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(54) Title: ANTIBODY-DRUG CONJUGATES COMPRISING AN ANTI-FOLR1 ANTIBODY

(57) Abstract: Provided, inter alia, are antibody drug conjugates (ADCs) which specifically bind Folate Receptor one (FOLR1). Further disclosed are pharmaceutical compositions, and methods for treating cancer.

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ANTIBODY-DRUG CONJUGATES COMPRISING AN ANTI-FOLR1 ANTIBODY

[0001] This application claims priority to International Application No. PCT/CN2022/091860, filed on May 10, 2022, and Chinese Application No. 202310390683.5, filed April 13, 2023, the disclosures of which are hereby incorporated by reference in their entireties.

[0002] Throughout this application various publications, patents, and/or patent applications are referenced. The disclosures of the publications, patents and/or patent applications are hereby incorporated by reference in their entireties into this application in order to more fully describe the state of the art to which this disclosure pertains.

TECHNICAL FIELD

[0003] The present disclosure relates to antibody drug conjugates (ADCs) comprising an anti-FOLR1 antibody and methods of making and using the same.

INTRODUCTION AND SUMMARY

[0004] Antibody-Drug Conjugates (ADCs) allow for the targeted delivery of a drug moiety to a tumor, and, in some embodiments intracellular accumulation therein, where systemic administration of unconjugated drugs may result in unacceptable levels of toxicity to normal cells (Polakis P. (2005) *Current Opinion in Pharmacology* 5:382-387). ADCs are targeted chemotherapeutic molecules which combine properties of both antibodies and cytotoxic drugs by targeting potent cytotoxic drugs to antigen-expressing tumor cells (Teicher, B.A. (2009) *Current Cancer Drug Targets* 9:982-1004), thereby enhancing the therapeutic index by maximizing efficacy and minimizing off-target toxicity (Carter, P.J. and Senter P.D. (2008) *The Cancer Jour.* 14(3):154-169; Chari, R.V. (2008) *Acc. Chem. Res.* 41:98-107.

[0005] The present disclosure provides ADCs comprising an anti-FOLR1 antibody conjugated to the drug moiety through linker moieties. In embodiments, the anti-FOLR1 antibody binds to FOLR1-expressing cancer cells and allows for selective uptake of the ADC into the cancer cells. In embodiments, the ADCs provided herein selectively deliver an effective

amount of drug moiety to tumor tissue and reduce the non-specific toxicity associated with related ADCs. The ADC compounds described herein include those with anticancer activity.

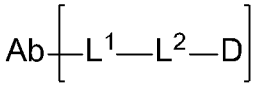
[0006] Folate Receptor 1 (FOLR1), also known as Folate Receptor-alpha (FRα), or Folate Binding Protein, (UniProt P15328), is a glycosylphosphatidylinositol (GPI)-anchored glycoprotein with a strong binding affinity for folic acid and reduced folic acid derivatives (Leung et al. (2013) *Clin. Biochem.* 46:1462- 1468). FOLR 1 has important functions relating to cell proliferation and survival (Kelemen L.E. (2006) *Int. J. Cancer* 119(2):2430250), and it mediates delivery of the physiological folate, 5-methyltetrahydrofolate, to the interior of cells. Expression of FOLR1 on normal tissues is restricted to the apical membrane of epithelial cells in the kidney proximal tubules, alveolar pneumocytes of the lung, bladder, testes, choroid plexus, and thyroid (Weitman S.D. et al. (1992) *Cancer Res.* 52:3396-3401; Antony A.C. (1996) *Ann. Rev. Nutr.* 16:501 -521; Kalli K.R. et al. (2008) *Gynecol. Oncol.* 108:619-626). FOLR1 is overexpressed in epithelial-derived tumors including ovarian, uterine, breast, endometrial, pancreatic, renal, lung, colorectal, and brain tumors. This expression pattern of FOLR1 makes it a desirable target for FOLR1-directed cancer therapy.

[0007] There is a need for improved methods of modulating the immune regulation of folate receptor alpha (FOLR1) and the downstream signaling processes activated by folate receptor alpha (FOLR1). Moreover, given the specific expression of folate receptor alpha (FOLR1) in cancer- and carcinoma-transformed cells and lower expression in non-cancer tissue, there is a need for improved therapeutics that can specifically target cells and tissues that overexpress folate receptor alpha (FOLR1). Antibody conjugates to FOLR1 could be used to deliver therapeutic or diagnostic payload moieties to target cells expressing folate receptor alpha for the treatment of such diseases. Thus, antibody drug conjugates (ADCs) where the drug is conjugated to anti-FOLR1 antibodies, can provide a very targeted and potent anti-tumor activity.

[0008] In one aspect, provided herein are antibody-drug conjugates (ADCs) comprising a monoclonal antibody. In another aspect, provided herein are antibody-drug conjugates (ADCs) comprising an anti-FOLR1 antibody. In another aspect, provided herein are methods for treating cancers, such as FOLR1-expressing cancers, using the ADCs disclosed herein.

[0009] In embodiments, the present disclosure provides an antibody drug conjugate (ADC) having an IgG antibody that binds to a FOLR1 target conjugated at the cysteine sites of the IgG antibody. In embodiments, the present disclosure provides an antibody drug conjugate (ADC) having an IgG antibody that binds to a FOLR1 target conjugated at the lysine sites of the IgG antibody. The present disclosure further provides a method for treating epithelial-derived tumors including ovarian, uterine, breast, endometrial, pancreatic, renal, lung, colorectal, and brain tumors, comprising providing an effective amount of a FOLR1 ADC.

[0010] In one aspect, provided herein is an antibody drug conjugate (ADC) of formula (I):



, or a pharmaceutically acceptable salt thereof, wherein Ab is an anti-FOLR1 antibody; m is an integer from 1 to 8; L^1 is a linker bound to the anti-FOLR1 antibody; L^2 is a bond, -C(O)-, -NH-, Amino Acid Unit, $-(CH_2CH_2O)_n-$, $-(CH_2)_n-$, –(4-aminobenzyloxycarbonyl)–, $-(C(O)CH_2CH_2NH)-$, or any combination thereof; wherein n is an integer from 1 to 24; and D is a drug moiety.

[0011] In an aspect, provided herein is a method of treating a FOLR1-expressing cancer in a subject in need thereof, said method including administering an ADC described herein (including in an aspect, embodiment, table, example, or claim), or a pharmaceutically acceptable salt thereof, to the subject.

[0012] In an aspect, provided herein is a method of treating a FOLR1-expressing cancer in a subject in need thereof, said method including administering an ADC described herein (including in an aspect, embodiment, table, example, or claim), or a pharmaceutically acceptable salt thereof, and further administering a therapeutically effective amount of one or more additional active agents. In embodiments, the additional active agent is a VEGF inhibitor.

[0013] In any embodiment disclosed herein, the monoclonal antibody can be an anti-FOLR1 antibody.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] FIGS. 1A-C show results of an *in vitro* efficacy study of 151-C-Lock-D5 and 151-K-Lock-D5 and the corresponding controls IgG-C-Lock-D5 and IgG-K-Lock-D5 using: FIG. 1A IGROV1 (high expression FOLR1) cells; FIG. 1B SKOV-3 (low expression FOLR1) cells; and FIG 1C A549 is FOLR1 negative (negative control).

[0015] FIG. 2A shows results of an *in vivo* efficacy study in IGROV1 xenograft in BALB/c nude mice treated with 151-K-Lock-D5 (3 mg/kg or 6 mg/kg), 151-C-Lock-D5 (1.5 mg/kg, 3 mg/kg, or 6 mg/kg), or a control IgG-C-Lock-D5 (6 mg/kg). FIG. 2B shows the tumor volume in mice on day 46 following treatments as described in FIG. 2A. FIG. 2C shows results of *in vivo* toxicity study in mice (following treatments as described in FIG. 2A). The figure shows body weight change in ADC treated mice.

[0016] FIG. 3A shows results of an *in vivo* efficacy study in SKOV-3 xenograft in BALB/c nude mice treated with two doses, on days 15 and 29, of 151-K-Lock-D5 (3 mg/kg or 6 mg/kg each dose), 151-C-Lock-D5 (1.5 mg/kg, 3 mg/kg, or 6 mg/kg each dose) and a control IgG-K-Lock-D5 (6 mg/kg each dose). FIG. 3B shows the tumor volume in mice on day 49 following treatments as described in FIG. 3A. FIG. 3C shows results of *in vivo* toxicity study in mice (following treatments as described in FIG. 3A). The figure shows body weight change in ADC treated mice.

[0017] FIG. 4A shows results of an in vivo antitumor efficacy study in PDX model LU-01-1618 PDX in BALB/c nude mice treated with a single dose of 151-K-Lock-D5 (1.5 mg/kg), 151-K-Lock-D5 (3 mg/kg), 151-K-Lock-D5 (6 mg/kg), 151-K-Lock-D5 (10 mg/kg), isotype ADC (10 mg/kg) and vehicle. FIG. 4B shows results of an in vivo toxicity study in mice (following treatments as described in FIG. 4A). The figure shows body weight change in mice.

[0018] FIG. 5 shows results of an *in vitro* efficacy study of 151-C-Lock-D5 and 151-GGFG-Dxd and a negative control 151-mAb using SKOV-3 (low expression FOLR1) cells.

[0019] FIG. 6A shows results of an *in vivo* efficacy study in SKOV-3 xenograft in BALB/c nude mice treated with two doses, on days 15 and 29, of 151-C-Lock-D5 (3 mg/kg or 6 mg/kg

each dose), 151-VC-MMAE (3 mg/kg or 6 mg/kg each dose) and a control IgG-VC-MMAE (6 mg/kg each dose). FIG. 6B shows results of *in vivo* toxicity study in mice (following treatments as described in FIG. 6A). The figure shows body weight change in ADC treated mice.

[0020] FIG. 7A shows results of an *in vivo* efficacy study in OVCAR-3 xenograft in BALB/c nude mice treated with a single dose of 151-K-Lock-D5 (5 mg/kg), bevacizumab (5 mg/kg), 151-K-Lock-D5/bevacizumab (5 mg/kg each; 151-K-Lock-D5 administered intravenously and bevacizumab intraperitoneally) and a control Isotype ADC (10 mg/kg). FIG. 7B shows the tumor volume in mice on day 48 following treatments as described in FIG. 7A. FIG. 7C shows results of *in vivo* toxicity study in mice (following treatments as described in FIG. 7A). The figure shows body weight change in ADC treated mice.

DETAILED DESCRIPTION OF THE INVENTION

Definitions:

[0021] Unless defined otherwise, technical and scientific terms used herein have meanings that are commonly understood by those of ordinary skill in the art. Generally, terminologies pertaining to techniques of cell and tissue culture, molecular biology, immunology, microbiology, genetics, transgenic cell production, protein chemistry and nucleic acid chemistry and hybridization described herein are well known and commonly used in the art. The methods and techniques provided herein are generally performed according to conventional procedures well known in the art and as described in various general and more specific references that are cited and discussed herein unless otherwise indicated. See, e.g., Sambrook et al. Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates (1992). A number of basic texts describe standard antibody production processes, including, Borrebaeck (ed) *Antibody Engineering, 2nd Edition* Freeman and Company, NY, 1995; McCafferty et al. *Antibody Engineering, A Practical Approach* IRL at Oxford Press, Oxford, England, 1996; and Paul (1995) *Antibody Engineering Protocols* Humana Press, Towata, N.J., 1995; Paul (ed.), *Fundamental Immunology*, Raven Press, N.Y, 1993; Coligan (1991) *Current Protocols in Immunology* Wiley/Greene, NY; Harlow and Lane (1989)

Antibodies: A Laboratory Manual Cold Spring Harbor Press, NY; Stites et al. (eds.) *Basic and Clinical Immunology* (4th ed.) Lange Medical Publications, Los Altos, Calif., and references cited therein; *Coding Monoclonal Antibodies: Principles and Practice* (2nd ed.) Academic Press, New York, N.Y., 1986, and Kohler and Milstein *Nature* 256: 495-497, 1975. All of the references cited herein are incorporated herein by reference in their entireties. Enzymatic reactions and enrichment/purification techniques are also well known and are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The terminology used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are well known and commonly used in the art. Standard techniques can be used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[0022] The headings provided herein are not limitations of the various aspects of the disclosure, which aspects can be understood by reference to the specification as a whole.

[0023] Unless otherwise required by context herein, singular terms shall include pluralities and plural terms shall include the singular. Singular forms "a", "an" and "the", and singular use of any word, include plural referents unless expressly and unequivocally limited on one referent.

[0024] It is understood the use of the alternative (e.g., "or") herein is taken to mean either one or both or any combination thereof of the alternatives.

[0025] The term "and/or" used herein is to be taken mean specific disclosure of each of the specified features or components with or without the other. For example, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "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" is intended to encompass each of the following aspects: 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).

[0026] "Any combination thereof" refers to any two or more, or all, of the preceding elements, and combinations further include those in which an element is repeated. Thus, "X, Y,

Z, or any combination thereof" encompasses X and Y; Y and Z; X and Z; and X, Y, and Z, as well as further combinations in which one or more of X, Y, and Z appear more than once.

[0027] As used herein, the term "about" refers to a value or composition that is within an acceptable error range for the particular value or composition as determined by one of ordinary skill in the art, which will depend in part on how the value or composition is measured or determined, i.e., the limitations of the measurement system. For example, "about" or "approximately" can mean within one or more than one standard deviation per the practice in the art. Alternatively, "about" or "approximately" can mean a range of up to 10% (i.e., ±10%) or more depending on the limitations of the measurement system. For example, about 5 mg can include any number between 4.5 mg and 5.5 mg. Furthermore, particularly with respect to biological systems or processes, the terms can mean up to an order of magnitude or up to 5-fold of a value. When particular values or compositions are provided in the instant disclosure, unless otherwise stated, the meaning of "about" or "approximately" should be assumed to be within an acceptable error range for that particular value or composition. In embodiments, about includes the specified value.

