete RPMI to provide final ADC concentrations between 300 nM and 0.0003 nM following addition to the 20 μL cell solution and incubated for 120 hours at 37° C. Untreated cells served as negative controls. Following the incubation, the assay plates were removed from the incubator and cooled to room temperature. Then, 40 μL of Cell Titer Glo 2.0 solution (Promega Corp.) was added to each assay well, followed by mixing the plates on an orbital shaker for two minutes at 500 revolutions per minute (rpm) and then placing the plates in the dark for 20 minutes. After 20 minutes, the plate covers were removed, and luminescence was read on an Envision plate reader.

[0516] For each treatment replicate, a mean value, standard deviation (SD), and %CV (percent inhibition) were calculated. Percent CV values greater than 15% and 20% were noted. A

mean value was calculated by averaging the RLUs and subtracting the values of the blank. In addition, IC50 and Amax values were calculated. Percent inhibition was calculated by the formula: 100 -(test mean/treatment control) x 100.

Indirect (piggyback) cytotoxicity assay

[0517] Briefly, the following low, medium, and high CEACAM5-expressing cell lines were plated into 384-well plates in 20 µl cell suspensions: 2,500 Ls174T cells (low); 1,100 BxPC-3 cells (medium); 250 HCT-CEA (stably transformed cells; high); and 2,000 MKN-45 (high). The cells were plated in complete RPMI media supplemented with 10% HI-FBS and 1% Pen/Strep and incubated for 20-24 hours at 37° C. Amounts of 4x solutions of anti-CEACAM-5 mAb dilutions (each diluted 3-fold relative to the preceding sample) were prepared so as to provide mAb concentrations between 60 nM and 0.01 nM following addition to a 4x stock solution of monomethyl auristatin E (MMAE) drug toxin-conjugated to the variable heavy domain of heavy chain (Vhh) at a concentration of 400 nM in complete RPMI media. The Vhh (also referred to as VHH) moiety is an IgG-binding secondary "nanobody" that specifically targets the Kappa LC of human IgGs. The resulting 2x mAb and MMAE-Vhh solutions in RPMI were allowed to precomplex for 15 minutes after which 20 µL of the 2x antibody/MMAE-Vhh conjugate solution was added to the 20 µL cell suspension and incubated for 120 hours at 37° C. Untreated cells and MMAE-Vhh alone served as negative controls. Following the incubation, the assay plates were removed from the incubator and cooled to room temperature. Then, 40 µL of Cell Titer Glo 2.0 solution (Promega Corp.) was added to each assay well, followed by mixing the plates on an orbital shaker for two minutes at 500 revolutions per minute (rpm) and then placing the plates in the dark for 20 minutes. After 20 minutes, the plate covers were removed, and luminescence was read on an Envision machine.

[0518] For each treatment replicate, a mean value, standard deviation (SD), and %CV (percent inhibition) were calculated. Percent CV values greater than 15% and 20% were noted. A mean value was calculated by averaging the RLUs and subtracting the values of the blank. In addition, IC50 and Amax values were calculated. Percent inhibition was calculated by the formula: 100 -(test mean/treatment control) x 100.

Results

[0519] HIC and MS analyses show that the tested MMAE ADCs and DXd ADCs were stable in the tested buffers and mouse serum.

[0520] **FIG. 3A** shows piggyback cytotoxicity reflected as % inhibition for selected anti-CEACAM5 mAbs with secondary VHH-MMAE and **FIG. 3B** shows internalization rates of selected antibodies in MKN45 cells. **FIG. 3C** shows cytotoxicity as a function of internalization in MKN45 cells. The internalization data for **FIG. 3B** and **FIG. 3C** are data for each mAb with a secondary anti-Fab pHrodo Red flurophore secondary. The cytotoxicity assay data in **FIG. 3C** depict percent inhibition for select CEACAM5 mAbs delivering the cytotoxic agent MMAE via a secondary antibody (Vhh) that binds to the kappa portion of the mAb. Cytotoxic inhibition and internalization data was also generated using CEACAM5-low expressing Ls174T cells (see **FIG. 4A** and **FIG. 4B**). Data show that the generated anti-CEACAM5 antibodies had cytotoxic activity and internalization ability in both CEACAM5-expressing cells with differential antigen density.

[0521] **FIGS. 5A-5H** show % growth inhibition data for selected antibody-conjugate-DXd treated cells compared to isotype and benchmark controls at the indicated antibody concentrations in: negative HCT-116 (**FIG. 5A** and **FIG. 5C**), BxPC-3, a medium CEACAM5 expressing cell line (**FIG. 5B** and **FIG. 5D**), and the engineered HCT-116 CEACAM5 expressing lines, HCT-CEA (**FIG. 5E** and **FIG. 5G**) and MKN45 (**FIG. 5F and FIG. 5H**). **FIG. 5C, FIG. 5D**, **FIG. 5G,** and **FIG. 5H** show IC50 and Amax values, where IC50 values reflect 50% growth inhibition relative to maximum growth inhibition, and where Amax values reflect maximal growth inhibition. **Table 15** summarizes the results obtained from the ADC (DXd) cytotoxicity assays across different CEACAM5-expressing cell lines. Bin 2 (Control #2) antibodies were used as controls.

Table 15. Summary of results obtained from ADC (DXd) cytotoxicity assay.

	IC50 (nM)				Amax			
Antibody	Ls174T	BxPC3	MKN45	HCT116-CEACAM5	Ls174T	BxPC3	MKN45	HCT116-CEACAM5
MBN001	26.9	13.3	1.72	0.32	66.9	69.6	75.5	90.5
Control-2	29.2	9.27	0.67	0.08	64.3	71.9	78.2	90.2

Table 16. Summary table of amino acid sequences and nucleic acid sequences

Description	Amino acid Sequence or nucleic acid sequence	SEQ ID NO.
H. sapiens CEACAM5 amino acid sequence GenBank: AAH34671.1	MESPSAPPHRWCIPWQRLLLTASLLTFWNPPTTAKLTIESTPFNVAEGKEVLL LVHNLPQHLFGYSWYKGERVDGNRQIIGYVIGTQQATPGPAYSGREIIYPNAS LLIQNIIQNDTGFYTLHVIKSDLVNEEATGQFRVYPELPKPSISSNNSKPVED KDAVAFTCEPETQDATYLWWVNNQSLPVSPRLQLSNGNRTLTLFNVTRNDSAS YKCETQNPVSARRSDSVILNVLYGPDAPTISPLNTSYRSGENLNLSCHAASNP PAQYSWFVNGTFQQSTQELFIPNITVNNSGSYTCQAHNSDTGLNRTTVTTITV YAEPPKPFITSNNSNPVEDEDAVALTCEPEIQNTTYLWWVNNQSLPVSPRLQL SNDNRTLTLLSVTRNDVGPYECGIQNELSVDHSDPVILNVLYGPDDPTISPSY	1

	TYYRPGVNLSLSCHAASNPPAQYSWLIDGNIQQHTQELFISNITEKNSGLYTC QANNSASGHSRTTVKTITVSAELPKPSISSNNSKPVEDKDAVAFTCEPEAQNT TYLWWVNGQSLPVSPRLQLSNGNRTLTLFNVTRNDARAYVCGIQNSVSANRSD PVTLDVLYGPDTPIISPPDSSYLSGANLNLSCHSASNPSPQYSWRINGIPQQH TQVLFIAKITPNNNGTYACFVSNLATGRNNSIVKSITVSASGTSPGLSAGATV GIMIGVLVGVALI	
H. sapiens CEACAM5 nucleotide sequence GenBank: BC034671.1	GGACAGCAGACCAGACAGTCACAGCAGCCTTGACAAAACGTTCCTGGAACTCA AGCTCTTCTCCACAGAGGAGGACAGAGCAGACAGCAGAGACCATGGAGTCTCC CTCGGCCCCTCCCCACAGATGGTGCATCCCCTGGCAGAGGCTCCTGCTCACAG CCTCACTTCTAACCTTCTGGAACCCGCCCACCACTGCCAAGCTCACTATTGAA TCCACGCCGTTCAATGTCGCAGAGGGGAAGGAGGTGCTTCTACTTGTCCACAA TCTGCCCCAGCATCTTTTTGGCTACAGCTGGTACAAAGGTGAAAGAGTGGATG GCAACCGTCAAATTATAGGATATGTAATAGGAACTCAACAAGCTACCCCAGGG CCCGCATACAGTGGTCGAGAGATAATATACCCCAATGCATCCCTGCTGATCCA GAACATCATCCAGAATGACACAGGATTCTACACCCTACACGTCATAAAGTCAG ATCTTGTGAATGAAGAAGCAACTGGCCAGTTCCGGGTATACCCGGAGCTGCCC AAGCCCTCCATCTCCAGCAACAACTCCAAACCCGTGGAGGACAAGGATGCTGT GGCCTTCACCTGTGAACCTGAGACTCAGGACGCAACCTACCTGTGGTGGGTAA ACAATCAGAGCCTCCCGGTCAGTCCCAGGCTGCAGCTGTCCAATGGCAACAGG ACCCTCACTCTATTCAATGTCACAAGAAATGACTCAGCAAGCTACAAATGTGA AACCCAGAACCCAGTGAGTGCCAGGCGCAGTGATTCAGTCATCCTGAATGTCC TCTATGGCCCGGATGCCCCCACCATTTCCCCTCTAAACACATCTTACAGATCA GGGGAAAATCTGAACCTCTCCTGCCACGCAGCCTCTAACCCACCTGCACAGTA CTCTTGGTTTGTCAATGGGACTTTCCAGCAATCCACCCAAGAGCTCTTTATCC CCAACATCACTGTGAATAATAGTGGATCCTATACGTGCCAAGCCCATAACTCA GACACTGGCCTCAATAGGACCACAGTCACGACGATCACAGTCTATGCAGAGCC ACCCAAACCCTTCATCACCAGCAACAACTCCAACCCCGTGGAGGATGAGGATG CTGTAGCCTTAACCTGTGAACCTGAGATTCAGAACACAACCTACCTGTGGTGG GTAAATAATCAGAGCCTCCCGGTCAGTCCCAGGCTGCAGCTGTCCAATGACAA CAGGACCCTCACTCTACTCAGTGTCACAAGGAATGATGTAGGACCCTATGAGT GTGGAATCCAGAACGAATTAAGTGTTGACCACAGCGACCCAGTCATCCTGAAT GTCCTCTATGGCCCAGACGACCCCACCATTTCCCCCTCATACACCTATTACCG TCCAGGGGTGAACCTCAGCCTCTCCTGCCATGCAGCCTCTAACCCACCTGCAC AGTATTCTTGGCTGATTGATGGGAACATCCAGCAACACACACAAGAGCTCTTT ATCTCCAACATCACTGAGAAGAACAGCGGACTCTATACCTGCCAGGCCAATAA CTCAGCCAGTGGCCACAGCAGGACTACAGTCAAGACAATCACAGTCTCTGCGG AGCTGCCCAAGCCCTCCATCTCCAGCAACAACTCCAAACCCGTGGAGGACAAG GATGCTGTGGCCTTCACCTGTGAACCTGAGGCTCAGAACACAACCTACCTGTG GTGGGTAAATGGTCAGAGCCTCCCAGTCAGTCCCAGGCTGCAGCTGTCCAATG GCAACAGGACCCTCACTCTATTCAATGTCACAAGAAATGACGCAAGAGCCTAT GTATGTGGAATCCAGAACTCAGTGAGTGCAAACCGCAGTGACCCAGTCACCCT GGATGTCCTCTATGGGCCGGACACCCCCATCATTTCCCCCCCAGACTCGTCTT ACCTTTCGGGAGCGAACCTCAACCTCTCCTGCCACTCGGCCTCTAACCCATCC CCGCAGTATTCTTGGCGTATCAATGGGATACCGCAGCAACACACACAAGTTCT CTTTATCGCCAAAATCACGCCAAATAATAACGGGACCTATGCCTGTTTTGTCT CTAACTTGGCTACTGGCCGCAATAATTCCATAGTCAAGAGCATCACAGTCTCT GCATCTGGAACTTCTCCTGGTCTCTCAGCTGGGGCCACTGTCGGCATCATGAT TGGAGTGCTGGTTGGGGTTGCTCTGATATAGCAGCCCTGGTGTAGTTTCTTCA TTTCAGGAAGACTGACAGTTGTTTTGCTTCTTCCTTAAAGCATTTGCAACAGC TACAGTCTAAAATTGCTTCTTTACCAAGGATATTTACAGAAAAGACTCTGACC AGAGATCGAGACCATCCTAGCCAACATCGTGAAACCCCATCTCTACTAAAAAT ACAAAAATGAGCTGGGCTTGGTGGCGCGCACCTGTAGTCCCAGTTACTCGGGA GGCTGAGGCAGGAGAATCGCTTGAACCCGGGAGGTGGAGATTGCAGTGAGCCC AGATCGCACCACTGCACTCCAGTCTGGCAACAGAGCAAGACTCCATCTCAAAA AGAAAAGAAAAGAAGACTCTGACCTGTACTCTTGAATACAAGTTTCTGATACC ACTGCACTGTCTGAGAATTTCCAAAACTTTAATGAACTAACTGACAGCTTCAT GAAACTGTCCCCCAAGATCAAGCAGAGAAAATAATTAATTTCATGGGACTAAA TGAACTAATGAGGATAATATTTTCATAATTTTTTATTTGAAATTTTGCTGATT CTTTAAATGTCTTGTTTCCCAGATTTCAGGAAACTTTTTTTCTTTTAAGCTAT	2

	CCACAGCTTACAGCAATTTGATAAAATATACTTTTGTGAACAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAA	
Macaca fascicularis CEACAM5 amino acid sequence NCBI XP_005589491.2	MGSPSAPLHRWCIPWQTLLLTASLLTFWNPPTTAQLTIESRPFNVAEGKEVLL LAHNVSQNLFGYIWYKGERVDASRRIGSCVIRTQQITPGPAHSGRETIDFNAS LLIHNVTQSDTGSYTIQVIKEDLVNEEATGQFRVYPELPKPYISSNNSNPVED KDAVALTCEPETQDTTYLWWVNNQSLPVSPRLELSSDNRTLTVFNIPRNDTTS YKCETQNPVSVRRSDPVTLNVLYGPDAPTISPLNTPYRAGENLNLSCHAASNP AAQYSWFVNGTFQQSTQELFIPNITVNNSGSYMCQAHNSATGLNRTTVTAITV YAELPKPYITSNNSNPIEDKDAVTLTCEPETQDTTYLWWVNNQSLSVSSRLEL SNDNRTLTVFNIPRNDTTFYECETQNPVSVRRSDPVTLNVLYGPDAPTISPLN TPYRAGENLNLSCHAASNPAAQYSWFVNGTFQQSTQELFIPNITVNNSGSYMC QAHNSATGLNRTTVTAITVYVELPKPYISSNNSNPIEDKDAVTLTCEPVAENT TYLWWVNNQSLSVSPRLQLSNGNRILTLLSVTRNDTGPYECGIQNSESAKRSD PVTLNVTYGPDTPIISPPDLSYRSGANLNLSCHSDSNPSPQYSWLINGTLRQH TQVLFISKITSNNNGAYACFVSNLATGRNNSIVKNISVSSGDSAPGSSGLSAR ATVGIIIGMLVGVALM	3
Macaca fascicularis CEACAM5 nucleotide sequence NCBI: XM_005589434.3	GATGCCGAGAAGTACTCCTGCTGTAGGAGGAGACTCAGGACAGAGGGAGGAAG GACAGCAGACCAGGCAGTCACAGCTGCCCTGACAAGAGCGTTCCTAGAGCTCA GGATCTTCTCCACAGAAGAGGACAGAGCAGACAGCAGAGACCATGGGGTCTCC CTCGGCCCCTCTTCACAGATGGTGCATCCCCTGGCAGACGCTCCTGCTCACAG CCTCACTTCTAACCTTCTGGAACCCGCCCACCACTGCCCAGCTCACTATTGAA TCCAGGCCGTTCAATGTTGCAGAGGGGAAGGAGGTTCTTCTACTTGCCCACAA TGTGTCCCAGAATCTTTTTGGCTACATCTGGTACAAGGGAGAAAGAGTGGATG CCAGCCGTCGAATTGGATCATGTGTAATAAGAACTCAACAAATTACCCCAGGG CCCGCACACAGCGGTCGAGAGACAATAGACTTCAATGCATCCCTGCTGATCCA CAATGTCACCCAGAGTGACACAGGATCCTACACCATACAAGTCATAAAGGAAG ATCTTGTGAATGAAGAAGCAACTGGCCAGTTCCGGGTATACCCGGAGCTGCCC AAGCCCTACATCTCCAGCAACAACTCCAACCCTGTGGAGGACAAGGATGCTGT GGCCTTAACCTGTGAACCTGAGACTCAGGACACAACCTACCTGTGGTGGGTAA ACAATCAGAGCCTCCCGGTCAGTCCCAGGCTGGAGCTGTCCAGTGACAACAGG ACCCTCACTGTATTCAATATTCCAAGAAATGACACAACATCCTACAAATGTGA AACCCAGAACCCAGTGAGTGTCAGACGCAGCGACCCAGTCACCCTGAATGTCC TCTATGGCCCGGATGCGCCCACCATTTCCCCTCTAAACACACCTTACAGAGCA GGGGAAAATCTGAACCTCTCCTGCCACGCAGCCTCTAACCCAGCTGCACAGTA CTCTTGGTTTGTCAATGGGACGTTCCAGCAATCCACACAAGAGCTCTTTATAC CCAACATCACCGTGAATAATAGCGGATCCTATATGTGCCAAGCCCATAACTCA GCCACTGGCCTCAATAGGACCACAGTCACGGCGATCACAGTCTACGCGGAGCT GCCCAAGCCCTACATCACCAGCAACAACTCCAACCCCATAGAGGACAAGGATG CTGTGACCTTAACCTGTGAACCTGAGACTCAGGACACAACCTACCTGTGGTGG GTAAACAATCAGAGCCTCTCGGTCAGTTCCAGGCTGGAGCTGTCCAATGACAA CAGGACCCTCACTGTATTCAATATTCCAAGAAACGACACAACGTTCTACGAAT GTGAAACCCAGAACCCAGTGAGTGTCAGACGCAGCGACCCAGTCACCCTGAAT GTCCTCTATGGCCCGGATGCGCCCACCATTTCCCCTCTAAACACACCTTACAG AGCAGGGGAAAATCTGAACCTCTCCTGCCACGCAGCCTCTAACCCAGCTGCAC AGTACTCTTGGTTTGTCAATGGGACGTTCCAGCAATCCACACAAGAGCTCTTT ATACCCAACATCACCGTGAATAATAGCGGATCCTATATGTGCCAAGCCCATAA CTCAGCCACTGGCCTCAATAGGACCACAGTCACGGCGATCACAGTCTATGTGG AGCTGCCCAAGCCCTACATCTCCAGCAACAACTCCAACCCCATAGAGGACAAG GATGCTGTGACCTTAACCTGTGAACCTGTGGCTGAGAACACAACCTACCTGTG GTGGGTAAACAATCAGAGCCTCTCGGTCAGTCCCAGGCTGCAGCTCTCCAATG GCAACAGGATCCTCACTCTACTCAGTGTCACACGGAATGACACAGGACCCTAT GAATGTGGAATCCAGAACTCAGAGAGTGCAAAACGCAGTGACCCAGTCACCCT GAATGTCACCTATGGCCCAGACACCCCCATCATATCCCCCCCAGACTTGTCTT ACCGTTCGGGAGCAAACCTCAACCTCTCCTGCCACTCGGACTCTAACCCATCC CCGCAGTATTCTTGGCTTATCAATGGGACACTGCGGCAACACACACAAGTTCT CTTTATCTCCAAAATCACATCAAACAATAACGGGGCCTATGCCTGTTTTGTCT CTAACTTGGCTACTGGTCGCAATAACTCCATAGTCAAGAACATCTCAGTCTCC TCTGGCGATTCAGCACCTGGAAGTTCTGGTCTCTCAGCTAGGGCTACTGTCGG	4

	CATCATAATTGGAATGCTGGTTGGGGTTGCTCTGATGTAGCAGCCGTGGTGTA GTTTCTGCAATTAAGAAAGACTGACAGTTGTTTTGATTCTTCCTTAAAGCATT TGCAACAGCTACAGTCTAAAATTGCCTCTTTACCAAGGATATTTATAGAAAAG ACTCTGACCAGAGATCGAGACCATCCTAGCCAATATGGTGAAACCCCATCTCT ACGGAATTAGCTGGGCGTGGTGGTGTGCTCCTGTAGTCCCAGCTACTCGGGAG GCTGAGGCAGGAGAATCGCTTGAACCTGGGAAGCAGAGATTGCAGTGAGCCAA GATCGCGCCACTGCACTCCAGCCTGGCGACAGAGCAAGACTCCATATCAAAAA AAAAAAAAAAAAGTATATATATATATGAAAGAAAAGACTCTGACCTGTACTCT TGAATGAAAGTTTCTGATACCACTGGACTGTCTGAGAATTTCCAAAACGTAAT GAACAAACTGACAGCTTCATGAAACTGCCGACCAAGATCAAGCAAAGAAAATA ATTAATTTCATGGGACCAAATGAACTAATGAGGATAATATTTTCATAAATTTT TTTTGAAATTTTGCTGATTCTTTAAATGTCTTGTTTCCCAGATTTCAGGAAAC TTTTTTTCCTTTGCGCTATCTACAGCTTACAACAATTTGATAAAATATACTTT TGTGAATAAACATTGAGACATTTACATTTTCTCCCTATGTGGTCGCTCCAGAC TTGGGAAACTATTCATGAATATTTATATTGTATGGTAATATGTTTATTGTATA AGTTCAATAACTCTGCTCTTTGTATAACAGAA	
MBN001 HCDR1 amino acid sequence	SHGMH	14
MBN001 HCDR2 amino acid sequence	FISYDGSYKSYVDSVKG	15
MBN001 HCDR3 amino acid sequence	GLTGTGAFDI	16
MBN001 VH amino acid sequence	QVQLVETGGGVVQPGRSLRLSCAASGITFSSHGMHWVRQSPGKGLEWVTFISY DGSYKSYVDSVKGRFAISRDNSKNTLYLQMNSLRPEDTAVYYCATGLTGTGAF DIWGQGTMVTVSS	17
MBN001 VH nucleotide sequence	CAGGTGCAGCTGGTGGAGACTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCT GAGACTCTCCTGTGCAGCCTCTGGAATCACCTTCAGTAGCCATGGCATGCACT GGGTCCGCCAGTCTCCAGGCAAGGGGCTGGAGTGGGTGACATTTATATCATAT GATGGAAGTTATAAATCCTATGTCGACTCCGTGAAGGGCCGATTCGCCATCTC CAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGACCTG AGGACACGGCTGTGTATTACTGTGCGACCGGATTAACTGGAACTGGTGCTTTT GATATCTGGGGCCAAGGGACAATGGTCACCGTCTCCTCA	18
MBN001 LCDR1 amino acid sequence	RASQSVSSSYLA	19
MBN001 LCDR2 amino acid sequence	GASSRAT	20
MBN001 LCDR3 amino acid sequence	QQYGSSPYT	21
MBN001 VL amino acid sequence	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGAS SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPYTFGQGTKLE IK	22
MBN001 VL nucleotide sequence	GAAATTGTATTGACGCAGTCTCCAGGGACCCTGTCTTTGTCTCCAGGGGAAAG AGCCACCCTCTCCTGCCGGGCCAGTCAGAGTGTCTCCAGCTCGTACTTAGCCT GGTACCAACAGAAACCTGGCCAGGCCCCCCGACTCCTCATCTATGGTGCCTCC TCAAGGGCCACTGGAATCCCAGACAGATTCAGTGGGAGTGGGTCTGGGACAGA CTTCACTCTCACCATCTCAAGACTGGAGCCTGAAGATTTTGCCGTATATTACT	23

	GTCAACAGTATGGGAGCTCACCGTACACTTTTGGCCAGGGGACCAAGCTGGAG ATCAAA	
6xHis-A3-B3 huCEACAM5 amino acid sequence	HHHHHHETVRFQGPKPSISSNNSKPVEDKDAVAFTCEPEAQNTTYLWWVNGQS LPVSPRLQLSNGNRTLTLFNVTRNDARAYVCGIQNSVSANRSDPVTLDVLYGP DTPIISPPDSSYLSGANLNLSCHSASNPSPQYSWRINGIPQQHTQVLFIAKIT PNNNGTYACFVSNLATGRNNSIVKSITVSASGTSPGLSA	24
Human CEACAM5 (aa 569-587) amino acid sequence	CGIQNSVSANRSDPVTL	25
Human CEACAM5 (aa 626-640) amino acid sequence	SWRINGIPQQHTQVL	26
Human CEACAM5 (aa 674-685) amino acid sequence	VSASGTSPGLSA	27
Human CEACAM5 (aa 618-641) amino acid sequence	ASNPSPQYSWRINGIPQQHTQVLF	28
Human CEACAM5 (590-605) amino acid sequence	LYGPDTPIISPPDSSY	29
hIgG1.3f amino acid sequence	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKT HTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPG	30
Cleavable linker amino acid sequence	PVGVV	31
Human CEACAM5 (aa 618-625) amino acid sequence	ASNPSPQY	32
Human CEACAM5 (aa 607-613)	SGANLNL	33

amino acid sequence		
Human CEACAM5 (aa 629-641) amino acid sequence	INGIPQQHTQVLF	34
MBP001 HCDR1 amino acid sequence	SHGMH	35
MBP001 HCDR2 amino acid sequence	FISYDGSYKSYVDSVKG	36
MBP001 HCDR3 amino acid sequence	GLTGTGAFDI	37
MBP001 VH amino acid sequence	QVQLVESGGGVVQPGRSLRLSCAASGIYFSSHGMHWVRQAPGKGLEWVTFISY DGSYKSYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCATGLTGTGAF DIWGQGTMVTVSS	38
MBP001 VH nucleotide sequence	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCT GAGACTCTCCTGTGCAGCCTCTGGAATCTACTTCAGTAGCCATGGCATGCACT GGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGACATTTATATCATAT GATGGAAGTTATAAATCCTATGTCGACTCCGTGAAGGGCCGATTCACCATCTC CAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCTG AGGACACGGCTGTGTATTACTGTGCGACCGGATTAACTGGAACTGGTGCTTTT GATATCTGGGGCCAAGGGACAATGGTCACCGTCTCCTCA	39
MBP001 LCDR1 amino acid sequence	RASQSVSSSYLA	40
MBP001 LCDR2 amino acid sequence	GASSRAT	41
MBP001 LCDR3 amino acid sequence	QQYGSSPYT	42
MBP001 VL amino acid sequence	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGAS SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPYTFGQGTKLE IK	43
MBP001 VL nucleotide sequence	GAAATTGTATTGACGCAGTCTCCAGGGACCCTGTCTTTGTCTCCAGGGGAAAG AGCCACCCTCTCCTGCCGGGCCAGTCAGAGTGTCTCCAGCTCGTACTTAGCCT GGTACCAACAGAAACCTGGCCAGGCCCCCCGACTCCTCATCTATGGTGCCTCC TCAAGGGCCACTGGAATCCCAGACAGATTCAGTGGGAGTGGGTCTGGGACAGA CTTCACTCTCACCATCTCAAGACTGGAGCCTGAAGATTTTGCCGTATATTACT	44

	GTCAACAGTATGGGAGCTCACCGTACACTTTTGGCCAGGGGACCAAGCTGGAG ATCAAA	
MBP001 HC amino acid sequence	QVQLVESGGGVVQPGRSLRLSCAASGIYFSSHGMHWVRQAPGKGLEWVTFISY DGSYKSYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCATGLTGTGAF DIWGQGTMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPG	45
MBP001 LC amino acid sequence	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGAS SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPYTFGQGTKLE IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC	46
MBP001 HC nucleotide sequence	CAGGTCCAGCTCGTGGAATCCGGAGGCGGAGTGGTGCAGCCGGGGAAGATCACT GCGCCTGTCATGCGCAGCCTCGGGGATCTACTTTTCGTCCCACGGAATGCATT GGGTCCGCCAAGCTCCCGGAAAGGGTTTGGAATGGGTCACCTTCATTAGCTAC GACGGCTCCTACAAGTCGTACGTGGACTCCGTGAAGGGGAGGTTCACTATCTC CCGGGACAACAGCAAGAACACGCTGTACCTCCAAATGAACTCCCTTCGGGCCG AGGATACCGCCGTGTACTATTGTGCCACCGGTCTGACCGGCACTGGAGCGTTC GATATCTGGGGCCAGGGCACTATGGTCACCGTGTCCAGCGCTAGCACCAAGGG CCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAG CGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCG TGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACA GTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCT TGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAG GTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACC GTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAA AACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTG GTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGG CGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCA CGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGC AAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAA AACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGC CCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTC AAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCC GGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCT TCCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTC TTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGTC ACTCTCCCTGTCCCCGGGT	47
MBP001 LC nucleotide sequence	GAAATTGTATTGACGCAGTCTCCAGGGACCCTGTCTTTGTCTCCAGGGGAAAG AGCCACCCTCTCCTGCCGGGCCAGTCAGAGTGTCTCCAGCTCGTACTTAGCCT GGTACCAACAGAAACCTGGCCAGGCCCCCCGACTCCTCATCTATGGTGCCTCC TCAAGGGCCACTGGAATCCCAGACAGATTCAGTGGGAGTGGGTCTGGGACAGA CTTCACTCTCACCATCTCAAGACTGGAGCCTGAAGATTTTGCCGTATATTACT GTCAACAGTATGGGAGCTCACCGTACACTTTTGGCCAGGGGACCAAGCTGGAG ATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGA GCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATC CCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAAC TCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAG CAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCT GCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGG GGAGAGTGT	48