[0028] In this disclosure, "comprises," "comprising," "containing" and "having" and the like can have the meaning ascribed to them in U.S. Patent law and can mean "includes," "including," and the like. "Consisting essentially of" or "consists essentially" likewise has the meaning ascribed in U.S. Patent law and the term is open-ended, allowing for the presence of more than that which is recited so long as basic or novel characteristics of that which is recited is not changed by the presence of more than that which is recited, but excludes prior art embodiments.

[0029] The terms "polypeptide," "peptide" and "protein" and other related terms used herein are used interchangeably to refer to a polymer of amino acid residues, wherein the polymer may in embodiments be conjugated to a moiety that does not consist of amino acids. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers. A "fusion protein" refers to a chimeric protein containing sequences from two or more separate proteins, e.g., that are recombinantly expressed, synthesized, or conjugated to form parts of a single moiety.

Polypeptides include mature molecules that have undergone cleavage. These terms encompass native and artificial proteins, protein fragments and polypeptide analogs (such as muteins, variants, chimeric proteins and fusion proteins) of a protein sequence as well as post-translationally, or otherwise covalently or non-covalently, modified proteins. Two or more polypeptides (e.g., 3 polypeptide chains) can associate with each other, via covalent and/or non-covalent association, to form a multimeric polypeptide complex (e.g., multi-specific antigen binding protein complex). Association of the polypeptide chains can also include peptide folding. Thus, a polypeptide complex can be dimeric, trimeric, tetrameric, or higher order complexes depending on the number of polypeptide chains that form the complex.

[0030] As used herein, the terms "cancer," "neoplasm," and "tumor" are used interchangeably and, in either the singular or plural form, refer to cells that have undergone a malignant transformation that makes them pathological to the host organism. In embodiments, the cancer is a cancer that overexpresses FOLR1. Primary cancer cells can be readily distinguished from non-cancerous cells by well-established techniques, particularly histological examination. The definition of a cancer cell, as used herein, includes not only a primary cancer cell, but any cell derived from a cancer cell ancestor. This includes metastasized cancer cells, and in vitro cultures and cell lines derived from cancer cells. When referring to a type of cancer that normally manifests as a solid tumor, a "clinically detectable" tumor is one that is detectable on the basis of tumor mass; e.g., by procedures such as computed tomography (CT) scan, magnetic resonance imaging (MRI), X-ray, ultrasound or palpation on physical examination, and/or which is detectable because of the expression of one or more cancer-specific antigens in a sample obtainable from a patient. Tumors may be a hematopoietic (or hematologic or hematological or blood-related) cancer, for example, cancers derived from blood cells or immune cells, which may be referred to as "liquid tumors." Specific examples of clinical conditions based on hematologic tumors include leukemias such as chronic myelocytic leukemia, acute myelocytic leukemia, chronic lymphocytic leukemia and acute lymphocytic leukemia; plasma cell malignancies such as multiple myeloma, MGUS and Waldenstrom's macroglobulinemia; lymphomas such as non-Hodgkin's lymphoma, Hodgkin's lymphoma; and the like.

[0031] The cancer may be any cancer in which an abnormal number of blast cells or unwanted cell proliferation is present or that is diagnosed as a hematological cancer, including both lymphoid and myeloid malignancies. Myeloid malignancies include, but are not limited to, acute myeloid (or myelocytic or myelogenous or myeloblastic) leukemia (undifferentiated or differentiated), acute promyeloid (or promyelocytic or promyelogenous or promyeloblastic) leukemia, acute myelomonocytic (or myelomonoblastic) leukemia, acute monocytic (or monoblastic) leukemia, erythroleukemia and megakaryocytic (or megakaryoblastic) leukemia. These leukemias may be referred together as acute myeloid (or myelocytic or myelogenous) leukemia (AML). Myeloid malignancies also include myeloproliferative disorders (MPD) which include, but are not limited to, chronic myelogenous (or myeloid) leukemia (CML), chronic myelomonocytic leukemia (CMML), essential thrombocythemia (or thrombocytosis), and polycythemia vera (PCV). Myeloid malignancies also include myelodysplasia (or myelodysplastic syndrome or MDS), which may be referred to as refractory anemia (RA), refractory anemia with excess blasts (RAEB), and refractory anemia with excess blasts in transformation (RAEBT); as well as myelofibrosis (MFS) with or without agnogenic myeloid metaplasia.

[0032] Hematopoietic cancers also include lymphoid malignancies, which may affect the lymph nodes, spleens, bone marrow, peripheral blood, and/or extranodal sites. Lymphoid cancers include B-cell malignancies, which include, but are not limited to, B-cell non-Hodgkin's lymphomas (B-NHLs). B-NHLs may be low-grade (or indolent), intermediate-grade (or aggressive) or high-grade (very aggressive). Indolent Bcell lymphomas include follicular lymphoma (FL); small lymphocytic lymphoma (SLL); marginal zone lymphoma (MZL) including nodal MZL, extranodal MZL, splenic MZL and splenic MZL with villous lymphocytes; lymphoplasmacytic lymphoma (LPL); and mucosa-associated-lymphoid tissue (MALT or extranodal marginal zone) lymphoma. Intermediate-grade B-NHLs include mantle cell lymphoma (MCL) with or without leukemic involvement, diffuse large cell lymphoma (DLBCL), follicular large cell (or grade 3 or grade 3B) lymphoma, and primary mediastinal lymphoma (PML). High-grade B-NHLs include Burkitt's lymphoma (BL), Burkitt-like lymphoma, small non-cleaved cell lymphoma (SNCCL) and lymphoblastic lymphoma. Other B-NHLs include immunoblastic lymphoma (or immunocytoma), primary effusion lymphoma, HIV

associated (or AIDS related) lymphomas, and post-transplant lymphoproliferative disorder (PTLD) or lymphoma. B-cell malignancies also include, but are not limited to, chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), Waldenstrom's macroglobulinemia (WM), hairy cell leukemia (HCL), large granular lymphocyte (LGL) leukemia, acute lymphoid (or lymphocytic or lymphoblastic) leukemia, and Castleman's disease. NHL may also include T-cell non-Hodgkin's lymphoma s (T-NHLs), which include, but are not limited to T-cell non-Hodgkin's lymphoma not otherwise specified (NOS), peripheral T-cell lymphoma (PTCL), anaplastic large cell lymphoma (ALCL), angioimmunoblastic lymphoid disorder (AILD), nasal natural killer (NK) cell/T-cell lymphoma, gamma/delta lymphoma, cutaneous T cell lymphoma, mycosis fungoides, and Sezary syndrome.

[0033] Hematopoietic cancers also include Hodgkin's lymphoma (or disease) including classical Hodgkin's lymphoma, nodular sclerosing Hodgkin's lymphoma, mixed cellularity Hodgkin's lymphoma, lymphocyte predominant (LP) Hodgkin's lymphoma, nodular LP Hodgkin's lymphoma, and lymphocyte depleted Hodgkin's lymphoma. Hematopoietic cancers also include plasma cell diseases or cancers such as multiple myeloma (MM) including smoldering MM, monoclonal gammopathy of undetermined (or unknown or unclear) significance (MGUS), plasmacytoma (bone, extramedullary), lymphoplasmacytic lymphoma (LPL), Waldenstrom's Macroglobulinemia, plasma cell leukemia, and primary amyloidosis (AL). Hematopoietic cancers may also include other cancers of additional hematopoietic cells, including polymorphonuclear leukocytes (or neutrophils), basophils, eosinophils, dendritic cells, platelets, erythrocytes and natural killer cells. Tissues which include hematopoietic cells referred herein to as "hematopoietic cell tissues" include bone marrow; peripheral blood; thymus; and peripheral lymphoid tissues, such as spleen, lymph nodes, lymphoid tissues associated with mucosa (such as the gut-associated lymphoid tissues), tonsils, Peyer's patches and appendix, and lymphoid tissues associated with other mucosa, for example, the bronchial linings.

[0034] Exemplary cancers that may be treated with a compound or method provided herein include brain cancer, glioma, glioblastoma, neuroblastoma, prostate cancer, colorectal cancer, pancreatic cancer, Medulloblastoma, melanoma, cervical cancer, gastric cancer, ovarian cancer, lung cancer, cancer of the head, Hodgkin's Disease, and Non-Hodgkin's Lymphomas.

Exemplary cancers that may be treated with a compound or method provided herein include cancer of the thyroid, endocrine system, brain, breast, cervix, colon, head & neck, liver, kidney, lung, ovary, pancreas, rectum, stomach, and uterus. Additional examples include, thyroid carcinoma, cholangiocarcinoma, pancreatic adenocarcinoma, skin cutaneous melanoma, colon adenocarcinoma, rectum adenocarcinoma, stomach adenocarcinoma, esophageal carcinoma, head and neck squamous cell carcinoma, breast invasive carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, non-small cell lung carcinoma, mesothelioma, multiple myeloma, neuroblastoma, glioma, glioblastoma multiforme, ovarian cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, primary brain tumors, malignant pancreatic insulanoma, malignant carcinoid, urinary bladder cancer, premalignant skin lesions, testicular cancer, thyroid cancer, neuroblastoma, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, endometrial cancer, adrenal cortical cancer, neoplasms of the endocrine or exocrine pancreas, medullary thyroid cancer, medullary thyroid carcinoma, melanoma, colorectal cancer, papillary thyroid cancer, hepatocellular carcinoma, or prostate cancer.

[0035] In embodiments, the cancers that may be treated with an immunoconjugate or method provided herein include epithelial-derived tumors including ovarian, uterine, breast, endometrial, pancreatic, renal, lung, colorectal, and brain tumors. In embodiments, the cancers that may be treated with an immunoconjugate or a method provided herein include serous and endometrioid epithelial ovarian cancer, endometrial adenocarcinoma, non-small cell lung carcinoma (NSCLC) of the adenocarcinoma subtype, and triple-negative breast cancer (TNBC).

[0036] An "advanced" cancer is one which has spread outside the site or organ of origin, either by local invasion or metastasis. The term "advanced" cancer includes both locally advanced and metastatic disease. "Metastatic" cancer refers to cancer that has spread from one part of the body to another part of the body. A "refractory" cancer is one that progresses even though an anti-tumor treatment, such as a chemotherapy, is administered to the cancer patient. An example of a refractory cancer is one which is platinum refractory. A "recurrent" cancer is one that has regrown, either at the initial site or at a distant site, after a response to initial therapy.

[0037] An "antibody" and "antibodies" and related terms used herein refers to an intact immunoglobulin or to an antigen binding portion thereof that binds specifically to an antigen. Antigen binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. Antigen binding portions include, *inter alia*, Fab, Fab', $F(ab')_2$, Fv, domain antibodies (dAbs), and complementarity determining region (CDR) fragments, single-chain antibodies (scFv), chimeric antibodies, diabodies, triabodies, tetrabodies, and polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the polypeptide.

[0038] Antibodies include recombinantly produced antibodies and antigen binding portions. Antibodies include non-human, chimeric, humanized and fully human antibodies. Antibodies include monospecific, multispecific (e.g., bispecific, trispecific and higher order specificities). Antibodies include tetrameric antibodies, light chain monomers, heavy chain monomers, light chain dimers, heavy chain dimers. Antibodies include $F(ab')_2$ fragments, Fab' fragments and Fab fragments. Antibodies include single domain antibodies, monovalent antibodies, single chain antibodies, single chain variable fragment (scFv), camelized antibodies, affibodies, disulfide-linked Fvs (sdFv), anti-idiotypic antibodies (anti-Id), minibodies. Antibodies include monoclonal and polyclonal populations. Anti-FOLR1 antibodies are described herein.

[0039] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not

limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

[0040] An "epitope" and related terms as used herein refers to a portion of an antigen that is bound by an antigen binding protein (e.g., by an antibody or an antigen binding portion thereof). An epitope can comprise portions of two or more antigens that are bound by an antigen binding protein. An epitope can comprise non-contiguous portions of an antigen or of two or more antigens (e.g., amino acid residues that are not contiguous in an antigen's primary sequence but that, in the context of the antigen's tertiary and quaternary structure, are near enough to each other to be bound by an antigen binding protein). Generally, the variable regions, particularly the CDRs, of an antibody interact with the epitope. Anti-FOLR1 antibodies, and antigen binding proteins thereof, that bind an epitope of a FOLR1 polypeptide are described herein.

[0041] An "antibody fragment", "antibody portion", "antigen-binding fragment of an antibody", or "antigen-binding portion of an antibody" and other related terms used herein refer to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include, but are not limited to, Fv, Fab, Fab', Fab'-SH, $F(ab')_2$; Fd; and Fv fragments, as well as dAb; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); polypeptides that contain at least a portion of an antibody that is sufficient to confer specific antigen binding to the polypeptide. Antigen binding portions of an antibody may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. Antigen binding portions include, inter alia, Fab, Fab', F(ab')2, Fv, domain antibodies (dAbs), and complementarity determining region (CDR) fragments, chimeric antibodies, diabodies, triabodies, tetrabodies, and polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer antigen binding properties to the antibody fragment. Antigen-binding fragments of anti-FOLR1 antibodies are described herein.

[0042] An antigen binding protein can have, for example, the structure of an immunoglobulin. In one embodiment, an "immunoglobulin" refers to a tetrameric molecule. Each tetrameric molecule is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa or lambda light chains. In embodiments, the light chains are kappa. In embodiments, the light chains are lambda. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 or more amino acids. See generally, Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair form the antibody binding site such that an intact immunoglobulin has two antigen binding sites. In one embodiment, an antigen binding protein can be a synthetic molecule having a structure that differs from a tetrameric immunoglobulin molecule but still binds a target antigen or binds two or more target antigens. For example, a synthetic antigen binding protein can comprise antibody fragments, 1-6 or more polypeptide chains, asymmetrical assemblies of polypeptides, or other synthetic molecules. The terms "variable heavy chain," "V_H," or "VH" refer to the variable region of an immunoglobulin heavy chain, including an Fv, scFv , dsFv or Fab; while the terms "variable light chain," "V_L" or "VL" refer to the variable region of an immunoglobulin light chain, including of an Fv, scFv , dsFv or Fab. "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (See, e.g., Kindt et al. Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to

screen a library of complementary VL or VH domains, respectively. See, e.g., Portolano et al., J. Immunol. 150:880-887 (1993); Clarkson et al., Nature 352:624-628 (1991). Antigen binding proteins having immunoglobulin-like properties that bind specifically to FOLR1 are described herein.

[0043] Examples of antibody functional fragments include, but are not limited to, complete antibody molecules, antibody fragments, such as Fv, single chain Fv (scFv), complementarity determining regions (CDRs), VL (light chain variable region), VH (heavy chain variable region), Fab, F(ab)2' and any combination of those or any other functional portion of an immunoglobulin peptide capable of binding to target antigen (*see, e.g.*, FUNDAMENTAL IMMUNOLOGY (Paul ed., 4th ed. 2001). As appreciated by one of skill in the art, various antibody fragments can be obtained by a variety of methods, for example, digestion of an intact antibody with an enzyme, such as pepsin; or de novo synthesis. Antibody fragments are often synthesized de novo either chemically or by using recombinant DNA methodology. Thus, the term antibody, as used herein, includes antibody fragments either produced by the modification of whole antibodies, or those synthesized de novo using recombinant DNA methodologies (*e.g.*, single chain Fv) or those identified using phage display libraries (see, *e.g.*, McCafferty *et al.*, (1990) *Nature* 348:552). The term "antibody" also includes bivalent or bispecific molecules, diabodies, triabodies, and tetrabodies. Bivalent and bispecific molecules are described in, *e.g.*, Kostelny *et al.* (1992) *J. Immunol.* 148:1547, Pack and Pluckthun (1992) *Biochemistry* 31:1579, Hollinger *et al.*(1993), *PNAS. USA* 90:6444, Gruber *et al.* (1994) *J Immunol.* 152:5368, Zhu *et al.* (1997) *Protein Sci.* 6:781, Hu *et al.* (1996) *Cancer Res.* 56:3055, Adams *et al.* (1993) *Cancer Res.* 53:4026, and McCartney, *et al.* (1995) *Protein Eng.* 8:301.

[0044] The terms “antigen binding protein” “antigen binding domain,” “antigen binding region,” or “antigen binding site” and related terms used herein refers to a protein comprising a portion that binds to an antigen and, optionally, a scaffold or framework portion that allows the antigen binding portion to adopt a conformation that promotes binding of the antigen binding protein to the antigen. Examples of antigen binding proteins include antibodies, antibody fragments (e.g., an antigen binding portion of an antibody), antibody derivatives, and antibody analogs. The antigen binding protein can comprise, for example, an alternative protein scaffold

or artificial scaffold with grafted CDRs or CDR derivatives. Such scaffolds include, but are not limited to, antibody-derived scaffolds comprising mutations introduced to, for example, stabilize the three-dimensional structure of the antigen binding protein as well as wholly synthetic scaffolds comprising, for example, a biocompatible polymer. See, for example, Korndorfer et al., 2003, Proteins: Structure, Function, and Bioinformatics, Volume 53, Issue 1:121-129; Roque et al., 2004, Biotechnol. Prog. 20:639-654. In addition, peptide antibody mimetics ("PAMs") can be used, as well as scaffolds based on antibody mimetics utilizing fibronectin components as a scaffold. Antigen binding proteins that bind FOLR1 are described herein.

[0045] In one embodiment, a dissociation constant (K_D) can be measured using a BIACORE surface plasmon resonance (SPR) assay. Surface plasmon resonance refers to an optical phenomenon that allows for the analysis of real-time interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIACORE system (Biacore Life Sciences division of GE Healthcare, Piscataway, NJ).

[0046] "Specifically binds" as used throughout the present specification in relation to anti-FOLR1 antigen binding proteins means that the antigen binding protein binds human FOLR1 (h FOLR1) with no or insignificant binding to other human proteins. The term however does not exclude the fact that antigen binding proteins of the invention may also be cross-reactive with other forms of FOLR1, for example primate FOLR1. In embodiments, an antibody specifically binds to a target antigen if it binds to the antigen with a dissociation constant K_D of 10^{-5} M or less, or 10^{-6} M or less, or 10^{-7} M or less, or 10^{-8} M or less, or 10^{-9} M or less, or 10^{-10} M or less.

[0047] The term "FOLR1," as used herein, refers to any native FOLR1 from any vertebrate source, including mammals such as primates (e.g. humans, cynomolgus monkey (cyno)) and rodents (e.g., mice and rats), unless otherwise indicated. FOLR1 is also referred to as "human folate receptor 1," "folate receptor alpha (FR-α)," and "FRα". FOLR1 is a single chain membrane protein capable of binding to folic acid and its derivatives. The term encompasses "full-length," unprocessed FOLR1 as well as any form of FOLR1 that results from processing in the cell. The term also encompasses naturally occurring variants of FOLR1, e.g., splice variants, allelic variants, and isoforms. Human FOLR 1 sequences are known and include, for example,

the sequences publicly available at UniProtKB Accession No. P 15328 (including isoforms). The amino acid sequence of an exemplary human FOLR1 protein is shown in SEQ ID NO: 9.

[0048] The term "FOLR1-expressing cancer" refers to a cancer comprising cells that express FOLR on their surface.

[0049] The term "increased expression" or "overexpression" of FOLR1 in a particular tumor, tissue, or cell sample refers to FOLR 1 (a FOLR1 polypeptide or a nucleic acid encoding such a polypeptide) that is present at a level higher than that which is present in a healthy or non-diseased (native, wild type) tissue or cells of the same type or origin. Such increased expression or overexpression can be caused, for example, by mutation, gene amplification, increased transcription, increased translation, or increased protein stability.

[0050] The terms "anti-FOLR1 antibody" and "an antibody that binds to FOLR1" refer to an antibody that is capable of binding FOLR1 with sufficient affinity such that the antibody is useful as a therapeutic agent in targeting FOLR1. In one embodiment, the extent of binding of an anti-FOLR1 antibody to an unrelated, non-FOLR1 protein is less than about 10% of the binding of the antibody to FOLR1 as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to FOLR1 has a dissociation constant (Kd) of $\leq 1\mu M$, ≤ 100 nM, ≤ 10 nM, , ≤ 5 nM , ≤ 4 nM, ≤ 3 nM, ≤ 2 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g., 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M). In certain embodiments, an anti-FOLR1 antibody binds to an epitope of FOLR1 that is conserved among FOLR1 from different species.

[0051] The term "chimeric antibody" and related terms used herein refers to an antibody that contains one or more regions from a first antibody and one or more regions from one or more other antibodies. In one embodiment, one or more of the CDRs are derived from a human antibody. In another embodiment, all of the CDRs are derived from a human antibody. In another embodiment, the CDRs from more than one human antibody are mixed and matched in a chimeric antibody. For instance, a chimeric antibody may comprise a CDR1 from the light chain of a first human antibody, a CDR2 and a CDR3 from the light chain of a second human antibody, and the CDRs from the heavy chain from a third antibody. In another example, the CDRs

originate from different species such as human and mouse, or human and rabbit, or human and goat. One skilled in the art will appreciate that other combinations are possible.

[0052] Further, the framework regions may be derived from one of the same antibodies, from one or more different antibodies, such as a human antibody, or from a humanized antibody. In one example of a chimeric antibody, a portion of the heavy and/or light chain is identical with, homologous to, or derived from an antibody from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is/are identical with, homologous to, or derived from an antibody (-ies) from another species or belonging to another antibody class or subclass. Also included are fragments of such antibodies that exhibit the desired biological activity (i.e., the ability to specifically bind a target antigen). Chimeric antibodies can be prepared from portions of any of the anti-FOLR1 antibodies described herein.

[0053] "Effector functions" refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor); and B cell activation.

[0054] The term "Fc" or "Fc region" as used herein refers to the portion of an antibody heavy chain constant region beginning in or after the hinge region and ending at the C-terminus of the heavy chain. The Fc region comprises at least a portion of the CH and CH3 regions, and may or may not include a portion of the hinge region. Two polypeptide chains each carrying a half Fc region can dimerize to form an Fc region. An Fc region can bind Fc cell surface receptors and some proteins of the immune complement system. An Fc region exhibits effector function, including any one or any combination of two or more activities including complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent phagocytosis (ADP), opsonization and/or cell binding. An Fc region can bind an Fc receptor, including FcγRI (e.g., CD64), FcγRII (e.g, CD32) and/or FcγRIII (e.g., CD16a).

[0055] "Humanized antibody" refers to an antibody having a sequence that differs from the sequence of an antibody derived from a non-human species by one or more amino acid substitutions, deletions, and/or additions, such that the humanized antibody is less likely to

induce an immune response, and/or induces a less severe immune response, as compared to the non-human species antibody, when it is administered to a human subject. In one embodiment, certain amino acids in the framework and constant domains of the heavy and/or light chains of the non-human species antibody are mutated to produce the humanized antibody. In another embodiment, the constant domain(s) from a human antibody are fused to the variable domain(s) of a non-human species. In another embodiment, one or more amino acid residues in one or more CDR sequences of a non-human antibody are changed to reduce the likely immunogenicity of the non-human antibody when it is administered to a human subject, wherein the changed amino acid residues either are not critical for immunospecific binding of the antibody to its antigen, or the changes to the amino acid sequence that are made are conservative changes, such that the binding of the humanized antibody to the antigen is not significantly worse than the binding of the non-human antibody to the antigen. Examples of how to make humanized antibodies may be found in U.S. Pat. Nos. 6,054,297, 5,886,152 and 5,877,293.

[0056] The term “human antibody” refers to antibodies that have one or more variable and constant regions derived from human immunoglobulin sequences. In one embodiment, all of the variable and constant domains are derived from human immunoglobulin sequences (e.g., a fully human antibody). These antibodies may be prepared in a variety of ways, examples of which are described below, including through recombinant methodologies or through immunization with an antigen of interest of a mouse that is genetically modified to express antibodies derived from human heavy and/or light chain-encoding genes. Fully human anti-FOLR1 antibodies and antigen binding proteins thereof are described herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

[0057] The term "isolated", means altered “by the hand of man” from its natural state, has been changed or removed from its original environment, or both. When the term “isolated” is applied to a nucleic acid or protein, denotes that the nucleic acid or protein is essentially free of other cellular components with which it is associated in the natural state. It can be, for example, in a homogeneous state and may be in either a dry or aqueous solution. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis, high-performance liquid chromatography or mass spectrophotometry. A protein that is the predominant species present in a preparation is substantially purified. For example, a

polynucleotide or a polypeptide naturally present in a living organism is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", including but not limited to when such polynucleotide or polypeptide is introduced back into a cell, even if the cell is of the same species or type as that from which the polynucleotide or polypeptide was separated.

[0058] "CDRs" are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable domains of immunoglobulin heavy and light chains. There are three heavy chain and three light chain CDRs (or CDR regions) in the variable portion of an immunoglobulin. Thus, "CDRs" as used herein may refer to all three heavy chain CDRs, or all three light chain CDRs (or both all heavy and all light chain CDRs, if appropriate).

[0059] CDRs provide the majority of contact residues for the binding of the antibody to the antigen or epitope. CDRs of interest in this invention are derived from donor antibody variable heavy and light chain sequences, and include analogs of the naturally occurring CDRs, which analogs also share or retain the same antigen binding specificity and/or neutralizing ability as the donor antibody from which they were derived.

[0060] The CDR sequences of antibodies can be determined by the Kabat numbering system (Kabat et al; (Sequences of proteins of Immunological Interest NIH, 1987); alternatively they can be determined using the Chothia numbering system (Al-Lazikani et al., (1997) JMB 273, 927-948), the contact definition method (MacCallum R. M., and Martin A. C. R. and Thornton J. M, (1996), Journal of Molecular Biology, 262 (5), 732-745) or any other established method for numbering the residues in an antibody and determining CDRs known to the skilled man in the art.

[0061] Other numbering conventions for CDR sequences available to a skilled person include "AbM" (University of Bath) and "contact" (University College London) methods. The minimum overlapping region using at least two of the Kabat, Chothia, AbM and contact methods can be determined to provide the "minimum binding unit". The minimum binding unit may be a sub-portion of a CDR.

[0062] "Affinity" refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

[0063] An "affinity matured" antibody refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.

[0064] As used herein, the term "variant" polypeptides and "variants" of polypeptides refers to a polypeptide comprising an amino acid sequence with one or more amino acid residues inserted into, deleted from and/or substituted into the amino acid sequence relative to a reference polypeptide sequence. Polypeptide variants include fusion proteins. In the same manner, a variant polynucleotide comprises a nucleotide sequence with one or more nucleotides inserted into, deleted from and/or substituted into the nucleotide sequence relative to another polynucleotide sequence. Polynucleotide variants include fusion polynucleotides.

[0065] As used herein the term "domain" refers to a folded protein structure which has tertiary structure independent of the rest of the protein. Generally, domains are responsible for discrete functional properties of proteins and in many cases may be added, removed or transferred to other proteins without loss of function of the remainder of the protein and/or of the domain. An "antibody single variable domain" is a folded polypeptide domain comprising sequences characteristic of antibody variable domains. It therefore includes complete antibody variable domains and modified variable domains, for example, in which one or more loops have been replaced by sequences which are not characteristic of antibody variable domains, or antibody variable domains which have been truncated or comprise N- or C-terminal

extensions, as well as folded fragments of variable domains which retain at least the binding activity and specificity of the full-length domain.