MBP002 VH amino acid sequence	QVQLVESGGGVVQPGRSLRLSCAASGITFSSHGMHWVRQAPGKGLEWVTFISY DGSYKSYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCATGLTGTNAL DIWGQGTMVTVSS	49
MBP002 VL amino acid sequence	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYYAS SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGRSPYTFGQGTKLE IK	50
MBP003 VH amino acid sequence	QVQLVESGGGVVQPGRSLRLSCAASGITFSSHGMHWVRQAPGKGLEWVTFISY DGSYKSYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCATGLTGTGAF DIWGQGTMVTVSS	51
MBP003 VL amino acid sequence	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGAS SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGRSPYTFGQGTKLE IK	52
MBP002 VH nucleotide sequence	CAGGTCCAACTGGTGGAAAGCGGCGGTGGAGTGGTGCAGCCTGGACGGTCCCT GAGACTCTCATGTGCCGCCAGCGGAATCACCTTTTCGTCCCATGGCATGCACT GGGTCCGCCAAGCACCGGGAAAGGGGCTGGAATGGGTCACCTTCATTTCCTAC GATGGCTCGTACAAGTCCTACGTGGACTCAGTGAAAGGGAGGTTCACGATCTC CCGCGACAACTCGAAGAACACCCTGTACTTGCAAATGAACAGCCTGCGGGCTG AGGATACTGCCGTGTACTATTGCGCCACCGGACTTACCGGAACTAATGCGCTC GACATCTGGGGCCAGGGTACCATGGTCACTGTGTCCTCC	53
MBP002 VL nucleotide sequence	GAAATTGTATTGACGCAGTCTCCAGGGACCCTGTCTTTGTCTCCAGGGGAAAG AGCCACCCTCTCCTGCCGGGCCAGTCAGAGTGTCTCCAGCTCGTACTTAGCCT GGTACCAACAGAAACCTGGCCAGGCCCCCCGACTCCTCATCTATTACGCCTCC TCAAGGGCCACTGGAATCCCAGACAGATTCAGTGGGAGTGGGTCTGGGACAGA CTTCACTCTCACCATCTCAAGACTGGAGCCTGAAGATTTTGCCGTATATTACT GTCAACAGTATGGGCGTTCACCGTACACTTTTGGCCAGGGGACCAAGCTGGAG ATCAAA	54
MBP003 VH nucleotide sequence	CAAGTGCAGTTGGTGGAAAGCGGAGGCGGAGTGGTGCAGCCCGGAAGAAGCCT GCGCCTTTCTTGTGCCGCTAGCGGTATCACGTTCTCCTCACATGGGATGCACT GGGTCCGCCAAGCACCGGGAAAGGGCCTGGAATGGGTCACCTTCATCTCGTAC GACGGTTCATATAAGTCGTACGTGGATTCCGTGAAAGGGCGGTTCACTATTTC CCGGGACAACTCCAAGAACACCCTCTACCTCCAAATGAACTCCCTGAGGGCCG AGGATACCGCCGTGTACTACTGCGCGACCGGACTGACCGGAACTGGCGCCTTT GACATCTGGGGCCAGGGCACTATGGTCACCGTGTCGTCC	55
MBP003 VL nucleotide sequence	GAAATTGTATTGACGCAGTCTCCAGGGACCCTGTCTTTGTCTCCAGGGGAAAG AGCCACCCTCTCCTGCCGGGCCAGTCAGAGTGTCTCCAGCTCGTACTTAGCCT GGTACCAACAGAAACCTGGCCAGGCCCCCCGACTCCTCATCTATGGTGCCTCC TCAAGGGCCACTGGAATCCCAGACAGATTCAGTGGGAGTGGGTCTGGGACAGA CTTCACTCTCACCATCTCAAGACTGGAGCCTGAAGATTTTGCCGTATATTACT GTCAACAGTATGGGCGTTCACCGTACACTTTTGGCCAGGGGACCAAGCTGGAG ATCAAA	56
aa 571-587 of SEQ ID NO: 24	YVCGIQNSVSANRSDPVTL	57
aa 626-640 of SEQ ID NO: 24	SWRINGIPQQHTQVL	58
aa 590-605 of SEQ ID NO: 24	DVLYGPDTPIISPPDSSY	59

(aa 674-685 of SEQ ID NO: 24)	ITVSASGTSPGLSA	60
MBN001 Mutational Scan HCDR1 amino acid sequence	GITFSSH	61
MBN001 Mutational Scan HCDR2 amino acid sequence	TFISYDGSYKSYVDSVKG	62
MBN001 Mutational Scan HCDR3 amino acid sequence	TGLTGTGAFDI	63
MBN001 Mutational Scan LCDR1 amino acid sequence	RASQSVSSSYLA	64
MBN001 Mutational Scan LCDR2 amino acid sequence	GASSRAT	65
MBN001 Mutational Scan LCDR3 amino acid sequence	QQYGSSPY	66
MBP004 VH amino acid sequence	QVQLVESGGGVVQPGRSLRLSCAASGITFSSHGMHWVRQAPGKGLEWVTFISY DGSYKSYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCATGLTGTGAF DIWGQGTMVTVSS	67
MBP004 VL amino acid sequence	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGAS SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGRSPYTFGQGTKLE IK	68
MBP005 VH amino acid sequence	QVQLVESGGGVVQPGRSLRLSCAASGIYFSSHGMHWVRQAPGKGLEWVTFISY DGSYKSYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCATGLTGTGAF DIWGQGTMVTVSS	69
MBP005 VL amino acid sequence	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGAS SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPYTFGQGTKLE IK	70
MBP006 VH amino acid sequence	QVQLVESGGGVVQPGRSLRLSCAASGITFSSHGMHWVRQAPGKGLEWVTFISY DGSYISYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCATGLTGTGAN DIWGQGTMVTVSS	71

MBP006 VL amino acid sequence	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGAS SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYKSSPYTFGQGTKLE IK	72
MBP007 VH amino acid sequence	QVQLVESGGGVVQPGRSLRLSCAASGIYFSSHGMHWVRQAPGKGLEWVTFISY DGSYKSYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCATGLTGTGAN DIWGQGTMVTVSS	73
MBP007 VL amino acid sequence	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGAS SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQNKPSPYTFGQGTKLE IK	74
MBP008 VH amino acid sequence	QVQLVESGGGVVQPGRSLRLSCAASGITFSSHGMHWVRQAPGKGLEWVTFISY DGSYKSYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCATGLTGTNAL DIWGQGTMVTVSS	75
MBP008 VL amino acid sequence	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYYAS SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGRSPYTFGQGTKLE IK	76
MBP009 VH amino acid sequence	QVQLVESGGGVVQPGRSLRLSCAASGIYFSSHGMHWVRQAPGKGLEWVTFISY DGSYKSYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCATGLTGTGAF DIWGQGTMVTVSS	77
MBP009 VL amino acid sequence	EIVLTQSPGTLSLSPGERATLSCRASQSVSSIYLAWYQQKPGQAPRLLIYYAS SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPYTFGQGTKLE IK	78
MBP010 VH amino acid sequence	QVQLVESGGGVVQPGRSLRLSCAASGITFSSHGMHWVRQAPGKGLEWVTFISY DSSYKSYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCATGLTGTGAF DIWGQGTMVTVSS	79
MBP010 VL amino acid sequence	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGAS SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQNKRSPYTFGQGTKLE IK	80
MBP011 VH amino acid sequence	QVQLVESGGGVVQPGRSLRLSCAASGITFSSHGMHWVRQAPGKGLEWVTFISY DGSIKSYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCATGLTGTGAF DIWGQGTMVTVSS	81
MBP011 VL amino acid sequence	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGAS SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYKRSPYTFGQGTKLE IK	82
MBP012 VH amino acid sequence	QVQLVESGGGVVQPGRSLRLSCAASGITFSSHGMHWVRQAPGKGLEWVTFASY DGSYKQYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCATGLTGTGAF DIWGQGTMVTVSS	83
MBP012 VL amino acid sequence	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGAS SRNTGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYKRSPYTFGQGTKLE IK	84
MBP013 VH amino acid sequence	QVQLVESGGGVVQPGRSLRLSCAASGIYFSSHGMHWVRQAPGKGLEWVTFISY DGSIKSYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCATGLTGTNAF DIWGQGTMVTVSS	85
MBP013 VL amino acid sequence	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYYAS SRNTGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPYTFGQGTKLE IK	86
MBP014 VH amino acid sequence	QVQLVESGGGVVQPGRSLRLSCAASGITFSSHGMHWVRQAPGKGLEWVTFISY DSSYKLYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCATGLTGTGAF DIWGQGTMVTVSS	87

MBP014 VL amino acid sequence	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYYAS SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYGSSPYTFGQGTKLE IK	88
MBP015 VH amino acid sequence	QVQLVESGGGVVQPGRSLRLSCAASGIYFSSHGMHWVRQAPGKGLEWVTFITY DGSYKSYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCATGLTGTGAF DIWGQGTMVTVSS	89
MBP015 VL amino acid sequence	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYYAS SRNTGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPYTFGQGTKLE IK	90
MBP016 VH amino acid sequence	QVQLVESGGGVVQPGRSLRLSCAASGITFSSHGMHWVRQAPGKGLEWVTFSSY DGSYKSYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCATGLTGTNAF DIWGQGTMVTVSS	91
MBP016 VL amino acid sequence	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGAS SRNTGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYKSSPYTFGQGTKLE IK	92
MBP017 VH amino acid sequence	QVQLVESGGGVVQPGRSLRLSCAASGITFSSHGMHWVRQAPGKGLEWVTFISY DGSYKSYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCATGLTGTGAN DIWGQGTMVTVSS	93
MBP017 VL amino acid sequence	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYYAS SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPYTFGQGTKLE IK	94

Example 13: Mutational scan and optimization of anti-CEACAM5 mAb MBN001

[0522] Mutational scanning was performed to identify variants of MBN001 with improved affinity for human and/or cynomolgus CEACAM5, as described in **FIG. 6A**. A single chain variable fragment (scFv) library was created that allows for single amino acid substitutions in the CDRs using NNK oligos. For each CDR, multiple oligos were designed that incorporated an NNK codon at each position (N=A, C, T, G and K=G, T) to allow for the encoding of all 20 amino acids plus a stop codon at each position. The Kabat definition was used for all CDRs, except for HCDR1, where the AbM definition was used. In some cases, CDR residues were omitted from the scan or additional non-germline Vernier zone amino acids were included in the scan. In particular, Vernier zone residues in the VH at positions 49 and 94 were included in the library design. Positions 33-35 in HCDR1 as well as position 97 in LCDR3 were omitted from the scan. Additionally, the following framework residues in the VH were reverted to germline in the library design: T7S, S40A, A68T, P84A. The diversified positions (MBN001 mutational scan for the analysis of CDR positions) are detailed in **FIG. 6B.**

[0523] The library was expressed in an mRNA display system (Xu et.al. (2002) *Chemistry & Biology* 9: 933-942; Roberts and Szostak (1997) *Proc. Natl. Acad. Sci* 94: 12297-12302) and

taken through a single round of selection against human and cynomolgus CEACAM5. Briefly, the DNA library was subjected to transcription and translation in a manner that fuses the scFv protein to the encoding mRNA via a puromycin linkage. The scFv-mRNA fusions were exposed to biotinylated human CEACAM5 and biotinylated cynomolgus CEACAM5 in separate selections. scFvs that bound the targets were captured by streptavidin beads, eluted, and amplified by PCR. The captured antibodies were sequenced by NGS. The frequency of each sequence in the post-selection population was divided by the frequency of each selection in the starting population to calculate an enrichment ratio. This enrichment ratio was normalized to the enrichment ratio of the parent antibody sequence to generate a normalized enrichment ratio:

$$Normalized\ enrichment\ ratio = \frac{(frequency\ after\ selection)/(frequency\ in\ starting\ library)}{(frequency\ of\ parent\ antibody\ after\ selection)/(frequency\ of\ parent\ antibody\ in\ starting\ library)}$$

[0524] Using these normalized enrichment ratios (ERs), heat maps were generated to assess the effect of every single amino acid substitution upon the binding to human and cynomolgus CEACAM5. The error in the method was approximately 2-fold, and so ER values from 0.5 to 2 were considered to be neutral, while values over 2 were considered to be favorable, and values under 0.5 were considered to be unfavorable. This analysis provided a rich set of information about the effect of single amino acid substitutions, as is shown in **FIG. 6C** (MBN001 HCDR1 Human CEACAM5), **FIG. 6D** (MBN001 HCDR1 Cynomolgus CEACAM5), **FIG. 6E** (MBN001 HCDR2 Human CEACAM5), **FIG. 6F** (MBN001 HCDR2 Cynomolgus CEACAM5), **FIG. 6G** (MBN001 HCDR3 Human CEACAM5), **FIG. 6H** (MBN001 HCDR3 Cynomolgus CEACAM5), **FIG. 6I** (MBN001 LCDR1 Human CEACAM5), **FIG. 6J** (MBN001 LCDR1 Cynomolgus CEACAM5), **FIG. 6K** (MBN001 LCDR2 Human CEACAM5), **FIG. 6L** (MBN001 LCDR2 Cynomolgus CEACAM5), **FIG. 6M** (MBN001 LCDR3 Human CEACAM5), and **FIG. 6N** (MBN001 LCDR3 Cynomolgus CEACAM5), and as summarized in **Table 17.** Using the deep mutational scanning data, it is shown that many CDR positions (for example as shown for HCDR1-3 and LCDR1-3 in **FIG. 6B)** were tolerant to mutation, meaning that these substitutions can be made in the identified CDR position/amino acid sequence and maintain the desired of the antibody or antigen binding portion ability to bind human CEACAM5 and cynomolgus CEACAM5.