[0066] The term "cytotoxic agent" or "payload" as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to, radioactive isotopes (e.g., ^{211}At, ^{131}I, ^{125}I, ^{90}Y, ^{186}Re, ^{188}Re, ^{153}Sm, ^{212}Bi, ^{32}P, ^{212}Pb and radioactive isotopes of Lu); chemotherapeutic agents or drugs (e.g., methotrexate, adriamycin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof; and the various antitumor or anticancer agents disclosed below.

[0067] A "chemotherapeutic agent" is a chemical compound useful in the treatment of a cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclosphosphamide (CYTOXAN®); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scopolectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; auristatin; duostatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and

ranimnustine; antibiotics such as the enediyne antibiotics (e. g., calicheamicin, especially calicheamicin gamma1I and calicheamicin omegaI1 (see, e.g., Agnew, Chem Intl. Ed. Engl., 33: 183-186 (1994)); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores), aclacinomysins, actinomycin, anthramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); thiotepa; taxoids, e.g., paclitaxel (TAXOL®; Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANETM Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, Illinois), and docetaxel (TAXOTERE®; Rhône-Poulenc Rorer, Antony, France); chloranbucil; gemcitabine (GEMZAR®); 6-thioguanine;

mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine (VELBAN®); platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine (ONCOVIN®); oxaliplatin; leucovorin; vinorelbine (NAVELBINE®); novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine (XELODA®); pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone; CVP, an abbreviation for a combined therapy of cyclophosphamide, vincristine, and prednisolone; and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATINTM) combined with 5-FU and leucovorin.

[0068] An "antibody-drug conjugate" or "ADC" is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent. The antibody can be any antibody described herein. The cytotoxic agent can be any cytotoxic agent described herein. The antibody can be directly linked to the cytotoxic agent via a covalent bond, or the antibody can be linked to the cytotoxic agent indirectly via a linker. Typically, the linker is covalently bonded to the antibody and also covalently bonded to the cytotoxic agent. Such a linker may be a cleavable linker, for example, cleavable under certain pH condition (pH sensitive linker), cleavable by a protease (protease sensitive linker), or cleavable in the presence of glutathione (glutathione sensitive linker). In some examples, the linker comprises a protease cleavage site, which may contain 2-5 amino acid residues that are recognizable and/or cleavable by a suitable protease. Such a peptide may comprise naturally-occurring amino acid residues, non-naturally occurring amino acid residues, or a combination thereof. In one example, the peptide linker can be a dipeptide linker. Examples include a valine-citrulline (val-cit) linker, a phenylalanine-lysine (phe-lys) linker, or maleimidocapronic-valine-citruline-p-aminobenzyloxycarbonyl (vc) linker. Alternatively, the linker may be non-cleavable, e.g., a linker comprising optionally substituted alkane or thioether. In some examples, the linker may comprise a functional group that can form a covalent bond with the antibody. Exemplary functional groups include, but are not limited to, a maleimide group, an iodoacetamide group, a vinyl sulfone group, an acrylate group, an acrylamide group, an acrylonitrile group, or a

methacrylate group. The term "antibody drug conjugate" or "ADC" refers to a conjugate wherein at least one cytotoxic agent is a therapeutic moiety such as a drug.

[0069] As used herein, the term "conjugated" when referring to two moieties means the two moieties are bonded, wherein the bond or bonds connecting the two moieties may be covalent or non-covalent. In embodiments, the two moieties are covalently bonded to each other (e.g. directly or through a covalently bonded intermediary). In embodiments, the two moieties are non-covalently bonded (e.g. through ionic bond(s), van der waal's bond(s)/interactions, hydrogen bond(s), polar bond(s), or combinations or mixtures thereof).

[0070] An "individual" or "subject" is a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human. In certain embodiments, the subject is an adult, an adolescent, a child, or an infant. In some embodiments, the terms "individual" or "patient" are used and are intended to be interchangeable with "subject".

[0071] "Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, with the aid of the local homology algorithm by Smith and Waterman, 1981, Ads App. Math. 2, 482, with the aid of the local homology algorithm by Needleman and Wunsch, 1970, J. Mol. Biol. 48, 443, with the aid of the similarity search algorithm by Pearson and Lipman, 1988, Proc. Natl Acad. Sci. USA 88, 2444, or with the aid of computer programs using said algorithms (e.g., EMBOSS Needle or EMBOSS Water, available at www.ebi.ac.uk/Tools/psa/). Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. "Percentage of sequence identity" or "percent (%) [sequence] identity", as used herein, is determined by

comparing two optimally locally aligned sequences over a comparison window defined by the length of the local alignment between the two sequences. (This may also be considered percentage of homology or "percent (%) homology".) The amino acid sequence in the comparison window may comprise additions or deletions (e.g., gaps or overhangs) as compared to the reference sequence for optimal alignment of the two sequences. Local alignment between two sequences only includes segments of each sequence that are deemed to be sufficiently similar according to a criterion that depends on the algorithm used to perform the alignment (e.g., EMBOSS Water). "identical" or percent "identity," refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (i.e., about 60% identity, preferably 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region, when compared and aligned for maximum correspondence over a comparison window or designated region). The percentage identity is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman (Add. APL. Math. 2:482, 1981), by the global homology alignment algorithm of Needleman and Wunsch (J. Mol. Biol. 48:443, 1970), by the search for similarity method of Pearson and Lipman (Proc. Natl. Acad. Sci. USA 85: 2444, 1988), or by inspection. GAP and BESTFIT, as additional examples, can be employed to determine the optimal alignment of two sequences that have been identified for comparison. Typically, the default values of 5.00 for gap weight and 0.30 for gap weight length are used.

[0072] A comparison of the sequences and determination of the percent identity between two polypeptide sequences, or between two polynucleotide sequences, may be accomplished using a mathematical algorithm. For example, the "percent identity" or "percent homology" of two polypeptide or two polynucleotide sequences may be determined by comparing the sequences using the GAP computer program (a part of the GCG Wisconsin Package, version 10.3 (Accelrys, San Diego, Calif.)) using its default parameters. Expressions such as “comprises a sequence with at least X% identity to Y” with respect to a test sequence mean that, when aligned

to sequence Y as described above, the test sequence comprises residues identical to at least X% of the residues of Y.

[0073] In one embodiment, the amino acid sequence of a test antibody may be similar but not identical to any of the amino acid sequences of the polypeptides that make up the multi-specific antigen binding protein complexes described herein. The similarities between the test antibody and the polypeptides can be at least 95%, or at least 96% identical, or at least 97% identical, or at least 98% identical, or at least 99% identical, to any of the polypeptides that make up the multi-specific antigen binding protein complexes described herein. In one embodiment, similar polypeptides can contain amino acid substitutions within a heavy and/or light chain. In one embodiment, the amino acid substitutions comprise one or more conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. See, e.g., Pearson (1994) Methods Mol. Biol. 24: 307-331, herein incorporated by reference in its entirety. Examples of groups of amino acids that have side chains with similar chemical properties include (1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; (2) aliphatic-hydroxyl side chains: serine and threonine; (3) amide-containing side chains: asparagine and glutamine; (4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; (5) basic side chains: lysine, arginine, and histidine; (6) acidic side chains: aspartate and glutamate, and (7) sulfur-containing side chains: cysteine and methionine.

[0074] Antibodies can be obtained from sources such as serum or plasma that contain immunoglobulins having varied antigenic specificity. If such antibodies are subjected to affinity purification, they can be enriched for a particular antigenic specificity. Such enriched preparations of antibodies usually are made of less than about 10% antibody having specific binding activity for the particular antigen. Subjecting these preparations to several rounds of

affinity purification can increase the proportion of antibody having specific binding activity for the antigen. Antibodies prepared in this manner are often referred to as "monospecific." Monospecific antibody preparations can be made up of about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 99.9% antibody having specific binding activity for the particular antigen. Antibodies can be produced using recombinant nucleic acid technology as described below.

[0075] The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

[0076] The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

[0077] The term "pharmaceutically acceptable salts" is meant to include salts of the active compounds that are prepared with relatively nontoxic acids or bases, depending on the particular substituents found on the compounds described herein. When compounds of the present disclosure contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable base addition salts include sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt. When compounds of the present disclosure contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of

pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, oxalic, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galacturonic acids and the like (*see*, for example, Berge *et al.*, "Pharmaceutical Salts", *Journal of Pharmaceutical Science*, **1977**, *66*, 1-19). Certain specific compounds of the present disclosure contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

[0078] Thus, the compounds of the present disclosure may exist as salts, such as with pharmaceutically acceptable acids. The present disclosure includes such salts. Non-limiting examples of such salts include hydrochlorides, hydrobromides, phosphates, sulfates, methanesulfonates, nitrates, maleates, acetates, citrates, fumarates, proprionates, tartrates (e.g., (+)-tartrates, (-)-tartrates, or mixtures thereof including racemic mixtures), succinates, benzoates, and salts with amino acids such as glutamic acid, and quaternary ammonium salts (e.g. methyl iodide, ethyl iodide, and the like). These salts may be prepared by methods known to those skilled in the art.

[0079] The neutral forms of the compounds are preferably regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound may differ from the various salt forms in certain physical properties, such as solubility in polar solvents.

[0080] In addition to salt forms, the present disclosure provides compounds, which are in a prodrug form. Prodrugs of the compounds described herein are those compounds that readily undergo chemical changes under physiological conditions to provide the compounds of the present disclosure. Prodrugs of the compounds described herein may be converted *in vivo* after administration. Additionally, prodrugs can be converted to the compounds of the present

disclosure by chemical or biochemical methods in an *ex vivo* environment, such as, for example, when contacted with a suitable enzyme or chemical reagent.

[0081] Certain compounds of the present disclosure can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are encompassed within the scope of the present disclosure. Certain compounds of the present disclosure may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present disclosure and are intended to be within the scope of the present disclosure.

[0082] "Pharmaceutically acceptable excipient" and "pharmaceutically acceptable carrier" refer to a substance that aids the administration of an active agent to and absorption by a subject and can be included in the compositions of the present disclosure without causing a significant adverse toxicological effect on the patient. Non-limiting examples of pharmaceutically acceptable excipients include water, NaCl, normal saline solutions, lactated Ringer's, normal sucrose, normal glucose, binders, fillers, disintegrants, lubricants, coatings, sweeteners, flavors, salt solutions (such as Ringer's solution), alcohols, oils, gelatins, carbohydrates such as lactose, amylose or starch, fatty acid esters, hydroxymethycellulose, polyvinyl pyrrolidine, and colors, and the like. Such preparations can be sterilized and, if desired, mixed with auxiliary agents such as lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, and/or aromatic substances and the like that do not deleteriously react with the compounds of the disclosure. One of skill in the art will recognize that other pharmaceutical excipients are useful in the present disclosure.

[0083] The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[0084] The term "administering", "administered" and grammatical variants refers to the physical introduction of an agent to a subject, using any of the various methods and delivery systems known to those skilled in the art. Exemplary routes of administration for the formulations disclosed herein include intravenous, intramuscular, subcutaneous, intraperitoneal,

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, intralymphatic, intralesional, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion, as well as in vivo electroporation. In some embodiments, the formulation is administered via a non-parenteral route, e.g., orally. Other non-parenteral routes include a topical, epidermal or mucosal route of administration, for example, intranasally, 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.

[0085] Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) or consecutive administration in any order. The combination therapy can provide "synergy" and prove "synergistic", i.e., the effect achieved when the active ingredients used together is greater than the sum of the effects that results from using the compounds separately. A synergistic effect can be attained when the active ingredients are: (1) co-formulated and administered or delivered simultaneously in a combined, unit dosage formulation; (2) delivered serially, by alternation, or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation therapy, a synergistic effect can be attained when the compounds are administered or delivered sequentially, e.g. , by different injections in separate syringes. A synergistic combination produces effects that are greater than the additive effects of the individual components of the combination.

[0086] An "effective amount" of an agent, e.g., a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

[0087] The abbreviations used herein have their conventional meaning within the chemical and biological arts. The chemical structures and formulae set forth herein are constructed according to the standard rules of chemical valency known in the chemical arts.

[0088] Descriptions of compounds of the present disclosure are limited by principles of chemical bonding known to those skilled in the art. Accordingly, where a group may be substituted by one or more of a number of substituents, such substitutions are selected so as to comply with principles of chemical bonding and to give compounds which are not inherently unstable and/or would be known to one of ordinary skill in the art as likely to be unstable under ambient conditions, such as aqueous, neutral, and several known physiological conditions. For example, a heterocycloalkyl or heteroaryl is attached to the remainder of the molecule via a ring heteroatom in compliance with principles of chemical bonding known to those skilled in the art thereby avoiding inherently unstable compounds.

[0089] Where substituent groups are specified by their conventional chemical formulae, written from left to right, they equally encompass the chemically identical substituents that would result from writing the structure from right to left, e.g., $-CH_2O-$ is equivalent to $-OCH_2-$.

[0090] The term saccharide means carbohydrate (or sugar). In embodiments, the saccharide is a monosaccharide. In embodiments, the saccharide is a polysaccharide. The most basic unit of saccharide is a monomer of carbohydrate. The general formula is $C_nH_{2n}O_n$. The term saccharide derivative means sugar molecules that have been modified with substituents other than hydroxyl groups. Examples include glycosylamines, sugar phosphates, and sugar esters. Other saccharide derivatives include for example beta-D-glucuronyl, D-galactosyl, and D-glucosyl.

[0091] The term "Charged Group" means a chemical group bearing a positive or a negative charge, such as for example phosphate, phosphonate, sulfate, sulfonate, nitrate, carboxylate, carbonate, etc. In some embodiments, a Charged Group is at least 50% ionized in aqueous solution at least one pH in the range of 5-9. In some embodiments, a Charged Group is an anionic Charged Group.