Table 18
Single site mutations in the CDRs of MBN001 that maintain or improve binding to human CEACAM5 and cynomolgus CEACAM5

CDR Region	Position	Amino Acid substitutions that increase affinity to huCEACAM5	Amino Acid substitutions that maintain binding to huCEACAM5	Amino Acid substitutions that increase affinity to cyCEACAM5	Amino Acid substitutions that maintain binding to cyCEACAM5
HCDR1	26		G (parent), T, K, N, E, Q		G (parent), S, T, K, H, Y, N, E
	27		L, I (parent)		L, I (parent)
	28	L, I, M, R, H, F, Y, D	G, A, C, S, T (parent), V, N, E, Q	C, L, I, M, H, F, Y, N	G, A, S, T (parent), V, R, W, D, E, Q
	29		F (parent), Y		F (parent), Y
	30	D	G, S (parent), Y, N, E	C, W	G, S (parent), T, V, L, R, D, E
	31	I, H, F, Y, W, D, E	C, S (parent), L, M, N, Q	H, F, Y, W, E	S (parent), T, V, L, I, M, K, R, N, D, Q
	32		P, L, H (parent), Y, N		P, L, H (parent), Y, N
	33	Not scanned			
	34	Not scanned			
	35	Not scanned			
HCDR2	49 (Vernier zone residue)		G, A, C, S, T (parent), V, L, I, M, F, Y, N, D, E		P, A, C, S, T (parent), V, L, I, M, K, H, F, Y, N, D, Q
	50		C, M, F (parent), Y, E		I, M, F (parent), Y, W
	51	A, S	G, T, V, L, I (parent), M, D, E	A, S	G, T, V, L, I (parent), M, F, D, E, Q
	52	T	S (parent), V	T	S (parent), V
	52a		Y (parent)		H, Y (parent)
	53		D (parent)		D (parent), Q
	54		G (parent), A, C, S, T, M, E, Q	S	G (parent), A, C, L, M, K, H, E, Q
	55		S (parent), T		S (parent), T
	56	I, M	L, Y (parent), W, Q	I, M	L, H, Y (parent), W
	57	P, V, L, I	A, C, S, T, M, K (parent), R, H, F, Y, W, N, D, E	V, L, I	P, A, C, S, T, M, K (parent), R, H, F, Y, W, N, E

	58	T, L, I, M, K, Q	G, A, C, S (parent), V, R, H, F, W, N	T, L, I, M, K, Q	P, G, A, C, S (parent), V, R, F, W, N
	59		G, A, C, S, T, V, L, I, M, K, R, H, F, Y (parent), W, N, D, E, Q		G, A, C, T, V, L, I, M, K, R, H, F, Y (parent), W, N, Q
	60		A, C, S, T, V (parent), L, I, M, F, Y, W, D, E		P, A, C, S, T, V (parent), L, I, K, F, W, D, E,
	61		P, G, A, C, S, T, V, L, I, M, K, R, F, Y, W, N, D (parent), E, Q	C	P, G, A, S, T, V, L, I, M, K, R, H, F, Y, W, N, D (parent), E, Q
	62		P, G, A, C, S (parent), T, V, L, I, M, K, R, H, F, Y, W, N, D, E, Q		P, G, A, C, S (parent), T, V, L, I, M, K, R, H, F, Y, W, D, Q
	63	T	G, A, C, S, V (parent), L, I, M, K, H, F, Y, W	T	P, G, A, C, S, V (parent), L, I, M, K, H, F, Y, W, N, D, E
	64		K (parent), R, H, Y		P, G, A, S, T, K (parent), R, H, Y, N
	65	W	P, G (parent), A, C, S, T, V, L, I, M, K, R, H, F, Y, N, D, E, Q	W	P, G (parent), A, C, S, T, V, L, I, M, K, R, H, F, Y, N, D, E, Q
HCDR3	94 (Vernier zone residue)		T (parent)		T (parent)
	95		G (parent)		G (parent)
	96		C, L (parent)	C	L (parent), Q
	97		T (parent)		T (parent)
	98		G (parent)		G (parent)
	99		T (parent)		T (parent)
	100	L, N	P, G (parent), C, V, I, D	N	P, G (parent), L, D
	100a		A (parent)		A (parent), H

	100b	S, V, L, M, K, H, Y, N	A, C, T, I, F (parent), W, D, Q	S, V, L, M, K, H, Y, N	A, C, T, I, F (parent), W, D, Q
	101		D (parent)		S, D (parent)
	102		V, I (parent)		V, I (parent), F, W
LCDR1	24		G, A, C, S, T, V, L, I, M, K, R (parent), H, F, Y, W, N, D, E, Q		P, G, A, C, S, T, V, L, I, M, R (parent), F, Y, W, N, D, E, Q
	25	P, C	G, A (parent), S, T, V, L, I, M, F, N, D, Q	P, C, L, I, M	G, A (parent), S, T, V, H, F, Y, W, N, E
	26		P, G, A, C, S (parent), T, V, L, I, M, K, R, H, F, Y, W, N, D, E, Q		P, G, A, C, S (parent), T, V, L, I, M, K, R, F, Y, W, N, D, E, Q
	27		P, G, A, C, S, T, V, L, I, M, K, R, H, F, Y, N, D, E, Q (parent)		P, G, C, S, T, V, L, I, M, K, R, H, Y, W, N, E, Q (parent)
	27a		P, G, A, C, S (parent), T, V, L, I, M, K, R, H, F, Y, W, N, D, E, Q	Y, W	P, G, A, C, S (parent), T, V, L, I, M, R, H, F, N, Q
	28	P, C, M, F	S, T, V (parent), L, I, K, Y, D, Q	T, M, F	P, C, V (parent), L, I, K, Y
	29		G, A, C, S (parent), L, H, Y, N, D, E, Q	G	P, C, S (parent), R, W, N, D, E
	30		P, A, S (parent), V, I, D	P	A, S (parent), T, V, I, W
	31	P, V, I, W, Q	G, A, C, S (parent), T, L, M, K, R, H, F, Y, E	V, I, K, W	P, A, C, S (parent), T, L, M, R, H, Y
	32	P, G	A, S, T, V, I, M, H, F, Y (parent), W, N, D		P, G, C, S, T, V, I, M, F, Y (parent), W, N, D, Q
	33	G, A, I	C, S, T, V, L (parent), M, K, R, F, Y, W, N, D, E, Q	I, M, Y, W	G, A, S, V, L (parent), K, R, F, N, E, Q

	34		A (parent), C, S		G, A (parent), C, S, T, M, Y
LCDR2	50	A, S, M, F, Y, W	G (parent), C, T, V, R, H, N, Q	A, C, S, V, M, F, Y, W	G (parent), L, R, H
	51	P, G, C	A (parent), S, T, L, M, H, W, N, Q	P, G, C, H	A (parent), S, T, L, M, K, R, W, Q
	52	Y, Q	G, A, C, S (parent), T, V, L, I, K, R, F, W, N, D, E	F, Y, W, Q	A, C, S (parent), V, L, I, M, E
	53	F, Y, W	P, G, A, C, S (parent), T, V, M, H, N, Q	W	P, A, S (parent), T, H, F, Y, N, Q
	54		P, A, C, T, V, L, I, M, K, R (parent), H, F, Y, W, N, E, Q		P, C, T, L, I, M, K, R (parent), F, W, N, Q
	55	L, H, D	P, G, A (parent), S, M, Y, W, N, Q	L, M, F, Y, N, D	P, G, A (parent), S, T, H
	56		P, C, S, T (parent), V, L, I, M, R, F, Y, W, N, D, Q	P, C, W, N	G, A, S, T (parent), L, I, K, R, H, F, Y
LCDR3	89	S, M, H, F, Y, N, D	G, A, K, E, Q (parent)	S, H, D	P, G, A, M, K, F, Y, N, E, Q (parent)
	90		Q (parent)		Q (parent)
	91	S, T, N	G, A, C, V, F, Y (parent), D, Q	G, C, S, T, N, Q	A, H, Y (parent), D
	92	C, V, L, I, M, K, R, F, Y, W	G (parent), A, S, T, H, N, D, E, Q	L, I, M, K, R, Y, W, N	G (parent), A, C, S, T, V, F, Q
	93	P, V, L, I, M, R, H, F, Y, Q	A, C, S (parent), T, K, W, N	P, C, T, V, L, I, M, K, R, H, F, Y, W, N, Q	A, S (parent)
	94	L, I, F, Y	P, G, A, C, S (parent), T, V, M, R, H, W, N, Q	C, L, I, M, F, Y, Q	G, A, S (parent), T, V, R, H, W
	95	V, M, K, R, H, Q	P (parent), A, C, T, L, I, F, Y, W, N, E	I, K	P (parent), A, C, T, V, L, M, R, H, F, W, N, E, Q
	96	S, T, V, I, M, H, W, N, D, Q	P, G, A, C, L, Y (parent), E	M, N, D, E	P, A, C, S, T, V, I, Y (parent), W, Q
	97	Not scanned			

Example 14: Production and analysis of anti-CEACAM5 mAb progeny of MBN001

[0525] This example describes the generation of progeny of anti-CEACAM5 antibody MBN001 and the characterization of the progeny antibodies. A subset of CDR amino acid substitutions that were predicted to improve MBN001 binding to human CEACAM5 or cynomolgus CEACAM5 based upon the deep mutational scanning data were selected for further analysis. Antibody genes with single amino acid substitutions and combinations of substitutions were synthesized in IgG expression vectors, transiently transfected into HEK cells, and purified via Protein A. In addition, the framework germline reversions described above were also incorporated into the sequences of the progeny. Purified IgG1.3 CEACAM5 mAb clones were characterized using the BIACORE® for high-throughput SPR-based monoclonal characterization.

[0526] The BIACORE® instrument was primed with 1X HBSP+ running buffer (Cytiva catalog no. BR100671). The CM5 chip (Cytiva catalog no. 29149604) was equilibrated to room temperature, and the BIACORE® instrument was re-primed. A human antibody Fc capture kit (Cytiva catalog no. 29234600) was utilized for this analysis. The human antibody Fc capture reagent was immobilized to both flow cells in all 8 flow channels of the CM5 chip via amine-coupling using the following conditions. Immobilization was performed at a temperature of 25°C. The anti-human Fc capture reagent was diluted to a concentration of 25 ug/mL in acetate pH5 buffer. The chip surface was activated by injecting a mixture of 1-ethyl-3- (-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxy succinimide (NHS) for 420 seconds at a rate of 10 microliters per minute (uL/min). A concentration (25 ug/mL) of anti-human Fc capture reagent was then injected on the surface for 420 seconds at a rate of 10 uL/min. The remaining chip surface was blocked by injecting ethanolamine for 420 seconds at a rate of 10 uL/min. This process yielded immobilization levels at approximately 9000RU.

[0527] The SPR kinetics against full-length human and cyno CEACAM5 were set up as follows. The antibody capture was performed by diluting the antibodies to a concentration of 10nM in HBSP+ buffer. The 10nM mAbs were captured for 20 seconds at 5 uL/min to achieve capture levels of approximately 100RU. Analyte binding was performed by first making a titration series of full-length human and cyno CEACAM5 that were prepared (from 500nM – 0.23nM) with three-fold dilutions in HBSP+ buffer. The association rate was 180 seconds at 30 uL/min. The dissociation rate was 600 seconds at 30 uL/min. Regeneration was performed using two injections of 3M magnesium chloride MgCl2 for 30 seconds at 30 uL/min. A data fit was performed using a

1:1 binding model with global Rmax. Rmax reflects the maximal response when all ligand is occupied.

[0528] SPR data demonstrating improvements in huCEACAM5 and cynoCEACAM5 affinities for progeny antibodies are shown in the isoaffinity plots in **FIG. 7A** and **FIG. 7B** for the progeny antibodies with values reported below in **Table 18A** and **Table 18B**, which shows that each of the tested antibodies bind both human CEACAM5 and cyno CEACAM5. Data showed that these progeny selectively bound human CEACAM5 and did not bind CEACAM1, CEACAM6, CEACAM7, and CEACAM8. Note that these progeny were also made in a hIgG1 form 1 as opposed to the hIgG1.3f form described in this Example. Both the hIgG1 form and the hIgG1.3f form shared the same heavy chain variable region and the same light chain variable region, i.e., only the CH2 regions of heavy chain were adapted, such that their binding characteristics would be expected to be similar. In fact, data show acceptable agreement (e.g., one-fold to two-fold similarity in human CEACAM5 and cyno CEACAM5 binding/SPR values) was observed between hIgG1 and hIgG1.3f versions of the progeny (data not shown). Data in **FIG. 8A**, **FIG. 8B**, and **FIG. 8C** confirm that the MBN001 progeny were effectively internalized into human CEACAM5 expressing cells and that these progeny could be used to effectively deliver cytotoxic agents. Data in **FIG. 9A** and **FIG. 9B** show absence of non-specific human CEACAM1 and human CEACAM6 cross-reactivity with MBN001 progeny mAbs MBP001, MBP003 and MBP002. **FIGs. 10A-10D** show that the FACS EC50 remained constant for each clone across cell lines and that the max MFI increases 10-fold from LS174T & BxPC3 to MKN45.

Table 18A. Binding data for hIgG1.3f progeny antibodies

Ab identifer	Class	IgG1.3f Ab	Parent Ab	huCEACAM5 ka (1/Ms)	huCEACAM5 kd(1/s)	huCEACAM5 KD (M)	cyCEACAM5 ka (1/Ms)	cyCEACAM5 (1/s)	cyCEACAM5 KD (M)
MBN001 parent	IgG1		MBN001	7.36E+04	5.79E-03	7.86E-08	7.99E+03	1.14E-02	1.42E-06
MBP004	IgG1		MBN001	7.60E+04	1.31E-03	1.73E-08	4.41E+04	1.79E-03	4.06E-08
MBP005	IgG1		MBN001	1.17E+05	1.70E-03	1.46E-08	4.92E+04	1.79E-03	3.65E-08
MBP006	IgG1		MBN001	8.51E+04	5.13E-04	6.03E-09	4.86E+04	7.93E-04	1.63E-08
MBP007	IgG1		MBN001	7.96E+04	1.71E-03	2.15E-08	4.27E+04	9.24E-04	2.17E-08
MBP008	IgG1		MBN001	8.92E+04	5.27E-04	5.91E-09	4.03E+04	7.84E-04	1.95E-08
MBP009	IgG1		MBN001	1.72E+05	4.80E-04	2.78E-09	5.03E+04	4.09E-04	8.14E-09
MBP010	IgG1		MBN001	5.00E+04	1.02E-03	2.04E-08	3.40E+04	7.06E-04	2.08E-08
MBP011	IgG1		MBN001	4.71E+04	6.71E-04	1.42E-08	4.26E+04	8.23E-04	1.93E-08

Table 18B. Binding data for hIgG1 progeny antibodies

Antibody identifier	Class	IgG1.3f antibody	Parent antibody	huCEACAM5 ka (1/Ms)	huCEACAM5 kd (1/s)	huCEACAM5 KD (M)	cyCEACAM5 ka (1/Ms)	cyCEACAM5 (1/s)	cyCEACAM5 KD (M)
MBP002	IgG1	MBP008	MBN001	4.33E+04	2.28E-04	5.26E-09	3.78E+04	5.02E-04	1.33E-08
MBP001	IgG1	MBP005	MBN001	4.52E+04	1.17E-03	2.58E-08	3.31E+04	1.24E-03	3.74E-08
MBP003	IgG1	MBP004	MBN001	4.20E+04	9.82E-04	2.34E-08	2.64E+04	1.14E-03	4.31E-08

Example 15: Internalization analysis of recombinant IgG1.3 progeny antibodies

[0529] Internalization experiments were conducted for hIgG1.3 MBN001 progeny antibodies, listed in Table 19A below. Antibodies were evaluated for internalization using HCT116-huCEACAM5, HCT116-cyCEACAM5, and MKN45 cell lines.

[0530] Additional internalization experiments were conducted for hIgG1 antibodies MBP001, MBP002, and MBP003 using the MKN45- and Ls174T cell lines. The MKN45 or Ls174T cellswere seeded at 10K cells/well (50uL).

[0531] Cell lines were first analyzed for cell density and viability using a Vi-cell viability machine. The cell lines were diluted to 0.2E6 vc/mL in growth media, and a volume (50uL) of each was dispensed to a flat-bottom 96-well plate to achieve cell counts of 10K cells/well. Ls174T media included MEM with 10% HI-FBS and 1% pen/strep. MKN45 media included RPMI (ATCC modification) with 10% HI-FBS and 1% pen/strep. HCT116 media included McCoy's 5a with 10% HI-FBS, 1% pen/strep, and 6 ug/mL Blasticidin. Cells were allowed to adhere to wells of the 96-well plate (Corning Cat. No. 3595) at 37°C/5% CO_2 for approximately 4 hours.

[0532] Each test mAb and control mAb was labeled by first pre-mixing the respective antibody with a pHrodo red (Fab conjugated to pH-sensitive; Thermo cat# Z25612) secondary reagent at a 3:1 dye:mAb molar ratio. A series of dilutions were prepared at 2X target concentration in growth media. The final mAb target concentration was 25nM. A volume (50uL) of labeled mAb samples were added to the plated cells to achieve a target mAb concentration (25nM).

[0533] Coated plates contacted with the labeled mAbs were incubated at 37°C for 30 minutes. Each plate was then read using an Incucyte apparatus, which collected images from the red and phase channels [10X objective; 3 images/well] for a 24-hour duration, with reads of plates every 30 minutes. The data are shown in **Table 19A** and **Table 19B**. Data show that the anti-CEACAM5 progeny antibodies -were internalized by the HCT-CEA cell line and MKN45 cell lines.

Table 19A. Internalization data for hIgG1.3 progeny antibodies

Antibody identifier	MKN45 24hr red/phase AUC (internalization)	HCT116-huCEACAM5 red/phase AUC (internalization)	HCT116-cyCEACAM5 red/phase AUC (internalization)
MBP004	3.79	1.14	2.50
MBP005	3.98	1.18	2.11
MBP006	3.55	1.14	2.40
MBP007	3.28	1.05	1.80
MBP008	4.15	1.13	2.54
MBP009	4.95	1.36	2.57
MBP010	3.11	1.00	2.55
MBP011	3.41	1.07	2.35

Table 19B. Internationalization data for hIgG1 progeny antibodies

Antibody identifier	Ls174T 24hr red/phase AUC (internalization)	MKN45 24hr red/phase AUC (internalization)
MBP001	0.69	2.42
MBP002	0.72	2.58
MBP003	0.67	2.62

[0534] MBN001 and certain progeny (e.g., MBP004 and MBP019) were analyzed for internalization to cynoCEACAM-5 expressing cells. It was observed that MBN001 and the progeny were efficientlyy internalized. Internalization of negative control mAbs was not observed.

Example 16: Cytotoxicity analysis of progeny antibodies

[0535] This example assessed the cytotoxicity of CEACAM5 targeting mAbs of MBN001 progeny mAbs conjugated to Compound B, as described above. Cytotoxicity was measured by IC50 value of cellular growth inhibition across CEACAM5-expressing cell lines Ls174T and MKN45 cell lines . Data were used (along with binding and internalization data) to select mAbs for in-vivo efficacy testing.

[0536] The Ls174T cells and MKN45 cells were harvested using a trypsin/EDTA (0.25%) solution (Gibco Cat # 25200-056). The media was removed and the cells were washed with 1x PBS (Ca+ and Mg free; Gibco Cat #14190-144). The cells were detached using the trypsin/EDTA solution. The trypsin/EDTA solution was neutralized with complete media. Cells were spun down at a speed of 1400 rpm for five minutes. The supernatant was removed and the cells were suspended in complete media corresponding to each cell line.

[0537] Cells were then counted and the cell concentration was adjusted to 1.0 x 10^6 cells/mL. Specific cells were diluted to the following concentrations: Ls174T: 0.125 x 10^6 cells/mL and MKN45: 0.1 x 10^6 cells/mL. A volume (20 µL) of the different cell suspensions to each well of a plate (PerkinElmer catalog no. 6007480). Plates were incubated for 20-24 hours. Dilutions of test reagents (e.g., antibody MBP001, MBP002and MBP003 conjugated to Compound B) were prepared and the dilutions were added to the plates and allowed to incubate for 120 hrs. at 37°C.

[0538] Cell viability was determined using a Cell TiterGlo (CTG) 2.0 cell viability assay (Promega catalog no. G9242). The CTG reagentss were removed from the refrigerator and allowed to equilibrate to room temperature. The assay plates were removed from the incubator and allowed to acclimate to room temperature. White backing adhesive was applied to the plate bottoms. A volume (40 µL) of CTG solution was added to each assay well and then mix on an orbital shaker for 2 minutes at a speed of 500 rpm. The assay plates were placed in the dark for 20 minutes. The plate covers were then removed and the luminescence was analyzed on an Envision plate reader. Data are shown in **Table 20**. Data show that the tested antibodies bound to the CEACAM5 on the different CEACAM5-expressing cells lines over 96 hours, such that the cytotoxic payloads were delivered to the cells for effective cytotoxic killing.

Table 20. Cytotoxicity data for progeny antibodies

Antibody identifier	MKN45 5-day cytotoxicity (IC50)	MKN45 5-day cytotoxicity (AUC)	Ls174T 5-day cytotoxicity (IC50)	Ls174T 5-day cytotoxicity (AUC)
MBP001	1.321	23852	2.031	18252
MBP002	1.194	24071	1.594	18858
MBP003	1.314	24378	2.208	18522

MBP004	0.0041	2381	1.027	865
MBP005	0.15	2348	10.97	964
MBP006	0.018	2439	2.831	902
MBP007	0.127	2074	24.9	834
MBP008	0.968	2203	2.6	1136.00
MBP009	0.08	2195	2.385	1037.00
MBP010	0.035	1363	2.548	909.90
MBP011	0.36	1770	4.468	938.90

Example 17. HDX epitope mapping of anti-CEACAM5 mAb MBN001

[0539] This Example analyzed the binding epitopes of human Carcinoembryonic antigen-related cell adhesion molecule 5 (hCEACAM5) upon interaction with anti-CEACAM5 mAb MBN001 Hydrogen Deuterium Exchange mass spectrometry (HDX-MS) probes protein conformation and conformational dynamics in solution by monitoring the rate and extent of deuterium exchange of backbone amide hydrogen atoms [Huang et al. 2014, Analytical and Bioanalytical Chemistry, 406, 6541-6558; Wei, et al., 2014, Drug Discovery Today, 19, 95-102]. The level of hydrogen to deuterium exchange depends on the solvent accessibility of backbone amide hydrogen atoms, protein hydrogen bonding, time and pH. HDX-MS provides a read out for both hydrogen bonding and solvent accessibility. The mass increase of the protein upon HDX can be precisely measured by MS. Comparing the rates of exchange between the bound and unbound states in HDX experiments can provide valuable insights into the conformational dynamics, binding, specificity, and stability of proteins. Regions of the protein that exhibit slower rate of hydrogen exchange in bound vs unbound state (protection) are indicative of potential binding sites, or structural stabilization. In the context of an antigen/antibody interactions, the regions of the antigen where hydrogen exchange slows in the presence of the antibody are identified as potential epitopes. Protein regions that show faster hydrogen exchange in bound vs. unbound state of the protein (de-protection) signify structural destabilization. Hydrogen exchange rates are also sensitive to allosteric effects which can complicate the interpretation of the results.

Methods

[0540] For purposes of designing peptide constructs involving subdomains of CEACAM5, a full atom three-dimensional model of CEACAM5 was constructed using MOE software (Molecular Operating Environment (MOE) 2022.02 Chemical Computing Group ULC, 910-1010 Sherbrooke St. W., Montreal, QC H3A 2R7, Canada, 2023.) using the Cα coordinates from solution scattering data (Boehm, M.K. and Perkins, S.J. FEBS Lett 475, 11-16, (2000), PDB code 1E07). A ribbon representation of the 3D model of human CEACAM5 along with the separate structural domains is shown in **FIG. 13A**. The separate domains in the protein sequence corresponding to the 3D model are shown in **FIG. 13B**. The seven shaded areas in **FIG. 13B** correspond to the different structural domains in **FIG. 13A**.

[0541] Prior to epitope mapping experiments, non-deuterated experiments were carried out to generate a list of common peptides for recombinant hCEACAM5-A3-B3 construct (SEQ ID NO: 24), and protein complexes of hCEACAM5-A3-B3 construct and Fab of mAb MBN001 at 15 μM, 1:1 molar ratio. In the HDX-MS experiment, a volume (5 μL) of each sample was diluted into 55 μL of D_2O buffer (10 mM phosphate buffer, D_2O, pD 7.0) to start the labeling reactions. The reactions were carried out for different periods of time: 20 sec, 1 min, 10 min and 60 min. By the end of each labeling reaction period, the reaction was quenched by adding quenching buffer (100 mM phosphate buffer with 4 M GdnCl and 0.4 M TCEP, pH 2.5, 1:1, v/v) and 50 μL of quenched sample was injected into Waters HDX-MS system for analysis. The deuterium uptake levels of common peptic peptides were monitored in the absence/presence of Fab. Differences in hydrogen exchange rates between bound and free antigen were identified subtracting the mean deuteration value of each peptide in the bound antigen from the mean deuteration of the same peptide in the free antigen state. Changes considered significant if exceeded three times the propagated pooled standard uncertainly.

Results

[0542] The hCEACAM5-A3-B3 construct was chosen for HDX experiments based on epitope binning data indicating binding of MBN001 to A3-B3 region of hCEACAM5. For the purpose of this report, the N-terminal residue numbering on the antigen starts at 488 to align hCEACAM5-A3-B3 construct numbering with the canonical sequence of hCEACAM5 (UniProt entry: P06731). The hCEACAM5 protein is a complex glycoprotein. Seven predicted N-linked glycosylation sites are present in the truncated hCEACAM5-A3-B3 construct used for this

analysis. The complexity and heterogeneity of hCEACAM5 posed a significant challenge for obtaining full sequence coverage. After method optimization, a sequence coverage of 60.1 % , and 3.45 redundancy were achieved for hCEACAM5-A3-B3 construct with HDX-MS platform (**FIG. 13C**).

[0543] In the presence of MBN001, a significant reduction in the rate of hydrogen exchange on hCEACAM5 in the peptide regions covering residues 588 to 606, and residues 668 to 685 was observed when compared to unbound hCEACAM5 antigen (**FIG. 13D**)

[0544] Further interpretation of HDX differences requires consideration of additional factors related to the HDX process [Bai et al., 1993 Proteins. 17(1):75-86]. First, proline residues do not possess an amide hydrogen and thus do not report on the HDX process. Next, following proteolysis, the N-terminal residue of each peptide is transformed from an amide to an amine. Amines undergo rapid deuterium loss during analysis. In addition, the first amide residue of each peptide (i.e., the second residue) also undergoes rapid deuterium loss due to the influence of the N-terminal amine. Finally, overlapping regions can be used to narrow down the results.

[0545] Peptide region 590-606 is covered by multiple overlapping peptides, all showing strong protection. Thus, suggesting high confidence that the area engages in binding. The amino acids with significant protection are further narrowed down to:

$$DVL^{590}Y^{591}G^{592}PD^{594}T^{595}PI^{597}I^{598}S^{599}PPD^{602}S^{603}S^{604}Y^{605}L^{606}.$$

The area was considered the primary epitope by HDX-MS.

[0546] The peptide region 668-685 was covered by a single peptide. After exclusion of the first two N-terminal amino acids and Proline residue at position 681, the protected region remains long, covering 15 amino acids, namely:

$$IVK^{670}SITVSASGTSPGLSA^{685}$$

No data was available for the following regions on hCEACAM5: 501-522, 546-574, 612-615, and 642-662, therefore no conclusions were made about these residues.

[0547] The overall HDX effects of MBN001 binding on hCEACAM5 are illustrated on **FIG. 13E**.

Conclusions

[0548] HDX-MS identified following residues and peptide regions as potential epitopes on hCEACAM5(A3B3) upon binding with MBN001:

L^{590}, Y^{591}, G^{592}, D^{594}, T^{595}, I^{597}, I^{598}, S^{599}, D^{602}, S^{603}, S^{604}, Y^{605}, L^{606}, K^{670}SITVSASGTS::GLSA^{685}

Example 18. Cryo-EM analysis of bin 1 mAb MBP001

[0549] This Example describes the cryo-EM analysis performed for bin 1 mab MBP001. The CEACAM5 construct is approximately 20 KDa in size and the Fab of MBP001 was approximately 50 KDa in size. Particles significantly smaller than ~120 kDa are harder to pick and align using cryo-EM analysis. Accordingly, an anti-CEACAM5 bin2 mAb (of approximately 50 KDa) was also generated and mixed along with the CEACAM5 construct and the MBP001 Fab, in order to produce a complex structure of approximately 120 kDa. This combined structure made the cryo-EM analysis much easier to perform and confirmed that bin 1 binder MBP001 bound a different epitope than a bin 2 mAb. Below is a description of the different steps and methods that were performed.

Design of human CEACAM5 A3-B3

[0550] CEACAM5, like other members of the CEA-related cell adhesion molecule (CEACAM) family of the immunoglobulin (Ig) gene superfamily, is a heavily glycosylated multidomain protein whose domain boundaries have been manually assigned in Uniprot using PROSITE annotation rules (ID # P06731; Uniprot: the Universal Protein Knowledgebase in 2023; The UniProt Consortium (2023) *Nucleic Acids Research* 51, D523–D531.

[0551] However, for purposes of designing peptide constructs involving subdomains of CEACAM5, a full atom three-dimensional model of CEACAM5 was constructed using MOE software (Molecular Operating Environment (MOE) 2022.02 Chemical Computing Group ULC, 910-1010 Sherbrooke St. W., Montreal, QC H3A 2R7, Canada, 2023.) using the Cα coordinates from solution scattering data (Boehm, M.K. and Perkins, S.J. *FEBS Lett* **475**, 11-16, (2000), PDB code 1E07). A ribbon representation of the 3D model of human CEACAM5 along with the separate structural domains is shown in **FIG. 13A**. The separate domains in the protein sequence corresponding to the 3D model are shown in **FIG. 13B**.

[0552] Designing the A3-B3 construct for purposes of screening antibodies further required the attachment of purification tags and a cleavage tag. For purification, a Histidine tag (amino sequence HHHHHH; SEQ ID NO: 95) and for protease cleavage the tobacco vein mottling virus (TVMV) tag (sequence ETVRFQG (SEQ ID NO: 102); Nallamsetty, *Protein Expr. Purif.* 38, 108-15, 2004) were selected. These were attached at the N-terminal end of the A3-B3 construct, the N-terminal rather than the C-terminal being chosen for this purpose because we were interested

in finding antibody-binding epitopes nearer to the C-terminus (i.e., B3 domain) in order to selectively bind the unshed rather than the shed or soluble form of CEACAM5 (shedding occurs near the C-terminus). The final construct is shown as SEQ ID No: 24.

Expression and purification of hCEACAM5 A3-B3 reagent

[0553] A human CEACAM5 A3-B3 domain protein (C-terminal region, 198 amino acid protein construct) shown below was constructed.

HHHHHHETVRFQGPKPSISSNNSKPVEDKDAVAFTCEPEAQNTTYLWWVNGQSLPVSPRLQLSNGNRTLTLFNVTRND
ARAYVCGIQNSVSANRSDPVTLDVLYGPDTPIISPPDSSYLSGANLNLSCHSASNPSPQYSWRINGIPQQHTQVLFIA
KITPNNNGTYACFVSNLATGRNNSIVKSITVSASGTSPGLSA (SEQ ID NO: 96)

[0554] The Human CEACAM5 A3-B3 domain protein was expressed by transient transfection of Expi293F™ GnTI- Cells (Thermo Fisher) with DNA encoding A3-B3 protein using ExpiFectamine™ 293 Transfection Kit (Thermo Fisher). After 24 hours, transfected cells were fed with enhancers provided in the kit and grown at 37°C, 8% CO_2 and 150 RPM for total 4 days. Supernatant was harvested by centrifugation and using 0.22um filtration (Corning).

[0555] Clarified medium of A3-B3 was purified from 5ml Histrap excel column (Cytiva) and eluted with 250mM imidazole-phosphate buffer. The eluate from the Histrap column was further purified with a preparative HiLoad Superdex 200 16/60 (Cytiva) to isolate A3-B3 monomer from aggregated material. Monomeric preparative size exclusion chromatography (SEC) fractions were pooled and filtered through a 0.22μm syringe filter (Pall) as the sample for CryoEM structure determination. Sample concentration was determined by A280 using a calculated molar extinction coefficient of 25,690 M^{-1} cm^{-1}. To assay the quality, the sample was checked by LC-MS, analytical SEC and SDS-PAGE.