[0092] The term "alkyl," by itself or as part of another substituent, means, unless otherwise stated, a straight (i.e., unbranched) or branched carbon chain (or carbon), or combination thereof, which may be fully saturated, mono- or polyunsaturated and can include mono-, di- and multivalent radicals. The alkyl may include a designated number of carbons (e.g., C_1-C_{10} means one to ten carbons). Alkyl is an uncyclized chain. Examples of saturated hydrocarbon radicals

include, but are not limited to, groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, methyl, homologs and isomers of, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like. An unsaturated alkyl group is one having one or more double bonds or triple bonds. Examples of unsaturated alkyl groups include, but are not limited to, vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butynyl, and the higher homologs and isomers. An alkoxy is an alkyl attached to the remainder of the molecule via an oxygen linker (-O-). An alkyl moiety may be an alkenyl moiety. An alkyl moiety may be an alkynyl moiety. An alkyl moiety may be fully saturated. An alkenyl may include more than one double bond and/or one or more triple bonds in addition to the one or more double bonds. An alkynyl may include more than one triple bond and/or one or more double bonds in addition to the one or more triple bonds.

[0093] The term "alkylene," by itself or as part of another substituent, means, unless otherwise stated, a divalent radical derived from an alkyl, as exemplified, but not limited by, $-CH_2CH_2CH_2CH_2-$. Typically, an alkyl (or alkylene) group will have from 1 to 24 carbon atoms, with those groups having 10 or fewer carbon atoms being preferred herein. A "lower alkyl" or "lower alkylene" is a shorter chain alkyl or alkylene group, generally having eight or fewer carbon atoms. The term "alkenylene," by itself or as part of another substituent, means, unless otherwise stated, a divalent radical derived from an alkene.

[0094] The term "heteroalkyl," by itself or in combination with another term, means, unless otherwise stated, a stable straight or branched chain, or any combination thereof, including at least one carbon atom and at least one heteroatom (e.g., O, N, P, Si, or S), and wherein the nitrogen and sulfur atoms may optionally be oxidized, and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) (e.g., O, N, S, Si, or P) may be placed at any interior position of the heteroalkyl group or at the position at which the alkyl group is attached to the remainder of the molecule. Heteroalkyl is an uncyclized chain. Examples include, but are not limited to: $-CH_2-CH_2-O-CH_3$, $-CH_2-CH_2-NH-CH_3$, $-CH_2-CH_2-N(CH_3)-CH_3$, $-CH_2-S-CH_2-CH_3$, $-CH_2-S-CH_2$, $-S(O)-CH_3$, $-CH_2-CH_2-S(O)_2-CH_3$, $-CH=CH-O-CH_3$, $-Si(CH_3)_3$, $-CH_2-CH=N-OCH_3$, $-CH=CH-N(CH_3)-CH_3$, $-O-CH_3$, $-O-CH_2-CH_3$, and -CN. Up to two or three heteroatoms may be consecutive, such as, for example, $-CH_2-NH-OCH_3$ and $-CH_2-O-Si(CH_3)_3$. A heteroalkyl

moiety may include one heteroatom (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include two optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include three optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include four optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include five optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include up to 8 optionally different heteroatoms (e.g., O, N, S, Si, or P). The term "heteroalkenyl," by itself or in combination with another term, means, unless otherwise stated, a heteroalkyl including at least one double bond. A heteroalkenyl may optionally include more than one double bond and/or one or more triple bonds in addition to the one or more double bonds. The term "heteroalkynyl," by itself or in combination with another term, means, unless otherwise stated, a heteroalkyl including at least one triple bond. A heteroalkynyl may optionally include more than one triple bond and/or one or more double bonds in addition to the one or more triple bonds.

[0095] Similarly, the term "heteroalkylene," by itself or as part of another substituent, means, unless otherwise stated, a divalent radical derived from heteroalkyl, as exemplified, but not limited by, $-CH_2-CH_2-S-CH_2-CH_2-$ and $-CH_2-S-CH_2-CH_2-NH-CH_2-$. For heteroalkylene groups, heteroatoms can also occupy either or both of the chain termini (e.g., alkyleneoxy, alkylenedioxy, alkyleneamino, alkylenediamino, and the like). Still further, for alkylene and heteroalkylene linking groups, no orientation of the linking group is implied by the direction in which the formula of the linking group is written. For example, the formula $-C(O)_2R'-$ represents both $-C(O)_2R'-$ and $-R'C(O)_2-$. As described above, heteroalkyl groups, as used herein, include those groups that are attached to the remainder of the molecule through a heteroatom, such as -C(O)R', -C(O)NR', -NR'R", -OR', -SR', and/or $-SO_2R'$. Where "heteroalkyl" is recited, followed by recitations of specific heteroalkyl groups, such as -NR'R" or the like, it will be understood that the terms heteroalkyl and -NR'R" are not redundant or mutually exclusive. Rather, the specific heteroalkyl groups are recited to add clarity. Thus, the term "heteroalkyl" should not be interpreted herein as excluding specific heteroalkyl groups, such as -NR'R" or the like.

[0096] The terms "cycloalkyl" and "heterocycloalkyl," by themselves or in combination with other terms, mean, unless otherwise stated, cyclic versions of "alkyl" and "heteroalkyl,"

respectively. Cycloalkyl and heterocycloalkyl are not aromatic. Additionally, for heterocycloalkyl, a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, and the like. Examples of heterocycloalkyl include, but are not limited to, 1-(1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholinyl, 3-morpholinyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1-piperazinyl, 2-piperazinyl, and the like. A "cycloalkylene" and a "heterocycloalkylene," alone or as part of another substituent, means a divalent radical derived from a cycloalkyl and heterocycloalkyl, respectively.

[0097] In embodiments, the term "cycloalkyl" means a monocyclic, bicyclic, or a multicyclic cycloalkyl ring system. In embodiments, monocyclic ring systems are cyclic hydrocarbon groups containing from 3 to 8 carbon atoms, where such groups can be saturated or unsaturated, but not aromatic. In embodiments, cycloalkyl groups are fully saturated. Examples of monocyclic cycloalkyls include cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl, cycloheptyl, and cyclooctyl. Bicyclic cycloalkyl ring systems are bridged monocyclic rings or fused bicyclic rings. In embodiments, bridged monocyclic rings contain a monocyclic cycloalkyl ring where two non adjacent carbon atoms of the monocyclic ring are linked by an alkylene bridge of between one and three additional carbon atoms (i.e., a bridging group of the form $(CH_2)_w$, where w is 1, 2, or 3). Representative examples of bicyclic ring systems include, but are not limited to, bicyclo[3.1.1]heptane, bicyclo[2.2.1]heptane, bicyclo[2.2.2]octane, bicyclo[3.2.2]nonane, bicyclo[3.3.1]nonane, and bicyclo[4.2.1]nonane. In embodiments, fused bicyclic cycloalkyl ring systems contain a monocyclic cycloalkyl ring fused to either a phenyl, a monocyclic cycloalkyl, a monocyclic cycloalkenyl, a monocyclic heterocyclyl, or a monocyclic heteroaryl. In embodiments, the bridged or fused bicyclic cycloalkyl is attached to the parent molecular moiety through any carbon atom contained within the monocyclic cycloalkyl ring. In embodiments, cycloalkyl groups are optionally substituted with one or two groups which are independently oxo or thia. In embodiments, the fused bicyclic cycloalkyl is a 5 or 6 membered monocyclic cycloalkyl ring fused to either a phenyl ring, a 5 or 6 membered monocyclic cycloalkyl, a 5 or 6 membered monocyclic cycloalkenyl, a 5 or 6

membered monocyclic heterocyclyl, or a 5 or 6 membered monocyclic heteroaryl, wherein the fused bicyclic cycloalkyl is optionally substituted by one or two groups which are independently oxo or thia. In embodiments, multicyclic cycloalkyl ring systems are a monocyclic cycloalkyl ring (base ring) fused to either (i) one ring system selected from the group consisting of a bicyclic aryl, a bicyclic heteroaryl, a bicyclic cycloalkyl, a bicyclic cycloalkenyl, and a bicyclic heterocyclyl; or (ii) two other ring systems independently selected from the group consisting of a phenyl, a bicyclic aryl, a monocyclic or bicyclic heteroaryl, a monocyclic or bicyclic cycloalkyl, a monocyclic or bicyclic cycloalkenyl, and a monocyclic or bicyclic heterocyclyl. In embodiments, the multicyclic cycloalkyl is attached to the parent molecular moiety through any carbon atom contained within the base ring. In embodiments, multicyclic cycloalkyl ring systems are a monocyclic cycloalkyl ring (base ring) fused to either (i) one ring system selected from the group consisting of a bicyclic aryl, a bicyclic heteroaryl, a bicyclic cycloalkyl, a bicyclic cycloalkenyl, and a bicyclic heterocyclyl; or (ii) two other ring systems independently selected from the group consisting of a phenyl, a monocyclic heteroaryl, a monocyclic cycloalkyl, a monocyclic cycloalkenyl, and a monocyclic heterocyclyl. Examples of multicyclic cycloalkyl groups include, but are not limited to tetradecahydrophenanthrenyl, perhydrophenothiazin-1-yl, and perhydrophenoxazin-1-yl.

[0098] In embodiments, a cycloalkyl is a cycloalkenyl. The term "cycloalkenyl" is used in accordance with its plain ordinary meaning. In embodiments, a cycloalkenyl is a monocyclic, bicyclic, or a multicyclic cycloalkenyl ring system. In embodiments, monocyclic cycloalkenyl ring systems are cyclic hydrocarbon groups containing from 3 to 8 carbon atoms, where such groups are unsaturated (i.e., containing at least one annular carbon carbon double bond), but not aromatic. Examples of monocyclic cycloalkenyl ring systems include cyclopentenyl and cyclohexenyl. In embodiments, bicyclic cycloalkenyl rings are bridged monocyclic rings or a fused bicyclic rings. In embodiments, bridged monocyclic rings contain a monocyclic cycloalkenyl ring where two non adjacent carbon atoms of the monocyclic ring are linked by an alkylene bridge of between one and three additional carbon atoms (i.e., a bridging group of the form $(CH_2)_w$, where w is 1, 2, or 3). Representative examples of bicyclic cycloalkenyls include, but are not limited to, norbornenyl and bicyclo[2.2.2]oct 2 enyl. In embodiments, fused bicyclic cycloalkenyl ring systems contain a monocyclic cycloalkenyl ring fused to either a phenyl, a

monocyclic cycloalkyl, a monocyclic cycloalkenyl, a monocyclic heterocyclyl, or a monocyclic heteroaryl. In embodiments, the bridged or fused bicyclic cycloalkenyl is attached to the parent molecular moiety through any carbon atom contained within the monocyclic cycloalkenyl ring. In embodiments, cycloalkenyl groups are optionally substituted with one or two groups which are independently oxo or thia. In embodiments, multicyclic cycloalkenyl rings contain a monocyclic cycloalkenyl ring (base ring) fused to either (i) one ring system selected from the group consisting of a bicyclic aryl, a bicyclic heteroaryl, a bicyclic cycloalkyl, a bicyclic cycloalkenyl, and a bicyclic heterocyclyl; or (ii) two ring systems independently selected from the group consisting of a phenyl, a bicyclic aryl, a monocyclic or bicyclic heteroaryl, a monocyclic or bicyclic cycloalkyl, a monocyclic or bicyclic cycloalkenyl, and a monocyclic or bicyclic heterocyclyl. In embodiments, the multicyclic cycloalkenyl is attached to the parent molecular moiety through any carbon atom contained within the base ring. In embodiments, multicyclic cycloalkenyl rings contain a monocyclic cycloalkenyl ring (base ring) fused to either (i) one ring system selected from the group consisting of a bicyclic aryl, a bicyclic heteroaryl, a bicyclic cycloalkyl, a bicyclic cycloalkenyl, and a bicyclic heterocyclyl; or (ii) two ring systems independently selected from the group consisting of a phenyl, a monocyclic heteroaryl, a monocyclic cycloalkyl, a monocyclic cycloalkenyl, and a monocyclic heterocyclyl.

[0099] In embodiments, a heterocycloalkyl is a heterocyclyl. The term "heterocyclyl" as used herein, means a monocyclic, bicyclic, or multicyclic heterocycle. The heterocyclyl monocyclic heterocycle is a 3, 4, 5, 6 or 7 membered ring containing at least one heteroatom independently selected from the group consisting of O, N, and S where the ring is saturated or unsaturated, but not aromatic. The 3 or 4 membered ring contains 1 heteroatom selected from the group consisting of O, N and S. The 5 membered ring can contain zero or one double bond and one, two or three heteroatoms selected from the group consisting of O, N and S. The 6 or 7 membered ring contains zero, one or two double bonds and one, two or three heteroatoms selected from the group consisting of O, N and S. The heterocyclyl monocyclic heterocycle is connected to the parent molecular moiety through any carbon atom or any nitrogen atom contained within the heterocyclyl monocyclic heterocycle. Representative examples of heterocyclyl monocyclic heterocycles include, but are not limited to, azetidinyl, azepanyl, aziridinyl, diazepanyl, 1,3-dioxanyl, 1,3-dioxolanyl, 1,3-dithiolanyl, 1,3-dithianyl, imidazolinyl, imidazolidinyl,

isothiazolinyl, isothiazolidinyl, isoxazolinyl, isoxazolidinyl, morpholinyl, oxadiazolinyl, oxadiazolidinyl, oxazolinyl, oxazolidinyl, piperazinyl, piperidinyl, pyranyl, pyrazolinyl, pyrazolidinyl, pyrrolinyl, pyrrolidinyl, tetrahydrofuranyl, tetrahydrothienyl, thiadiazolinyl, thiadiazolidinyl, thiazolinyl, thiazolidinyl, thiomorpholinyl, 1,1-dioxidothiomorpholinyl (thiomorpholine sulfone), thiopyranyl, and trithianyl. The heterocyclyl bicyclic heterocycle is a monocyclic heterocycle fused to either a phenyl, a monocyclic cycloalkyl, a monocyclic cycloalkenyl, a monocyclic heterocycle, or a monocyclic heteroaryl. The heterocyclyl bicyclic heterocycle is connected to the parent molecular moiety through any carbon atom or any nitrogen atom contained within the monocyclic heterocycle portion of the bicyclic ring system. Representative examples of bicyclic heterocyclyls include, but are not limited to, 2,3-dihydrobenzofuran-2-yl, 2,3-dihydrobenzofuran-3-yl, indolin-1-yl, indolin-2-yl, indolin-3-yl, 2,3-dihydrobenzothien-2-yl, decahydroquinolinyl, decahydroisoquinolinyl, octahydro-1H-indolyl, and octahydrobenzofuranyl. In embodiments, heterocyclyl groups are optionally substituted with one or two groups which are independently oxo or thia. In certain embodiments, the bicyclic heterocyclyl is a 5 or 6 membered monocyclic heterocyclyl ring fused to a phenyl ring, a 5 or 6 membered monocyclic cycloalkyl, a 5 or 6 membered monocyclic cycloalkenyl, a 5 or 6 membered monocyclic heterocyclyl, or a 5 or 6 membered monocyclic heteroaryl, wherein the bicyclic heterocyclyl is optionally substituted by one or two groups which are independently oxo or thia. Multicyclic heterocyclyl ring systems are a monocyclic heterocyclyl ring (base ring) fused to either (i) one ring system selected from the group consisting of a bicyclic aryl, a bicyclic heteroaryl, a bicyclic cycloalkyl, a bicyclic cycloalkenyl, and a bicyclic heterocyclyl; or (ii) two other ring systems independently selected from the group consisting of a phenyl, a bicyclic aryl, a monocyclic or bicyclic heteroaryl, a monocyclic or bicyclic cycloalkyl, a monocyclic or bicyclic cycloalkenyl, and a monocyclic or bicyclic heterocyclyl. The multicyclic heterocyclyl is attached to the parent molecular moiety through any carbon atom or nitrogen atom contained within the base ring. In embodiments, multicyclic heterocyclyl ring systems are a monocyclic heterocyclyl ring (base ring) fused to either (i) one ring system selected from the group consisting of a bicyclic aryl, a bicyclic heteroaryl, a bicyclic cycloalkyl, a bicyclic cycloalkenyl, and a bicyclic heterocyclyl; or (ii) two other ring systems independently selected from the group consisting of a phenyl, a monocyclic heteroaryl, a monocyclic cycloalkyl, a monocyclic

cycloalkenyl, and a monocyclic heterocyclyl. Examples of multicyclic heterocyclyl groups include, but are not limited to 10H-phenothiazin-10-yl, 9,10-dihydroacridin-9-yl, 9,10-dihydroacridin-10-yl, 10H-phenoxazin-10-yl, 10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl, 1,2,3,4-tetrahydropyrido[4,3-g]isoquinolin-2-yl, 12H-benzo[b]phenoxazin-12-yl, and dodecahydro-1H-carbazol-9-yl.

[00100] The terms "halo" or "halogen," by themselves or as part of another substituent, mean, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom. Additionally, terms such as "haloalkyl" are meant to include monohaloalkyl and polyhaloalkyl. For example, the term "halo(C_1-C_4)alkyl" includes, but is not limited to, fluoromethyl, difluoromethyl, trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like.

[00101] The term "acyl" means, unless otherwise stated, -C(O)R where R is a substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.

[00102] The term "aryl" means, unless otherwise stated, a polyunsaturated, aromatic, hydrocarbon substituent, which can be a single ring or multiple rings (preferably from 1 to 3 rings) that are fused together (i.e., a fused ring aryl) or linked covalently. A fused ring aryl refers to multiple rings fused together wherein at least one of the fused rings is an aryl ring. The term "heteroaryl" refers to aryl groups (or rings) that contain at least one heteroatom such as N, O, or S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. Thus, the term "heteroaryl" includes fused ring heteroaryl groups (i.e., multiple rings fused together wherein at least one of the fused rings is a heteroaromatic ring). A 5,6-fused ring heteroarylene refers to two rings fused together, wherein one ring has 5 members and the other ring has 6 members, and wherein at least one ring is a heteroaryl ring. Likewise, a 6,6-fused ring heteroarylene refers to two rings fused together, wherein one ring has 6 members and the other ring has 6 members, and wherein at least one ring is a heteroaryl ring. And a 6,5-fused ring heteroarylene refers to two rings fused together, wherein one ring has 6 members and the other ring has 5 members, and wherein at least one ring is a heteroaryl ring. A heteroaryl group can be attached to the remainder of the molecule through a carbon or heteroatom. Non-

limiting examples of aryl and heteroaryl groups include phenyl, naphthyl, pyrrolyl, pyrazolyl, pyridazinyl, triazinyl, pyrimidinyl, imidazolyl, pyrazinyl, purinyl, oxazolyl, isoxazolyl, thiazolyl, furyl, thienyl, pyridyl, pyrimidyl, benzothiazolyl, benzoxazoyl, benzimidazolyl, benzofuran, isobenzofuranyl, indolyl, isoindolyl, benzothiophenyl, isoquinolyl, quinoxalinyl, quinolyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxalinyl, 5-quinoxalinyl, 3-quinolyl, and 6-quinolyl. Substituents for each of the above noted aryl and heteroaryl ring systems are selected from the group of acceptable substituents described below. An "arylene" and a "heteroarylene," alone or as part of another substituent, mean a divalent radical derived from an aryl and heteroaryl, respectively. A heteroaryl group substituent may be -O- bonded to a ring heteroatom nitrogen.

[00103] A fused ring heterocyloalkyl-aryl is an aryl fused to a heterocycloalkyl. A fused ring heterocycloalkyl-heteroaryl is a heteroaryl fused to a heterocycloalkyl. A fused ring heterocycloalkyl-cycloalkyl is a heterocycloalkyl fused to a cycloalkyl. A fused ring heterocycloalkyl-heterocycloalkyl is a heterocycloalkyl fused to another heterocycloalkyl. Fused ring heterocycloalkyl-aryl, fused ring heterocycloalkyl-heteroaryl, fused ring heterocycloalkyl-cycloalkyl, or fused ring heterocycloalkyl-heterocycloalkyl may each independently be unsubstituted or substituted with one or more of the substitutents described herein.

[00104] Spirocyclic rings are two or more rings wherein adjacent rings are attached through a single atom. The individual rings within spirocyclic rings may be identical or different. Individual rings in spirocyclic rings may be substituted or unsubstituted and may have different substituents from other individual rings within a set of spirocyclic rings. Possible substituents for individual rings within spirocyclic rings are the possible substituents for the same ring when not part of spirocyclic rings (e.g. substituents for cycloalkyl or heterocycloalkyl rings). Spirocylic rings may be substituted or unsubstituted cycloalkyl, substituted or unsubstituted cycloalkylene, substituted or unsubstituted heterocycloalkyl or substituted or unsubstituted heterocycloalkylene

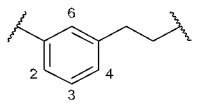
and individual rings within a spirocyclic ring group may be any of the immediately previous list, including having all rings of one type (e.g. all rings being substituted heterocycloalkylene wherein each ring may be the same or different substituted heterocycloalkylene). When referring to a spirocyclic ring system, heterocyclic spirocyclic rings means a spirocyclic rings wherein at least one ring is a heterocyclic ring and wherein each ring may be a different ring. When referring to a spirocyclic ring system, substituted spirocyclic rings means that at least one ring is substituted and each substituent may optionally be different.

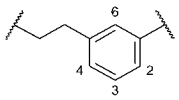
[00105] The symbol "〰" (a wavy line) denotes the point of attachment of a chemical moiety to the remainder of a molecule or chemical formula.

[00106] The term "oxo," as used herein, means an oxygen that is double bonded to a carbon atom.

[00107] The term "alkylsulfonyl," as used herein, means a moiety having the formula $-S(O_2)-R'$, where R' is a substituted or unsubstituted alkyl group as defined above. R' may have a specified number of carbons (e.g., "C_1-C_4 alkylsulfonyl").

[00108] The term "alkylarylene" as an arylene moiety covalently bonded to an alkylene moiety (also referred to herein as an alkylene linker). In embodiments, the alkylarylene group has the formula:



or


.

[00109] An alkylarylene moiety may be substituted on the alkylene moiety or the arylene linker (e.g. at carbons 2, 3, 4, or 6) with a substituent group （e.g. halogen, oxo, $-N_3$, $-CF_3$, $-CCl_3$, $-CBr_3$, $-CI_3$, -CN, -CHO, -OH, $-NH_2$, -COOH, $-CONH_2$, $-NO_2$, -SH, $-SO_2CH_3$ $-SO_3H$, , $-OSO_3H$, $-SO_2NH_2$, $-NHNH_2$, $-ONH_2$, $-NHC(O)NHNH_2$, substituted or unsubstituted C_1-C_5 alkyl or substituted or unsubstituted 2 to 5 membered heteroalkyl). In embodiments, the alkylarylene is unsubstituted.

[00110] Each of the above terms (e.g., "alkyl," "heteroalkyl," "cycloalkyl," "heterocycloalkyl," "aryl," and "heteroaryl") includes both substituted and unsubstituted forms of the indicated radical. Preferred substituents for each type of radical are provided below.

[00111] Substituents for the alkyl and heteroalkyl radicals (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) can be one or more of a variety of groups selected from, but not limited to, -OR', =O, =NR', =N-OR', -NR'R", -SR', -halogen, -SiR'R"R"', -OC(O)R', -C(O)R', -CO_2R', -CONR'R", -OC(O)NR'R", -NR"C(O)R', -NR'-C(O)NR"R"', -NR"$C(O)_2$R', -NR-C(NR'R"R"')=NR"", -NR-C(NR'R")=NR"', -S(O)R', -$S(O)_2$R', -$S(O)_2$NR'R", -$NRSO_2$R', –NR'NR"R"', –ONR'R", –NR'C(O)NR"NR"'R"", -CN, -NO_2, -$NR'SO_2$R", -NR'C(O)R", -NR'C(O)-OR", -NR'OR", in a number ranging from zero to (2m'+1), where m' is the total number of carbon atoms in such radical. R, R', R", R"', and R"" each preferably independently refer to hydrogen, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl (e.g., aryl substituted with 1-3 halogens), substituted or unsubstituted heteroaryl, substituted or unsubstituted alkyl, alkoxy, or thioalkoxy groups, or arylalkyl groups. When a compound described herein includes more than one R group, for example, each of the R groups is independently selected as are each R', R", R"', and R"" group when more than one of these groups is present. When R' and R" are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 4-, 5-, 6-, or 7-membered ring. For example, -NR'R" includes, but is not limited to, 1-pyrrolidinyl and 4-morpholinyl. From the above discussion of substituents, one of skill in the art will understand that the term "alkyl" is meant to include groups including carbon atoms bound to groups other than hydrogen groups, such as haloalkyl (e.g., -CF_3 and -CH_2CF_3) and acyl (e.g., -$C(O)CH_3$, -$C(O)CF_3$, -$C(O)CH_2OCH_3$, and the like).

[00112] Similar to the substituents described for the alkyl radical, substituents for the aryl and heteroaryl groups are varied and are selected from, for example: -OR', -NR'R", -SR', -halogen, -SiR'R"R"', -OC(O)R', -C(O)R', -CO_2R', -CONR'R", -OC(O)NR'R", -NR"C(O)R', -NR'-C(O)NR"R"', -NR"$C(O)_2$R', -NR-C(NR'R"R"')=NR"", -NR-C(NR'R")=NR"', -S(O)R', -$S(O)_2$R', -$S(O)_2$NR'R", -$NRSO_2$R', –NR'NR"R"', –ONR'R", –NR'C(O)NR"NR"'R"", -CN, -NO_2, -R', -N_3, -

$CH(Ph)_2$, fluoro(C_1-C_4)alkoxy, and fluoro(C_1-C_4)alkyl, -NR'SO_2R", -NR'C(O)R", -NR'C(O)-OR", -NR'OR", in a number ranging from zero to the total number of open valences on the aromatic ring system; and where R', R", R"', and R"" are preferably independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl. When a compound described herein includes more than one R group, for example, each of the R groups is independently selected as are each R', R", R"', and R"" groups when more than one of these groups is present.

[00113] Substituents for rings (e.g. cycloalkyl, heterocycloalkyl, aryl, heteroaryl, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene) may be depicted as substituents on the ring rather than on a specific atom of a ring (commonly referred to as a floating substituent). In such a case, the substituent may be attached to any of the ring atoms (obeying the rules of chemical valency) and in the case of fused rings or spirocyclic rings, a substituent depicted as associated with one member of the fused rings or spirocyclic rings (a floating substituent on a single ring), may be a substituent on any of the fused rings or spirocyclic rings (a floating substituent on multiple rings). When a substituent is attached to a ring, but not a specific atom (a floating substituent), and a subscript for the substituent is an integer greater than one, the multiple substituents may be on the same atom, same ring, different atoms, different fused rings, different spirocyclic rings, and each substituent may optionally be different. Where a point of attachment of a ring to the remainder of a molecule is not limited to a single atom (a floating substituent), the attachment point may be any atom of the ring and in the case of a fused ring or spirocyclic ring, any atom of any of the fused rings or spirocyclic rings while obeying the rules of chemical valency. Where a ring, fused rings, or spirocyclic rings contain one or more ring heteroatoms and the ring, fused rings, or spirocyclic rings are shown with one or more floating substituents (including, but not limited to, points of attachment to the remainder of the molecule), the floating substituents may be bonded to the heteroatoms. Where the ring heteroatoms are shown bound to one or more hydrogens (e.g. a ring nitrogen with two bonds to ring atoms and a third bond to a hydrogen) in the structure or formula with the floating substituent, when the heteroatom is bonded to the floating substituent, the substituent will be understood to replace the hydrogen, while obeying the rules of chemical valency.