Expression and purification of anti-CEACAM5 Fabs

[0556] Each Fab heavy chain (HC) generated shared the identical variable region (VH) and constant region (CH1) with its parental mAb. Two amino acids, GG, were added to the C-terminus of Fab HC. Fab Light chain (LC) remained the same as the parental mAb LC. DNA of Fab HC and LC was synthesized for expression.

Fab of MBP001:

Light Chain:

EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISR
LEPEDFAVYYCQQYGSSPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO 46)

Heavy Chain:

QVQLVESGGGVVQPGRSLRLSCAASGIYFSSHGMHWVRQAPGKGLEWVTFISYDGSYKSYVDSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCATGLTGTGAFDIWGQGTMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV
TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCGG (SEQ ID
NO: 100) (comprising the VH of SEQ ID NO: 45)

Fab of bin 2 mAb:

Light Chain:

DIQLTQSPSFLSASVGDRVTITCRASQGISTYLAWYQQKPGKAPKFLIYAEKTLQSGVPSRFSGSGSGTEFTLTISSL
QPEDFATYYCQQLASYPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQS
GNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 97)

Heavy Chain:

QVQLQESGPGLVKPSGTLSLTCAVSGGSISSSDWWSWVRQPPGKGLEWIGEIYHQGSTNYNPSLKSRVTISVDKSKNQ
FSLKLSSVTAADTAVYYCARASSSGYYGHDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCGG (SEQ ID
NO: 98)

[0557] The Fab of MBP001 and the Fab of the bin2 mAb were expressed in Expi293 cells (Thermo Fisher) by transient transfection with the Fab heavy chain (HC) and Fab light chain (LC) DNA using the same method described above for CEACAM5.

[0558] Each clarified medium of Fab was purified from 5ml Hitrap Mabselect (Protein L) VL column (Cytiva) and eluted with glycine pH 3 buffer neutralized with Tris pH 8. To remove the free light chain, the Mabselect eluate was added over a 5ml CaptureSelect CH1 XL column (Thermo Fisher) and eluted with glycine pH 3 solution. Finally, neutralized CH1 eluate was run over a HiLoad Superdex 75 16/60 (Cytiva) column to obtain highly monomeric Fab samples for Cryo EM. To assay the quality, the purified Fab samples were checked by LC-MS, analytical SEC and SDS-PAGE.

Complexing of + CEACAM5 A3-B3 + MBP001 Fab + Fab of bin 2 mAb

[0559] Purified CEACAM5 was complexed with both MBP001 Fab and the Fab of the bin2 mAb using a ~1:1:1 molar ratio. Specifically, 1 mg of CEACAM5 (263 µl) was mixed with 2 mg of the MBP001 Fab (344 µl) and with 2 mg of the Fab of the bin2 mAb (384 µl) and incubated on ice for approximately 2.5 hours. After the incubation period, 50 µl of complexing reaction solution was passed through a Bio-Spin 30 Size-exclusion chromatography column (30kDa MWCO, BioRad) to remove any contaminants and Apo-CEACAM5. Prior to use, Bio-Spin 30 columns were equilibrated with 1X PBS, and samples were applied according to the manufacturer's protocol. The final concentration of complexing reaction solution after passing through the Bio-Spin 30 column was 5.1 mg/ml, which was then used for preparation of cryoEM grids.

CryoEM Grid Preparation and data collection of CEACAM5 + Fabs complex

[0560] Prior to applying to cryoEM grids, 9 µl of CEACAM5 + Fabs complexing reaction solution was combined with 1 µl of 0.1% lauryl maltose neopentyl glycol (LMNG) to yield a final concentration of 0.01% LMNG. The presence of LMNG reduced sample interactions with the air-water-interface allowing for a reduction in particle degradation and an increase in observable particle orientations on grids (D'Imprima, *et al. eLife*. 8:e42747, 2019). After adding LMNG, 3.2 µl of sample was applied to plasma cleaned (22 secs at 20 mA) Quantifoil 1.2/1.3 Au 300 mesh grids and subsequently blotted and plunge frozen using an FEI Vitrobot machine (Thermofisher) following standard operating procedures. Grids were blotted for 5 secs with a blot force of 0 prior to plunge freezing. A total of 12 grids were made from the CEACAM5 + Fabs complexing reaction and used for CryoEM screening and data collection. Data were collected using a G4 Titan Krios machine equipped with a Falcon 4i direct election detector and with EPU data acquisition software (Thermofisher). Movies s (9538 number of movies; 242 frames per stack in EER mode) were collected at 300 keV, using a 75,000 X magnification (1.05 Å/pixel), and a total dose of 40 e/Å^2. A defocus range of -0.8 to -2.2 µm was used during data collection with 2 acquisition areas per hole.

Determination of the Structure by CryoEM of the CEACAM5 A3-B3 domains bound to Two Fabs

[0561] During data collection, cryoEM movies were processed using cryoSPARC Live (Punjani *et al.*, *Nat. Methods* 14, 290-296, 2017) using a standard workflow which included patch motion correction, patch CTF correction, blob auto-picking, and followed by initial particle

curation using 2D classification. Templates generated from three selected classes from the initial 2D averages were used for a refined particle picking strategy with particles subsequently curated using a single round of 2D classification. Following template picking, 2D classes containing the best selected particles (24 classes) were used for *ab initio* reconstruction with an output of four 3D classes (Punjani *et al.*, *Nat. Methods* 14, 290-296, 2017). The largest 3D class containing 415,405 particles was subjected to homogeneous refinement, which yielded a Gold Standard Fourier Shell Correlation (GSFSC) of 3.14 Å. This was followed by non-uniform refinement (Punjani *et al. Nat. Methods* 17, 1214-1221, 2020), which improved the resolution to yield a GSFSC of 3.08 Å. The resulting half maps were subjected to post-processing with DeepEMhancer (Sanchez-Garcia *et al. Commun. Biol.* 4, 874, 2021) and local resolution of the final map was calculated with cryoSPARC. The final refined map resulting from post-processing was used for initial model building in Chimera (Pettersen *et al.*, *J. Comput. Chem.* 25, 1605-1612, 2004). Generic Fvs, CL:CH1 domain dimers and the B3 domain of CEACAM5 derived from an AlphaFold model (Jumper et al., *Nature* 596, 583–589, 2021) were manually fitted to the density and then model positions refined in Chimera. Further model building was performed using COOT (Emsley & Cowtan, *Acta Crystallogr Sect. D* 60, 2126-2132, 2004; Emsley *et al.*, *Acta Crystallogr Sect. D* 66, 486-501, 2010). Real-space refinement was performed using PHENIX (Liebschner, *et al.*, *Acta Crystallogr. Sect. D* 75, 861-877, 2019).

[0562] While much of the model building was straightforward at several places in the B3 domain a residue register shift was observed compared to the AlphaFold model. Following placement of the B3 domain, the A3 domain was fitted. The final model included:

1. CEACAM5 residues 505-529, 532-574, 579-676 and N-acetylglucosamine residues attached to Asn 508, Asn 529, Asn 553, Asn 560, Asn 612, Asn 650, and Asn 665.
2. The Fab of MBP001: light chain: 1-212 (Kabat numbering including residue 27A); heavy chain: 1-128 and 136-228 (Kabat numbering including residues 52A, 82A, 82B, 82C, 100A, and 100B).
3. The Fab of the bin 2 mAb: light chain: 1-55 and 59-213 (Kabat numbering = sequential numbering); heavy chain: 1-229 (Kabat numbering including 35A, 82A, 82B, 82C, 100A, 100B, and 100C).

[0563] **FIG. 14A** is a final cryoEM map of the Fab of bin 2 mAb, CEACAM5 and the MBP001 Fab showing local resolution. The local resolution in the vicinity of the binding sites for the two Fabs to the B3 domain of CEACAM5 is 3 Å or better.

[0564] **FIG. 14B** is a ribbon diagram of the final model. Light chains of the two Fabs are in light gray. Heavy chains of the two Fabs are in dark gray, and CEACAM5 is in black. Arrow shaped ribbons indicate β-strands and dashes indicate uninterpreted regions, which include CEACAM5 A3 domain residues 530-531 and 575-578, MBP001 heavy chain residues 129-135, and Fab of Bin 2 mAb heavy chain residues 130-135 and light chain residues 56-58 (adjacent to CDR-L2). Epitope of MBP001 on the surface of CEACAM5 domain B3. In contact residues (Sheriff et al. *J. Mol. Biol.* **197**, 273-296, 1987; Sheriff, *Immunomethods* **3**, 191-196, 1993) is a narrower definition of the epitope. Buried residues (Connolly, *J. Appl. Crystallogr.* **16**, 548-558, 1983) is a broader definition of the epitope.

[0565] Epitope of MBP001 on the surface of CEACAM5 domain B3:

[0566] In contact residues:

Ile 597, Ile 598, Asn 630, Phe 656, Gly 663, Arg 664, Asn 665, Asn 666, Ser 667, Val 669, Lys 670, NAG 865 (attached to Asn 665)

[0567] Buried CEACAM5 residues with contacting residues underlined:

Glu 514, Asp 515, Arg 563, Gly 592, Pro 593, Asp 594, Thr 595, Pro 596, Ile 597, Ile 598, Pro 601, Ser 603, Ala 618, Arg 628, Asn 630, Gly 631, Thr 652, Ala 654, Phe 656, Val 657, Ser 658, Gly 663, Arg 664, Asn 665, Asn 666, Ser 667, Ile 668, Val 669, Lys 670, Ser 671, NAG 760 (attached to Asn 560), NAG 865 (attached to Asn 665)

[0568] Paratope of CEACAM5 domain B3 on the surface of MBP001:

[0569] In contact residues (Kabat Numbering):

MBP001 VL: Ser 31

MBP001 VH: Tyr 28, Ser 31, His 32, Tyr 52A, Asp 53, Gly 54, Ser 55, Thr 97, Gly 98

[0570] Buried residues (Kabat Numbering) with in-contact residues underlined:

MBP001 VL: Ser 31, Tyr 32, Tyr 49, Ser 53, Thr 56, Tyr 91, Glu 92, Ser 93

MBP001 VH: Tyr 28, Ser 30, Ser 31, His 32, Gly 33, Phe 50, Ser 52, Tyr 52A, Asp 53, Gly 54, Ser 55, Tyr 56, Lys 57, Asp 61, Lys 64, Leu 96, Thr 97, Gly 98, Thr 99, Gly 100

[0571] **FIG. 14C** is a distinct binding analysis from **FIG. 14B** that shows contacting residues (epitopes and paratopes) defined as residues whose heavy atoms (CEACAM5/MBP001) lie within 4.5A of each other. In the figure they are colored in black, whereas in the sequences they are bolded. The sequences encompassing CDRs in MBP001 are underlined.

[0572] Paratope In contact residues (Kabat numbering) to go with **FIG. 14C**:

6xHis-A3-B3 huCEACAM5 (MBP001 epitope residues bolded)
HHHHHHETVRFQGPKPSISSNNSKPVEDKDAVAFTCEPEAQNTTYLWWVNGQSLPVSPRLQLSNGNRTLTLFNVTRNDARAYVCGIQNSVSANRSDPVTLDVLYGPDTPIISPPDSSY
LSGANLNLSCHSASNPSPQYSWRINGIPQQHTQVLFIAKITPNNNGTYACFVSNLATGRNNSIVKSITVSASGTSPGLSA (SEQ ID NO: 24)

MBP001 VL (paratope residues bolded; CDRs underlined)
EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPYTFGQGTKLEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 46)

MBP001 VH (paratope residues bolded; CDRs underlined)
QVQLVESGGGVVQPGRSLRLSCAASGIYFSSHGMHWVRQAPGKGLEWVTFISYDGSYKSYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCATGLTGTGAFDIWGQGTMV
TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCGG (SEQ ID NO:
100)

[0573] In contact residues (Kabat Numbering):

MBP001 VL: Ser 31, Tyr 49, Ser 53, Gly 92

MBP001 VH: Tyr 28, Ser 31, His 32, Phe 50, Ser 52, Tyr 52A, Asp 53, Ser 55, Ty r56, Leu 96, Thr 97, Gly 98, Thr 99

Example 19. Testing the in vivo anti-tumor efficacy of the anti-CEACAM5 ADCs

[0574] This Example analyzed the in vivo anti-tumor efficacy of anti-CEACAM5 ADCs in the cell line-derived xenograft models (MKN45). $2X10^6$ MKN45 cells were inoculated subcutaneously into the right flank of four to six weeks old immunodeficient female Athymic Nude mice (CRL 490, Charles River) or NSG mice (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ, stock 005557, Jackson Laboratory). Animals with tumor volume between $150mm^3$~$250mm^3$ were randomized into treatment groups with mean tumor volume of 180 mm^3 (N=7). Tumor bearing animals were treated with a single intravenous injection of buffer control (Phosphate-buffered saline (PBS)) or

with ADCs. Tumor length (L) and width (W) were measured with calipers and tumor volumes were calculated using the formulation L*(W^2)/2.

[0575] Anti-tumor activity of ADCs comprising DXd conjugated anti-CEACAM5 MBN001 from epitope bin 1 (MBN001 ADC) and MBN002 from epitope bin 2 (MBN002 ADC) were evaluated in MKN45 model. Tumor bearing animals received an intravenous bolus injection of ADCs at 3 mg/kg on day 10 post implantation. Data show that the bin 1 lead MBN001 ADC at 3 mg/kg dose level was more effective as an antitumor agent in the MKN45 model compared to the bin 2 lead MBN002 ADC at the same dose level (**FIG. 12**). Plasma exposures of total ADCs were comparable among these three ADCs. Free payload plasma exposure of these ADCs was below the limit of quantification (data not shown).

[0576] In all the aforementioned in vivo efficacy studies, no significant effect on body weight was observed (data not shown). Thus, it appears that treatment of these anti-CEACAM5 ADCs was well tolerated through the course of treatment.

[0577] Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents of the specific aspects disclosed herein. Such equivalents are intended to be encompassed by the following claims.

WHAT IS CLAIMED:

1. An isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising:

(a) a heavy chain variable region (VH) comprising complementarity determining region (CDR)1, CDR2, and CDR3 regions comprising the amino acid sequences set forth in SEQ ID NOs: 14, 15, and 16, respectively, and a light chain variable region (VL) comprising CDR1, CDR2, and CDR3 regions comprising the amino acid sequences set forth in SEQ ID NOs: 19, 20, and 21, or;

(b) a VH comprising CDR1, CDR2, and CDR3 regions which have at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to the amino acid sequences set forth in SEQ ID NOs: 14, 15, and 16, respectively, and a VL comprising CDR1, CDR2, and CDR3 regions which have at least 85%, 90%, 95%, 98%, or 99% sequence identity to the amino acid sequences set forth in SEQ ID NOs: 19, 20, and 21, respectively.

2. The antibody, or antigen binding portion thereof, of claim 1, wherein the VH comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 38, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, or SEQ ID NO: 93.

3. The antibody, or antigen binding portion thereof, of claim 1, wherein the VH comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 17.

4. The antibody, or antigen binding portion thereof, of claim 1, wherein the VH comprises the amino acid sequence set forth in SEQ ID NO: 38, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, or SEQ ID NO: 93.

5. The antibody, or antigen binding portion thereof, of claim 1, wherein the VH comprises the amino acid sequence set forth in SEQ ID NO: 17.

6. The antibody, or antigen binding portion thereof, of any one of claims 1-5, wherein the VL comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence

identity to an amino acid sequence set forth in SEQ ID NO: 43, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 92, or SEQ ID NO: 94.

7. The antibody, or antigen binding portion thereof, of any one of claims 1-5, wherein the VL comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to an amino acid sequence set forth in SEQ ID NO: 22.

8. The antibody, or antigen binding portion thereof, of claim 1, wherein the VL comprises the amino acid sequence set forth in SEQ ID NO: 43, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 92, or SEQ ID NO: 94.

9. The antibody, or antigen binding portion thereof, of claim 1, wherein the VL comprises the amino acid sequence set forth in SEQ ID NO: 22.

10. The antibody, or antigen binding portion thereof, of claim 1, wherein the VH and the VL have at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to:

(a) the amino acid sequence set forth in SEQ ID NOs: 38 and the amino acid sequence set forth in SEQ ID NO 43, respectively;

(b) the amino acid sequence set forth in SEQ ID NO: 49 and the amino acid sequence set forth in SEQ ID NO: 50; respectively;

(c) the amino acid sequence set forth in SEQ ID NO: 51 and the amino acid sequence set forth in SEQ ID NO: 52, respectively;

(d) the amino acid sequence set forth in SEQ ID NO: 67 and the amino acid sequence set forth in SEQ ID NO: 68, respectively;

(e) the amino acid sequence set forth in SEQ ID NO: 69 and the amino acid sequence set forth in SEQ ID NO: 70, respectively;

(f) the amino acid sequence set forth in SEQ ID NO: 71 and the amino acid sequence set forth in SEQ ID NO: 72, respectively;

(g) the amino acid sequence set forth in SEQ ID NO: 73 and the amino acid sequence set forth in SEQ ID NO: 74, respectively;

(h) the amino acid sequence set forth in SEQ ID NO: 75 and the amino acid sequence set forth in SEQ ID NO: 76, respectively

(i) the amino acid sequence set forth in SEQ ID NO: 77 and the amino acid sequence set forth in SEQ ID NO: 78, respectively;

(j) the amino acid sequence set forth in SEQ ID NO: 79 and the amino acid sequence set forth in SEQ ID NO: 80, respectively;

(k) the amino acid sequence set forth in SEQ ID NO: 81 and the amino acid sequence set forth in SEQ ID NO: 82, respectively;

(l) the amino acid sequence set forth in SEQ ID NO: 83 and the amino acid sequence of SEQ ID NO: 84, respectively;

(m) the amino acid sequence set forth in SEQ ID NO: 85 and the amino acid sequence set forth in SEQ ID NO: 86, respectively;

(n) the amino acid sequence set forth in SEQ ID NO: 87 and the amino acid sequence set forth in SEQ ID NO: 88, respectively;

(o) the amino acid sequence set forth in SEQ ID NO: 89 and the amino acid sequence set forth in SEQ ID NO: 90, respectively;

(p) the amino acid sequence set forth in SEQ ID NO: 91 and the amino acid sequence set forth in SEQ ID NO: 92, respectively; or

(q) the amino acid sequence set forth in SEQ ID NO: 93 and the amino acid sequence set forth in SEQ ID NO: 94, respectively.

11. The antibody, or antigen binding portion thereof, of claim 1, wherein the VH and the VL have at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to: the amino acid sequence set forth in SEQ ID NOs: 17 and the amino acid sequence set forth in SEQ ID NO:22, respectively.

12. The antibody, or antigen binding portion thereof, of claim 1, wherein the VH and the VL comprises:

(a) the amino acid sequence set forth in SEQ ID NOs: 38 and the amino acid sequence set forth in SEQ ID NO 43, respectively;

(b) the amino acid sequence set forth in SEQ ID NO: 49 and the amino acid sequence set forth in SEQ ID NO: 50; respectively;

(c) the amino acid sequence set forth in SEQ ID NO: 51 and the amino acid sequence set forth in SEQ ID NO: 52, respectively;

(d) the amino acid sequence set forth in SEQ ID NO: 67 and the amino acid sequence set forth in SEQ ID NO: 68, respectively;

(e) the amino acid sequence set forth in SEQ ID NO: 69 and the amino acid sequence set forth in SEQ ID NO: 70, respectively;

(f) the amino acid sequence set forth in SEQ ID NO: 71 and the amino acid sequence set forth in SEQ ID NO: 72, respectively;

(g) the amino acid sequence set forth in SEQ ID NO: 73 and the amino acid sequence set forth in SEQ ID NO: 74, respectively;

(h) the amino acid sequence set forth in SEQ ID NO: 75 and the amino acid sequence set forth in SEQ ID NO: 76, respectively;

(i) the amino acid sequence set forth in SEQ ID NO: 77 and the amino acid sequence set forth in SEQ ID NO: 78, respectively;

(j) the amino acid sequence set forth in SEQ ID NO: 79 and the amino acid sequence set forth in SEQ ID NO: 80, respectively;

(k) the amino acid sequence set forth in SEQ ID NO: 81 and the amino acid sequence set forth in SEQ ID NO: 82, respectively;

(l) the amino acid sequence set forth in SEQ ID NO: 83 and the amino acid sequence set forth in SEQ ID NO: 84, respectively;

(m) the amino acid sequence set forth in SEQ ID NO: 85 and the amino acid sequence set forth in SEQ ID NO: 86, respectively;

(n) the amino acid sequence set forth in SEQ ID NO: 87 and the amino acid sequence set forth in SEQ ID NO: 88, respectively;

(o) the amino acid sequence set forth in SEQ ID NO: 89 and the amino acid sequence set forth in SEQ ID NO: 90, respectively;

(p) the amino acid sequence set forth in SEQ ID NO: 91 and the amino acid sequence set forth in SEQ ID NO: 92, respectively; or

(q) the amino acid sequence set forth in SEQ ID NO: 93 and the amino acid sequence set forth in SEQ ID NO: 94, respectively.

13. An isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NOs: 38 and the amino acid sequence set forth in SEQ ID NO 43, respectively.

14. An isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 49 and the amino acid sequence set forth in SEQ ID NO: 50; respectively.

15. An isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 51 and the amino acid sequence set forth in SEQ ID NO: 52, respectively.

16. An isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 67 and the amino acid sequence set forth in SEQ ID NO: 68, respectively.

17. An isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 69 and the amino acid sequence sest forth in SEQ ID NO: 70, respectively.

18. An isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 71 and the amino acid sequence set forth in SEQ ID NO: 72, respectively.

19. An isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 73 and the amino acid sequence set forth in SEQ ID NO: 74, respectively.

20. An isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 75 and the amino acid sequence set forth in SEQ ID NO: 76, respectively.

21. An isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 77 and the amino acid sequence set forth in SEQ ID NO: 78, respectively.

22. An isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 79 and the amino acid sequence set forth in SEQ ID NO: 80, respectively.

23. An isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 81 and the amino acid sequence set forth in SEQ ID NO: 82, respectively.

24. An isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 83 and the amino acid sequence set forth in SEQ ID NO: 84, respectively.

25. An isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 85 and the amino acid sequence set forth in SEQ ID NO: 86, respectively.

26. An isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 87 and the amino acid sequence set forth in SEQ ID NO: 88, respectively.

27. An isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 89 and the amino acid sequence set forth in SEQ ID NO: 90, respectively.

28. An isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 91 and the amino acid sequence set forth in SEQ ID NO: 92, respectively.

29. An isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a VL which comprise the amino acid sequence set forth in SEQ ID NO: 93 and the amino acid sequence set forth in SEQ ID NO: 94, respectively.

30. An isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5 comprising a VH and a light chain variable region which comprise the amino acid sequence set forth in SEQ ID NO: 17 and the amino acid sequence set forth in SEQ ID NO: 22, respectively.

31. An isolated antibody, or antigen binding portion thereof, which specifically binds to CEACAM5, comprising a heavy chain comprising the amino acid sequence as set forth in SEQ ID NO: 45 and a light chain comprising the amino acid sequence as set forth in SEQ ID NO: 46.

32. The antibody, or antigen binding portion thereof, of any one of claims 1-3 and 6-7, which binds human CEACAM5 and has at least one amino acid mutation described in **Table 17**.

33. The antibody, or antigen binding portion thereof, of any of the preceding claims, which specifically binds to CEACAM5 with a K_D less than $1x10^{-7}$ M, less than $1x10^{-8}$ M, less than $1x10^{-9}$ M, or a K_D described in **Table 8**, **Table 9, Table 10, Table 11**, **Table 18A**, or **Table 18B**.

34. The antibody, or antigen binding portion thereof, of any one of the preceding claims, which specifically binds to CEACAM5 with a K_D with about 100 nM or less, about 90 nM or less, about 80 nM or less, about 70 nM or less, about 60 nM or less, about 50 nM or less, about 40 nM or less, about 30 nM or less, about 20 nM or less, about 10 nM or less, about 5 nM or less, about 3 nM or less, about 1 nM or less, about 0.9 nM or less, about 0.8 nM or less, about 0.7 nM or less, about 0.6 nM or less, about 0.5 nM or less, about 0.4 nM or less, about 0.3 nM or less, about 0.2 nM or less, or about 0.1 nM or less.

35. The antibody, or antigen binding portion thereof, of any of the preceding claims, which internalizes into CEACAM5-expressing cells, for example the antibody, or antigen binding portion thereof internalizes with an AUC described in **Table 14**, **Table 19A**, or **Table 19B**.

36. The antibody, or antigen binding portion thereof, of any of the preceding claims, which binds to the A3-B3 domain of human CEACAM5 and in addition does not bind to human CEACAM1, human CEACAM6, human CEACAM7, human CEACAM8, or any combination thereof.

37. The antibody, or antigen binding portion thereof, of any of the preceding claims, which binds to an epitope of the A3-B3 domain of human CEACAM5 identified by HDX analysis in Example 17 and FIGs. 13A, 13B and 13C, for example, the epitope includes all or a portion of amino acids L^{590}, Y^{591}, G^{592}, D^{594}, T^{595}, I^{597}, I^{598}, S^{599}, D^{602}, S^{603}, S^{604}, Y^{605}, L^{606}, K^{670}SITVSASGTS::GLSA^{685} of human CEACAM5 (SEQ ID NO: 24).

38. The antibody, or antigen binding portion thereof, of any of the preceding claims, which binds to an epitope of the A3-B3 domain of human CEACAM5 identified by Cryo-EM analysis in Example 18 and FIGs. 14A, 14B and 14C, for example, the epitope includes all or a portion of amino acids residues 505-529, 532-574, 579-676 of human CEACAM5 (SEQ ID NO: 96) and N-acetylglucosamine residues attached to Asn 508, Asn 529, Asn 553, Asn 560, Asn 612, Asn 650, and Asn 665.

39. The antibody, or antigen binding portion thereof, of any of the preceding claims, which comprises an IgG1 constant region, IgG2 constant region, IgG3 constant region, IgG4 constant region, or a variant thereof.

40. The antibody, or antigen binding portion thereof, of any of the preceding claims, comprising an IgG1 antibody.

41. The antibody, or antigen binding portion thereof, of claim 40, comprising an IgG1.3f constant region.

42. The antibody of any of the preceding claims, which is a human, humanized, or chimeric antibody.

43. The antibody, or antigen binding portion thereof, of any of the preceding claims, wherein the antigen binding portion thereof comprises a Fab, Fab', (Fab')2, Fv, or scFv fragment.

44. An isolated antibody, or antigen binding portion thereof, which binds to the same epitope on human CEACAM5 as the antibody, or antigen binding portion thereof, of any of claims 1-43.

45. An isolated antibody, or antigen binding portion thereof, which competes for binding to human CEACAM5 with the antibody, or antigen binding portion thereof, of any of claims 1-43.

46. The antibody, or antigen binding portion thereof of any of the preceding claims, which competes for binding to human CEACAM 5 with a Bin 1 mAb.

47. The antibody, or antigen binding portion thereof of claim 46, which competes for binding to the A3-B3 domain of human CEACAM 5 with mAb MBN001.

48. The antibody, or antigen binding portion thereof, of claim 46, which competes for binding with MBP001, MBP002, MBP003, MBP004, MBP005, MBP006, MBP007, MBP008, MBP009, MBP010, MBP011, or any combination thereof.

49. The antibody, or antigen binding portion thereof, of claim 48, wherein the progeny mab is mAb MBP001.

50. A bispecific molecule comprising the antibody, or antigen binding portion thereof, of any of the preceding claims that binds human CEACAM5, and a second binding region that binds another antigen.

51. A multispecific molecule comprising the antibody, or antigen binding portion thereof, of any of the preceding claims and at least two binding regions, each of which binds other antigens.

52. A conjugate comprising the antibody, antigen binding portion thereof of any one of claims 1 to 49, the bispecific molecule of claim 50, or the multispecific molecule of claim 51.

53. The conjugate of claim 52, which is linked to a detectable moiety, a binding moiety, a labeling moiety, or a biologically active moiety.

54. The conjugate of claim 53, wherein the biologically active moiety comprises a cytotoxic moiety.

55. An antibody drug conjugate (ADC) comprising the antibody or antigen-binding fragment thereof of any one of claims 1 to 49, the bispecific molecule of claim 50, or the multispecific molecule of claim 51, and a cytotoxic moiety, which is conjugated to the antibody or antigen binding portion thereof.

56. The ADC of claim 55, wherein the cytotoxic moiety comprises an alkylating agent, an alkylating-like agent, an angiogenesis inhibitor, an antimetabolite agent, an antimitotic agent, an antitumor antibiotic, an apoptosis regulator, a DNA intercalating agent, a DNA replication and repair inhibitor, a histone deacetylase inhibitor, a hormonal agent, a kinase inhibitor, a mitochondria inhibitor, a nuclear export inhibitor, a proteasome inhibitor, a protein synthesis inhibitor, a radionuclide, a topoisomerase I inhibitor, a topoisomerase II inhibitor, or any combination thereof.

57. The ADC of claim 55, wherein the cytotoxic moiety comprises an anti-tubulin agent, which comprises dolastatins (*e.g.,* auristatin E (dolastatin-10); auristatin F (MMAE); and monomethyl auristatin (MMAF)); AFP; AEB; and AEVB); maytansinoids; taxanes (e.g., paclitaxel, docetaxel); T67 (Tularik); baccatin derivatives; taxane analogs (*e.g.*, epothilone A and B); nocodazole; colchicine and colcimid; estramustine; cryptophysins; cemadotin; combretastatins; discodermolide; or eleutherobin.