[00114] Two or more substituents may optionally be joined to form aryl, heteroaryl, cycloalkyl, or heterocycloalkyl groups. Such so-called ring-forming substituents are typically, though not necessarily, found attached to a cyclic base structure. In one embodiment, the ring-forming substituents are attached to adjacent members of the base structure. For example, two ring-forming substituents attached to adjacent members of a cyclic base structure create a fused ring structure. In another embodiment, the ring-forming substituents are attached to a single member of the base structure. For example, two ring-forming substituents attached to a single member of a cyclic base structure create a spirocyclic structure. In yet another embodiment, the ring-forming substituents are attached to non-adjacent members of the base structure.

[00115] Two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally form a ring of the formula -T-C(O)-$(CRR')_p$-U-, wherein T and U are independently -NR-, -O-, -CRR'-, or a single bond, and p is an integer of from 0 to 3. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -A-$(CH_2)_r$-B-, wherein A and B are independently -CRR'-, -O-, -NR-, -S-, -S(O) -, -$S(O)_2$-, -$S(O)_2$NR'-, or a single bond, and r is an integer of from 1 to 4. One of the single bonds of the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -$(CRR')_s$-X'-$(C"R"R"')_d$-, where s and d are independently integers of from 0 to 3, and X' is -O-, -NR'-, -S-, -S(O)-, -$S(O)_2$-, or -$S(O)_2$NR'-. The substituents R, R', R", and R"' are preferably independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl.

[00116] As used herein, the terms "heteroatom" or "ring heteroatom" are meant to include oxygen (O), nitrogen (N), sulfur (S), phosphorus (P), and silicon (Si).

[00117] A "substituent group," as used herein, means a group selected from the following moieties:

(A) oxo, halogen, -CCl_3, -CBr_3, -CF_3, -CI_3, -CH_2Cl, -CH_2Br, -CH_2F, -CH_2I, -$CHCl_2$, -$CHBr_2$, -CHF_2, -CHI_2, -CN, -OH, -NH_2, -COOH, -$CONH_2$, -NO_2, -SH, -SO_3H,

-SO_4H, -SO_2NH_2, –$NHNH_2$, –ONH_2, –$NHC(O)NHNH_2$, -$NHC(O)NH_2$, -$NHSO_2H$, -NHC(O)H, -NHC(O)OH, -NHOH, -$OCCl_3$, -OCF_3, -$OCBr_3$, -OCI_3,-$OCHCl_2$, -$OCHBr_2$, -$OCHI_2$, -$OCHF_2$, -N_3, unsubstituted alkyl (e.g., C_1-C_8 alkyl, C_1-C_6 alkyl, or C_1-C_4 alkyl), unsubstituted heteroalkyl (e.g., 2 to 8 membered heteroalkyl, 2 to 6 membered heteroalkyl, or 2 to 4 membered heteroalkyl), unsubstituted cycloalkyl (e.g., C_3-C_8 cycloalkyl, C_3-C_6 cycloalkyl, or C_5-C_6 cycloalkyl), unsubstituted heterocycloalkyl (e.g., 3 to 8 membered heterocycloalkyl, 3 to 6 membered heterocycloalkyl, or 5 to 6 membered heterocycloalkyl), unsubstituted aryl (e.g., C_6-C_{10} aryl, C_{10} aryl, or phenyl), or unsubstituted heteroaryl (e.g., 5 to 10 membered heteroaryl, 5 to 9 membered heteroaryl, or 5 to 6 membered heteroaryl), and

(B) alkyl (e.g., C_1-C_8 alkyl, C_1-C_6 alkyl, or C_1-C_4 alkyl), heteroalkyl (e.g., 2 to 8 membered heteroalkyl, 2 to 6 membered heteroalkyl, or 2 to 4 membered heteroalkyl), cycloalkyl (e.g., C_3-C_8 cycloalkyl, C_3-C_6 cycloalkyl, or C_5-C_6 cycloalkyl), heterocycloalkyl (e.g., 3 to 8 membered heterocycloalkyl, 3 to 6 membered heterocycloalkyl, or 5 to 6 membered heterocycloalkyl), aryl (e.g., C_6-C_{10} aryl, C_{10} aryl, or phenyl), heteroaryl (e.g., 5 to 10 membered heteroaryl, 5 to 9 membered heteroaryl, or 5 to 6 membered heteroaryl), substituted with at least one substituent selected from:

(i) oxo, halogen, -CCl_3, -CBr_3, -CF_3, -CI_3, -CH_2Cl, -CH_2Br, -CH_2F, -CH_2I, -$CHCl_2$, -$CHBr_2$, -CHF_2, -CHI_2, -CN, -OH, -NH_2, -COOH, -$CONH_2$, -NO_2, -SH, -SO_3H, -SO_4H, -SO_2NH_2, –$NHNH_2$, –ONH_2, –$NHC(O)NHNH_2$, -$NHC(O)NH_2$, -$NHSO_2H$, -NHC(O)H, -NHC(O)OH, -NHOH, -$OCCl_3$, -OCF_3, -$OCBr_3$, -OCI_3,-$OCHCl_2$, -$OCHBr_2$, -$OCHI_2$, -$OCHF_2$, -N_3, unsubstituted alkyl (e.g., C_1-C_8 alkyl, C_1-C_6 alkyl, or C_1-C_4 alkyl), unsubstituted heteroalkyl (e.g., 2 to 8 membered heteroalkyl, 2 to 6 membered heteroalkyl, or 2 to 4 membered heteroalkyl), unsubstituted cycloalkyl (e.g., C_3-C_8 cycloalkyl, C_3-C_6 cycloalkyl, or C_5-C_6 cycloalkyl), unsubstituted heterocycloalkyl (e.g., 3 to 8 membered heterocycloalkyl, 3 to 6 membered heterocycloalkyl, or 5 to 6 membered heterocycloalkyl), unsubstituted aryl (e.g., C_6-C_{10} aryl, C_{10} aryl, or phenyl), or unsubstituted heteroaryl (e.g., 5 to 10 membered heteroaryl, 5 to 9 membered heteroaryl, or 5 to 6 membered heteroaryl), and

(ii) alkyl (e.g., C_1-C_8 alkyl, C_1-C_6 alkyl, or C_1-C_4 alkyl), heteroalkyl (e.g., 2 to 8 membered heteroalkyl, 2 to 6 membered heteroalkyl, or 2 to 4 membered heteroalkyl),

cycloalkyl (e.g., C_3-C_8 cycloalkyl, C_3-C_6 cycloalkyl, or C_5-C_6 cycloalkyl), heterocycloalkyl (e.g., 3 to 8 membered heterocycloalkyl, 3 to 6 membered heterocycloalkyl, or 5 to 6 membered heterocycloalkyl), aryl (e.g., C_6-C_{10} aryl, C_{10} aryl, or phenyl), heteroaryl (e.g., 5 to 10 membered heteroaryl, 5 to 9 membered heteroaryl, or 5 to 6 membered heteroaryl), substituted with at least one substituent selected from:

(a) oxo, halogen, $-CCl_3$, $-CBr_3$, $-CF_3$, $-CI_3$, $-CH_2Cl$, $-CH_2Br$, $-CH_2F$, $-CH_2I$, $-CHCl_2$, $-CHBr_2$, $-CHF_2$, $-CHI_2$, -CN, -OH, $-NH_2$, -COOH, $-CONH_2$, $-NO_2$, -SH, $-SO_3H$, $-SO_4H$, $-SO_2NH_2$, $-NHNH_2$, $-ONH_2$, $-NHC(O)NHNH_2$, $-NHC(O)NH_2$, $-NHSO_2H$, -NHC(O)H, -NHC(O)OH, -NHOH, $-OCCl_3$, $-OCF_3$, $-OCBr_3$, $-OCI_3$, $-OCHCl_2$, $-OCHBr_2$, $-OCHI_2$, $-OCHF_2$, $-N_3$, unsubstituted alkyl (e.g., C_1-C_8 alkyl, C_1-C_6 alkyl, or C_1-C_4 alkyl), unsubstituted heteroalkyl (e.g., 2 to 8 membered heteroalkyl, 2 to 6 membered heteroalkyl, or 2 to 4 membered heteroalkyl), unsubstituted cycloalkyl (e.g., C_3-C_8 cycloalkyl, C_3-C_6 cycloalkyl, or C_5-C_6 cycloalkyl), unsubstituted heterocycloalkyl (e.g., 3 to 8 membered heterocycloalkyl, 3 to 6 membered heterocycloalkyl, or 5 to 6 membered heterocycloalkyl), unsubstituted aryl (e.g., C_6-C_{10} aryl, C_{10} aryl, or phenyl), or unsubstituted heteroaryl (e.g., 5 to 10 membered heteroaryl, 5 to 9 membered heteroaryl, or 5 to 6 membered heteroaryl), and

(b) alkyl (e.g., C_1-C_8 alkyl, C_1-C_6 alkyl, or C_1-C_4 alkyl), heteroalkyl (e.g., 2 to 8 membered heteroalkyl, 2 to 6 membered heteroalkyl, or 2 to 4 membered heteroalkyl), cycloalkyl (e.g., C_3-C_8 cycloalkyl, C_3-C_6 cycloalkyl, or C_5-C_6 cycloalkyl), heterocycloalkyl (e.g., 3 to 8 membered heterocycloalkyl, 3 to 6 membered heterocycloalkyl, or 5 to 6 membered heterocycloalkyl), aryl (e.g., C_6-C_{10} aryl, C_{10} aryl, or phenyl), heteroaryl (e.g., 5 to 10 membered heteroaryl, 5 to 9 membered heteroaryl, or 5 to 6 membered heteroaryl), substituted with at least one substituent selected from: oxo, halogen, $-CCl_3$, $-CBr_3$, $-CF_3$, $-CI_3$, $-CH_2Cl$, $-CH_2Br$, $-CH_2F$, $-CH_2I$, $-CHCl_2$, $-CHBr_2$, $-CHF_2$, $-CHI_2$, -CN, -OH, $-NH_2$, -COOH, $-CONH_2$, $-NO_2$, -SH, $-SO_3H$, $-SO_4H$, $-SO_2NH_2$, $-NHNH_2$, $-ONH_2$, $-NHC(O)NHNH_2$, $-NHC(O)NH_2$, $-NHSO_2H$, -NHC(O)H, -NHC(O)OH, -NHOH, $-OCCl_3$, $-OCF_3$, $-OCBr_3$, $-OCI_3$,$-OCHCl_2$, $-OCHBr_2$, $-OCHI_2$, $-OCHF_2$, $-N_3$, unsubstituted alkyl (e.g., C_1-C_8 alkyl, C_1-C_6 alkyl, or C_1-C_4 alkyl), unsubstituted heteroalkyl (e.g., 2 to 8 membered heteroalkyl, 2 to 6 membered heteroalkyl, or 2 to 4 membered heteroalkyl), unsubstituted cycloalkyl (e.g., C_3-C_8

cycloalkyl, C_3-C_6 cycloalkyl, or C_5-C_6 cycloalkyl), unsubstituted heterocycloalkyl (e.g., 3 to 8 membered heterocycloalkyl, 3 to 6 membered heterocycloalkyl, or 5 to 6 membered heterocycloalkyl), unsubstituted aryl (e.g., C_6-C_{10} aryl, C_{10} aryl, or phenyl), or unsubstituted heteroaryl (e.g., 5 to 10 membered heteroaryl, 5 to 9 membered heteroaryl, or 5 to 6 membered heteroaryl).

[00118] A "size-limited substituent" or " size-limited substituent group," as used herein, means a group selected from all of the substituents described above for a "substituent group," wherein each substituted or unsubstituted alkyl is a substituted or unsubstituted C_1-C_{20} alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 20 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C_3-C_8 cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 8 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C_6-C_{10} aryl, and each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 10 membered heteroaryl.

[00119] A "lower substituent" or " lower substituent group," as used herein, means a group selected from all of the substituents described above for a "substituent group," wherein each substituted or unsubstituted alkyl is a substituted or unsubstituted C_1-C_8 alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 8 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C_3-C_7 cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 7 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted phenyl, and each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 6 membered heteroaryl.

[00120] In some embodiments, each substituted group described in the compounds herein is substituted with at least one substituent group. More specifically, in some embodiments, each substituted alkyl, substituted heteroalkyl, substituted cycloalkyl, substituted heterocycloalkyl, substituted aryl, substituted heteroaryl, substituted alkylene, substituted heteroalkylene, substituted cycloalkylene, substituted heterocycloalkylene, substituted arylene, and/or substituted heteroarylene described in the compounds herein are substituted with at least one substituent

group. In other embodiments, at least one or all of these groups are substituted with at least one size-limited substituent group. In other embodiments, at least one or all of these groups are substituted with at least one lower substituent group.

[00121] In other embodiments of the compounds herein, each substituted or unsubstituted alkyl may be a substituted or unsubstituted C_1-C_{20} alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 20 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C_3-C_8 cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 8 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C_6-C_{10} aryl, and/or each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 10 membered heteroaryl. In some embodiments of the compounds herein, each substituted or unsubstituted alkylene is a substituted or unsubstituted C_1-C_{20} alkylene, each substituted or unsubstituted heteroalkylene is a substituted or unsubstituted 2 to 20 membered heteroalkylene, each substituted or unsubstituted cycloalkylene is a substituted or unsubstituted C_3-C_8 cycloalkylene, each substituted or unsubstituted heterocycloalkylene is a substituted or unsubstituted 3 to 8 membered heterocycloalkylene, each substituted or unsubstituted arylene is a substituted or unsubstituted C_6-C_{10} arylene, and/or each substituted or unsubstituted heteroarylene is a substituted or unsubstituted 5 to 10 membered heteroarylene.