58. The ADC of claim 55, wherein the cytotoxic moiety comprises an apoptosis regulator, which comprises AT-101 ((−)gossypol); G3139 or oblimersen (Bcl-2-targeting antisense oligonucleotide); IPI-194; IPI-565; N-(4-(4-((4′-chloro(1,1′-biphenyl)-2-yl)methyl)piperazin-1-ylbenzoyl)-4-(((1R)-3-(dimethylamino)-1-((phenylsulfanyl)methyl)propyl)amino)-3-nitrobenzenesulfonamide); N-(4-(4-((2-(4-chlorophenyl)-5,5-dimethyl-1-cyclohex-1-en-1-yl)methyl)piperazin-1-yl)benzoyl)-4-(((1R)-3-(morpholin-4-yl)-1-((phenylsulfanyl)methyl)propyl)amino)-3-((trifluoromethyl)sulfonyl)benzenesulfonamide; GX-070 (OBATOCLAX®; 1H-Indole, 2-(2-((3,5-dimethyl-1H-pyrrol-2-yl)methylene)-3-methoxy-2H-pyrrol-5-yl)−)); HGS1029; GDC-0145; GDC-0152; LCL-161; LBW-242; venetoclax; agents that target TRAIL or death receptors (*e.g.*, DR4 and DR5) such as ETR2-ST01, GDC0145, HGS-1029, LBY-135, PRO-1762; drugs that target caspases, caspase-regulators, BCL-2 family

members, death domain proteins, TNF family members, Toll family members, NF-kappa-B proteins, or any combination thereof.

59. The ADC of claim 55, wherein the cytotoxic moiety comprises a DNA intercalating agent, which comprises anthramycin; chicamycin A; enediynes; lexitropsins; tomaymycin; DC-81; sibiromycin; pyrrolobenzodiazepine derivative; SGD-1882 ((S)-2-(4-aminophenyl)-7-methoxy-8-(3 S)-7-methoxy-2-(4-methoxyphenyl)-5-oxo-5,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5(11aH)-one); or SG2000 (SJG-136; (11aS,11a′S)-8,8′-(propane-1,3-diylbis(oxy))bis(7-methoxy-2-methylene-2,3-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5(11 aH)-one)).

60. The ADC of claim 55, wherein the cytotoxic moiety comprises a DNA replication and repair inhibitor, which comprises Altretamine; Bleomycin; Dacarbazine; Dactinomycin; Mitobronitol; Mitomycin; Pingyangmycin; Plicamycin; Procarbazine; Temozolomide; ABT-888 (veliparib); olaparib; KU-59436; AZD-2281; AG-014699; BSI-201; BGP-15; INO-1001; ONO-2231; or any combination thereof.

61. The ADC of claim 55, wherein the cytotoxic moiety comprises a histone deacetylase inhibitor, which comprises vorinostat; romidepsin; chidamide; panobinostat; valproic acid; belinostat; mocetinostat; abexinostat; entinostat; SB939 (pracinostat); resminostat; givinostat; quisinostat; thioureidobutyronitrile (Kevetrin™); CUDC-10; CHR-2845 (tefinostat); CHR-3996; 4SC-202; CG200745; ACY-1215 (rocilinostat); ME-344; sulforaphane, or any combination thereof.

62. The ADC of claim 55, wherein the cytotoxic moiety comprises a hormonal agent, which comprises anastrozole; exemestane; arzoxifene; bicalutamide; cetrorelix; degarelix; deslorelin; trilostane; dexamethasone; flutamide; raloxifene; fadrozole; toremifene; fulvestrant; letrozole; formestane; glucocorticoids; doxercalciferol; sevelamer carbonate; lasofoxifene; leuprolide acetate; megesterol; mifepristone; nilutamide; tamoxifen citrate; abarelix; prednisone; finasteride; rilostane; buserelin; luteinizing hormone releasing hormone (LHRH); histrelin; trilostane or modrastane; fosrelin; goserelin, or any combination thereof.

63. The ADC of claim 55, wherein the cytotoxic moiety comprises a kinase inhibitor, which comprises afantinib; axitinib; binimetinib; bosutinib; crizotinib; dasatinib; erlotinib; fostamatinib; gefitinib; ibrutinib; imatinib; lapatinib; lestaurtinib; lenvatinib; mubritinib; nilotinib; pazopanib; pegaptanib; ruxolitinib; selemetinib; semaxanib; sildenib; sorafenib; SU6656; sunitinib;

vandetanib; vemurafenib; PI-103; PP242; PP30; Torin 1; XL019; INCB018424; ARRY-142886; ARRY-438162; PD-325901; PD-98059; AP-2357; CCI-779; everolimus; RAD-001; rapamycin; temsirolimus; LY294002; XL-147; CAL-120; ONC-21; AEZS-127; ETP-45658; PX-866; GDC-0941; BGT226; BEZ235; XL765; or any combination thereof.

64. The ADC of claim 55, wherein the cytotoxic moiety comprises a mitochondria inhibitor, which comprises pancratistatin; phenpanstatin; rhodamine-123; edelfosine; d-alpha-tocopherol succinate; compound 11β; aspirin; ellipticine; berberine; cerulenin; GX015-070 (Obatoclax®; 1H-Indole, 2-(2-((3,5-dimethyl-1H-pyrrol-2-yl)methylene)-3-methoxy-2H-pyrrol-5-yl)−); celastrol (tripterine); metformin; Brilliant green; ME-344; or any combination thereof.

65. The ADC of claim 55, wherein the cytotoxic moiety comprises a nuclear export inhibitor, which comprises callystatin A; delactonmycin; KPT-185 (propan-2-yl (Z)-3-[3-[3-methoxy-5-(trifluoromethyl)phenyl]-1,2,4-triazol-1-yl]prop-2-enoate); kazusamycin A; leptolstatin; leptofuranin A; leptomycin B; ratjadone; Verdinexor ((Z)-3-[3-[3,5-bis(trifluoromethyl)phenyl]-1,2,4-triazol-1-yl]-N-pyridin-2-ylprop-2-enehydrazide), or any combination thereof.

66. The ADC of claim 55, wherein the cytotoxic moiety comprises a proteasome inhibitor, which comprises Bortezomib; Carfilzomib; Epoxomicin; Ixazomib; Salinosporamide A, or any combination thereof.

67. The ADC of claim 55, wherein the cytotoxic moiety comprises a radionuclide agent, which comprises s I^{131}, In^{11}, Y^{90}, Lu^{177}, or any combination thereof.

68. The ADC of claim 55, wherein the cytotoxic moiety comprises a protein synthesis inhibitor, which comprises Streptomycin; Dihydrostreptomycin; Neomycin; Framycetin; Paromomycin; Ribostamycin; Kanamycin; Amikacin; Arbekacin; Bekanamycin; Dibekacin; Tobramycin; Spectinomycin; Hygromycin B; Paromomycin; Gentamicin; Netilmicin; Sisomicin; Isepamicin; Verdamicin; Astromicin; Tetracycline; Doxycycline; Chlortetracycline; Clomocycline; Demeclocycline; Lymecycline; Meclocycline; Metacycline; Minocycline; Oxytetracycline; Penimepicycline; Rolitetracycline; Tetracycline; Glycylcyclines; Tigecycline; Oxazolidinone; Eperezolid; Linezolid; Posizolid; Radezolid; Ranbezolid; Sutezolid; Tedizolid; Peptidyl transferase inhibitors; Chloramphenicol; Azidamfenicol; Thiamphenicol; Florfenicol; Pleuromutilins; Retapamulin; Tiamulin; Valnemulin; Azithromycin; Clarithromycin; Dirithromycin; Erythromycin; Flurithromycin; Josamycin; Midecamycin; Miocamycin; Oleandomycin; Rokitamycin; Roxithromycin; Spiramycin; Troleandomycin; Tylosin; Ketolides;

Telithromycin; Cethromycin; Solithromycin; Clindamycin; Lincomycin; Pirlimycin; Streptogramins; Pristinamycin; Quinupristin/dalfopristin; Virginiamycin, or any combination thereof.

69. The ADC of claim 55, wherein the cytotoxic moiety comprises a topoisomerase I inhibitor, which comprises camptothecin, derivatives thereof, or analogs thereof.

70. The ADC of claim 69, wherein the topoisomerase I inhibitor comprises topotecan, irinotecan, silatecan, cositecan, exatecan, lurtotecan, gimatecan, belotecan, rubitecan, NSC 100880, NSC 603071, NSC 107124, NSC 643833, NSC 629971, NSC 295500, NSC 249910, NSC 606985, NSC 74028, NSC 176323, NSC 295501, NSC 606172, NSC 606173, NSC 610458, NSC 618939, NSC 610457, NSC 610459, NSC 606499, NSC 610456, NSC 364830, NSC 606497, morpholinisoxorubicin, or SN-38, or derivative or analog thereof.

71. The ADC of claim 69, wherein the cytotoxic moiety comprises exatecan or derivative or analog thereof.

72. The ADC of any one of claims 55 to 71, wherein the cytotoxic moiety is conjugated to the antibody or antigen binding portion thereof by a linker.

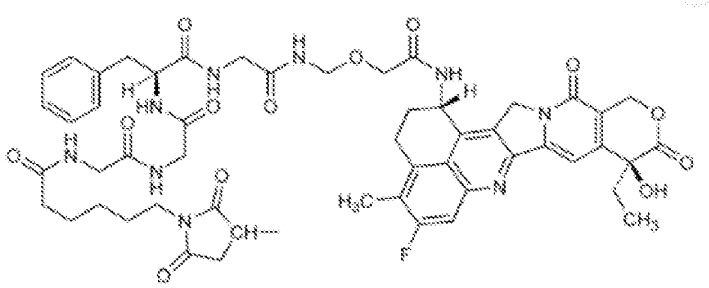
73. The ADC of claim 72, wherein the linker comprises a cleavable linker.

74. The ADC of claim 73, wherein the cleavable linker comprises a peptidyl linker, a disulfide linker, an acid labile linker, a photolabile linker, a peptidase labile linker, or any combination thereof.

75. The ADC of claim 74, wherein the peptidyl linker comprises Val-Cit, Ala-Val, Val-Ala-Val, Lys-Lys, Pro-Val-Gly-Val-Val (SEQ ID NO: 99), Ala-Asn-Val, Val-Leu-Lys, Ala-Ala-Asn, Cit-Cit, Val-Lys, Lys, Cit, Ser, Glu, or any combination thereof.

76. The ADC of claim 73, wherein the linker comprises a non-cleavable linker.

77. An antibody drug conjugate (ADC) comprising the antibody or antigen-binding fragment thereof of any one of claims 1 to 49, which is linked to the following linker-cytotoxic moiety:



[DXd Linker-exatecan],

an isomer thereof, or any pharmaceutical salt thereof.

78. A nucleic acid, or set of nucleic acids, comprising a nucleotide sequence that encodes the antibody, or antigen binding portion thereof, of any of claims 1 to 49, bispecific molecule of claim 50, or multispecific molecule of claim 51.

79. The nucleic acid of claim 78 comprising the pair nucleotide sequences that comprise: (1) SEQ ID NO: 18 and SEQ ID NO: 23; (2) SEQ ID NO: 39 and SEQ ID NO: 44; (3) SEQ ID NO: 53 and SEQ ID NO: 54; or (4) SEQ ID NO: 55 and SEQ ID NO: 56.

80. An expression vector comprising the nucleic acid, or set of nucleic acids, of claim 78 or claim 79.

81. A host cell cell comprising the nucleic acid, or set of nucleic acids, of claim 78 or claim 79 or the expression vector of claim 80.

82. An engineered cell comprising the nucleic acid, or set of nucleic acids, of claim 78 or claim 79 or the expression vector of claim 80, wherein the cell is engineered ex vivo to express the antibody, or antigen binding portion thereof, of any one of claims 1 to 49.

83. The engineered cell of claim 82, which is a cell expressing a chimeric antigen receptor comprising the antibody, or antigen binding portion thereof.

84. A pharmaceutical composition comprising the antibody, or antigen binding portion thereof, bispecific molecule, multispecific molecule, conjugate, ADC, nucleic acid or set of nucleic acids, expression vector, or engineered cell of any of the preceding claims, and a pharmaceutically acceptable carrier.

85. The pharmaceutical composition of claim 84, further comprising one or more additional therapeutic agents.

86. A kit comprising the antibody, or antigen binding portion thereof, bispecific molecule, multispecific molecule, conjugate, ADC, nucleic acid or set of nucleic acids, expression vector, or engineered cell of any of the preceding claims, and instructions for use.

87. Means for treating cancer in a patient in need thereof comprising a combination of an anti-CEACAM5 antibody, or antigen binding portion thereof and a cytotoxic moiety.

88. The means of claim 87, wherein the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises the antibody, or antigen binding portion thereof, of any one of claims 1-49.

89. Means for treating cancer in a subject in need thereof comprising a combination of (i) an anti-CEACAM5 antibody, or antigen binding portion thereof, bispecific molecule, multispecific molecule, conjugate, ADC, nucleic acid or a set of nucleic acids, expression vector, or engineered cell, and (ii) a pharmaceutically acceptable carrier.

90. The means of claim 89, wherein the anti-CEACAM5 antibody, or antigen binding portion thereof, comprises the antibody, or antigen binding portion thereof, bispecific molecule, multispecific molecule, conjugate, ADC, nucleic acid or a set of nucleic acids, expression vector, or engineered cell of the preceding claims.

91. A method of producing an anti-CEACAM5 antibody, or antigen binding portion thereof, a bispecific molecule, or a multispecific molecule comprising transfecting a cell with the nucleic acid of any one of claim 78 or claim 79 or the expression vector of claim 80 in a suitable condition.

92. The method of claim 91, further comprising isolating the antibody, or antigen binding portion thereof from the cell.

93. A method of treating cancer that expresses CEACAM5 in a subject in need thereof comprising administering to the subject a therapeutically effective amount of the antibody, or antigen binding portion thereof, bispecific molecule, multispecific molecule, conjugate, ADC, nucleic acid or a set of nucleic acids, expression vector, or engineered cell of any of the preceding claims.

94. The method of claim 93, wherein the cancer is selected from the group consisting of: colorectal cancer, breast cancer, lung cancer, ovarian cancer, pancreatic cancer, bladder cancer, uterine/cervical cancer, prostate cancer, testicular cancer, esophageal cancer, gastrointestinal cancer, colon cancer, kidney cancer, head and neck cancer, stomach cancer, germ cell cancer, bone

cancer, liver cancer, thyroid cancer, skin cancer, neoplasm of the central nervous system, lymphoma, leukemia, myeloma, sarcoma, and myelodysplastic syndromes.

95. The method of claim 93 or 94, further comprising administering one or more additional therapies.

96. The method of claim 95, wherein the one or more additional therapies comprises radiation therapy, chemotherapy, immune checkpoint inhibitor therapy, CAR-T therapy, immunosuppressive therapy, immunostimulatory therapy, cell therapy, or any combination thereof.

97. The method of claim 95, wherein the one or more additional therapies comprise an immune checkpoint inhibitor.

98. The method of claim 96, wherein the immune checkpoint inhibitor comprises an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-LAG-3 antibody, an anti-CTLA-4 antibody, an anti-TIGIT antibody, an anti-TIM3 antibody, or any combination thereof.

99. A method of detecting the absence or presence of human CEACAM5 in a sample comprising contacting the sample with the antibody, or antigen binding portion thereof, bispecific molecule, multispecific molecule, conjugate, ADC, nucleic acid or set of nucleic acids, expression vector, or engineered cell of any of the preceding claims, under conditions that allow for formation of a complex between the antibody and human CEACAM5; and detecting the formation of the complex.

100. A method of diagnosing a cancer that expresses human CEACAM5 comprising contacting a biological sample from a patient afflicted with or suspected to have the cancer with the antibody, or antigen binding portion thereof, bispecific molecule, multispecific molecule, conjugate, ADC, nucleic acid or set of nucleic acids, expression vector, or engineered cell of any of the preceding claims.