[00122] In some embodiments, each substituted or unsubstituted alkyl is a substituted or unsubstituted C_1-C_8 alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 8 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C_3-C_7 cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 7 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C_6-C_{10} aryl, and/or each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 9 membered heteroaryl. In some embodiments, each substituted or unsubstituted alkylene is a substituted or unsubstituted C_1-C_8 alkylene, each substituted or unsubstituted heteroalkylene is a substituted or unsubstituted 2 to 8 membered heteroalkylene, each substituted or unsubstituted cycloalkylene is a substituted or unsubstituted C_3-C_7 cycloalkylene, each substituted or unsubstituted heterocycloalkylene is a

substituted or unsubstituted 3 to 7 membered heterocycloalkylene, each substituted or unsubstituted arylene is a substituted or unsubstituted C_6-C_{10} arylene, and/or each substituted or unsubstituted heteroarylene is a substituted or unsubstituted 5 to 9 membered heteroarylene. In some embodiments, the compound is a chemical species set forth in the Examples section, figures, or tables below.

[00123] In embodiments, a substituted or unsubstituted moiety (e.g., substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted alkylene, substituted or unsubstituted heteroalkylene, substituted or unsubstituted cycloalkylene, substituted or unsubstituted heterocycloalkylene, substituted or unsubstituted arylene, and/or substituted or unsubstituted heteroarylene) is unsubstituted (e.g., is an unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, unsubstituted alkylene, unsubstituted heteroalkylene, unsubstituted cycloalkylene, unsubstituted heterocycloalkylene, unsubstituted arylene, and/or unsubstituted heteroarylene, respectively). In embodiments, a substituted or unsubstituted moiety (e.g., substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted alkylene, substituted or unsubstituted heteroalkylene, substituted or unsubstituted cycloalkylene, substituted or unsubstituted heterocycloalkylene, substituted or unsubstituted arylene, and/or substituted or unsubstituted heteroarylene) is substituted (e.g., is a substituted alkyl, substituted heteroalkyl, substituted cycloalkyl, substituted heterocycloalkyl, substituted aryl, substituted heteroaryl, substituted alkylene, substituted heteroalkylene, substituted cycloalkylene, substituted heterocycloalkylene, substituted arylene, and/or substituted heteroarylene, respectively).

[00124] In embodiments, a substituted moiety (e.g., substituted alkyl, substituted heteroalkyl, substituted cycloalkyl, substituted heterocycloalkyl, substituted aryl, substituted heteroaryl, substituted alkylene, substituted heteroalkylene, substituted cycloalkylene, substituted heterocycloalkylene, substituted arylene, and/or substituted heteroarylene) is substituted with at

least one substituent group, wherein if the substituted moiety is substituted with a plurality of substituent groups, each substituent group may optionally be different. In embodiments, if the substituted moiety is substituted with a plurality of substituent groups, each substituent group is different.

[00125] In embodiments, a substituted moiety (e.g., substituted alkyl, substituted heteroalkyl, substituted cycloalkyl, substituted heterocycloalkyl, substituted aryl, substituted heteroaryl, substituted alkylene, substituted heteroalkylene, substituted cycloalkylene, substituted heterocycloalkylene, substituted arylene, and/or substituted heteroarylene) is substituted with at least one size-limited substituent group, wherein if the substituted moiety is substituted with a plurality of size-limited substituent groups, each size-limited substituent group may optionally be different. In embodiments, if the substituted moiety is substituted with a plurality of size-limited substituent groups, each size-limited substituent group is different.

[00126] In embodiments, a substituted moiety (e.g., substituted alkyl, substituted heteroalkyl, substituted cycloalkyl, substituted heterocycloalkyl, substituted aryl, substituted heteroaryl, substituted alkylene, substituted heteroalkylene, substituted cycloalkylene, substituted heterocycloalkylene, substituted arylene, and/or substituted heteroarylene) is substituted with at least one lower substituent group, wherein if the substituted moiety is substituted with a plurality of lower substituent groups, each lower substituent group may optionally be different. In embodiments, if the substituted moiety is substituted with a plurality of lower substituent groups, each lower substituent group is different.

[00127] In embodiments, a substituted moiety (e.g., substituted alkyl, substituted heteroalkyl, substituted cycloalkyl, substituted heterocycloalkyl, substituted aryl, substituted heteroaryl, substituted alkylene, substituted heteroalkylene, substituted cycloalkylene, substituted heterocycloalkylene, substituted arylene, and/or substituted heteroarylene) is substituted with at least one substituent group, size-limited substituent group, or lower substituent group; wherein if the substituted moiety is substituted with a plurality of groups selected from substituent groups, size-limited substituent groups, and lower substituent groups; each substituent group, size-limited substituent group, and/or lower substituent group may optionally be different. In embodiments, if the substituted moiety is substituted with a plurality of groups selected from

substituent groups, size-limited substituent groups, and lower substituent groups; each substituent group, size-limited substituent group, and/or lower substituent group is different.

[00128] Certain compounds of the present disclosure possess asymmetric carbon atoms (optical or chiral centers) or double bonds; the enantiomers, racemates, diastereomers, tautomers, geometric isomers, stereoisometric forms that may be defined, in terms of absolute stereochemistry, as (R)-or (S)- or, as (D)- or (L)- for amino acids, and individual isomers are encompassed within the scope of the present disclosure. The compounds of the present disclosure do not include those that are known in art to be too unstable to synthesize and/or isolate. The present disclosure is meant to include compounds in racemic and optically pure forms. Optically active (R)- and (S)-isomers, or (D)- and (L)-isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the compounds described herein contain olefinic bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both E and Z geometric isomers.

[00129] As used herein, the term "isomers" refers to compounds having the same number and kind of atoms, and hence the same molecular weight, but differing in respect to the structural arrangement or configuration of the atoms.

[00130] The term "tautomer," as used herein, refers to one of two or more structural isomers which exist in equilibrium and which are readily converted from one isomeric form to another.

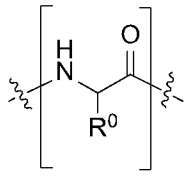
[00131] It will be apparent to one skilled in the art that certain compounds of this disclosure may exist in tautomeric forms, all such tautomeric forms of the compounds being within the scope of the disclosure.

[00132] Unless otherwise stated, structures depicted herein are also meant to include all stereochemical forms of the structure; i.e., the R and S configurations for each asymmetric center. Therefore, single stereochemical isomers as well as enantiomeric and diastereomeric mixtures of the present compounds are within the scope of the disclosure.

[00133] It should be noted that throughout the application that alternatives are written in Markush groups, for example, each amino acid position that contains more than one possible

amino acid. It is specifically contemplated that each member of the Markush group should be considered separately, thereby comprising another embodiment, and the Markush group is not to be read as a single unit.

[00134] "Linker" or "linker reagent" are used interchangeably and refer to a chemical moiety comprising a covalent bond or a chain of atoms that covalently attaches an antibody to a drug moiety. In various embodiments, linkers include a divalent radical. In various embodiments, linkers can comprise one or more amino acid residues.

[00135] "Amino Acid Unit" has the formula , where R^0 is hydrogen, methyl, isopropyl, isobutyl, sec-butyl, benzyl, p-hydroxybenzyl, —CH_2OH, —$CH(OH)CH_3$, —$CH_2CH_2SCH_3$, —CH_2CONH_2, —CH_2COOH, —$CH_2CH_2CONH_2$, —CH_2CH_2COOH, —$(CH_2)_3NHC(=NH)NH_2$, —$(CH_2)_3NH_2$, —$(CH_2)_3NHCOCH_3$, —$(CH_2)_3NHCHO$, —$(CH_2)_4NHC(=NH)NH_2$, —$(CH_2)_4NH_2$, —$(CH_2)_4NHCOCH_3$, —$(CH_2)_4NHCHO$, —$(CH_2)_3NHCONH_2$, —$(CH_2)_4NHCONH_2$, —$CH_2CH_2CH(OH)CH_2NH_2$, 2-pyridylmethyl-, 3-pyridylmethyl-, 4-pyridylmethyl-, phenyl, or cyclohexyl. In various embodiments, Amino Acid Unit includes not only naturally occurring amino acids but also minor amino acids, and non-naturally occurring amino acid analogs, such as citrulline, norleucine, selenomethionine, β-alanine, etc. An amino acid unit may be referred to by its standard three-letter code for the amino acid (e.g., Ala, Cys, Asp, Glu, Val, Phe, Lys, etc.).

[00136] As used herein, the terms "bioconjugate" and "bioconjugate linker" refers to the resulting association between atoms or molecules of "bioconjugate reactive groups" or "bioconjugate reactive moieties". The association can be direct or indirect. For example, a conjugate between a first bioconjugate reactive group (e.g., –NH_2, –C(O)OH, –N-hydroxysuccinimide, or –maleimide) and a second bioconjugate reactive group (e.g., sulfhydryl, sulfur-containing amino acid, amine, amine sidechain containing amino acid, or carboxylate) provided herein can be direct, e.g., by covalent bond or linker (e.g. a first linker or second linker), or indirect, e.g., by non-covalent bond (e.g. electrostatic interactions (e.g. ionic bond,

hydrogen bond, halogen bond), van der Waals interactions (e.g. dipole-dipole, dipole-induced dipole, London dispersion), ring stacking (pi effects), hydrophobic interactions and the like). In embodiments, bioconjugates or bioconjugate linkers are formed using bioconjugate chemistry (i.e. the association of two bioconjugate reactive groups) including, but are not limited to nucleophilic substitutions (e.g., reactions of amines and alcohols with acyl halides, active esters), electrophilic substitutions (e.g., enamine reactions) and additions to carbon-carbon and carbon-heteroatom multiple bonds (e.g., Michael reaction, Diels-Alder addition). These and other useful reactions are discussed in, for example, March, ADVANCED ORGANIC CHEMISTRY, 3rd Ed., John Wiley & Sons, New York, 1985; Hermanson, BIOCONJUGATE TECHNIQUES, Academic Press, San Diego, 1996; and Feeney et al., MODIFICATION OF PROTEINS; Advances in Chemistry Series, Vol. 198, American Chemical Society, Washington, D.C., 1982. In embodiments, the first bioconjugate reactive group (e.g., maleimide moiety) is covalently attached to the second bioconjugate reactive group (e.g. a sulfhydryl). In embodiments, the first bioconjugate reactive group (e.g., haloacetyl moiety) is covalently attached to the second bioconjugate reactive group (e.g. a sulfhydryl). In embodiments, the first bioconjugate reactive group (e.g., pyridyl moiety) is covalently attached to the second bioconjugate reactive group (e.g. a sulfhydryl). In embodiments, the first bioconjugate reactive group (e.g., –N-hydroxysuccinimide moiety) is covalently attached to the second bioconjugate reactive group (e.g. an amine). In embodiments, the first bioconjugate reactive group (e.g., fluorophenyl ester moiety) reacts with the second bioconjugate reactive group (e.g. an amine) to form a covalent bond. In embodiments, the first bioconjugate reactive group (e.g., –sulfo–N-hydroxysuccinimide moiety) reacts with the second bioconjugate reactive group (e.g. an amine) to form a covalent bond.

[00137] Useful bioconjugate reactive moieties used for bioconjugate chemistries herein include, for example:

(a) carboxyl groups and various derivatives thereof including, but not limited to, N-hydroxysuccinimide esters, N-hydroxybenztriazole esters, acid halides, acyl imidazoles, thioesters, p-nitrophenyl esters, alkyl, alkenyl, alkynyl and aromatic esters;

(b) hydroxyl groups which can be converted to esters, ethers, aldehydes, etc.

(c) haloalkyl groups wherein the halide can be later displaced with a nucleophilic group such as, for example, an amine, a carboxylate anion, thiol anion, carbanion, or an alkoxide ion, thereby resulting in the covalent attachment of a new group at the site of the halogen atom;

(d) dienophile groups which are capable of participating in Diels-Alder reactions such as, for example, maleimido or maleimide groups;

(e) aldehyde or ketone groups such that subsequent derivatization is possible via formation of carbonyl derivatives such as, for example, imines, hydrazones, semicarbazones or oximes, or via such mechanisms as Grignard addition or alkyllithium addition;

(f) sulfonyl halide groups for subsequent reaction with amines, for example, to form sulfonamides;

(g) thiol groups, which can be converted to disulfides, reacted with acyl halides, or bonded to metals such as gold, or react with maleimides;

(h) amine or sulfhydryl groups (e.g., present in cysteine), which can be, for example, acylated, alkylated or oxidized;

(i) alkenes, which can undergo, for example, cycloadditions, acylation, Michael addition, etc;

(j) epoxides, which can react with, for example, amines and hydroxyl compounds;

(k) phosphoramidites and other standard functional groups useful in nucleic acid synthesis;

(l) metal silicon oxide bonding; and

(m) metal bonding to reactive phosphorus groups (e.g. phosphines) to form, for example, phosphate diester bonds.

(n) azides coupled to alkynes using copper catalyzed cycloaddition click chemistry.

(o) biotin conjugate can react with avidin or strepavidin to form a avidin-biotin complex or streptavidin-biotin complex.

[00138] The bioconjugate reactive groups can be chosen such that they do not participate in, or interfere with, the chemical stability of the conjugate described herein. Alternatively, a reactive functional group can be protected from participating in the crosslinking reaction by the presence of a protecting group. In embodiments, the bioconjugate comprises a molecular entity derived from the reaction of an unsaturated bond, such as a maleimide, and a sulfhydryl group.

[00139] "Analog," or "analogue" is used in accordance with its plain ordinary meaning within Chemistry and Biology and refers to a chemical compound that is structurally similar to another compound (i.e., a so-called "reference" compound) but differs in composition, e.g., in the replacement of one atom by an atom of a different element, or in the presence of a particular functional group, or the replacement of one functional group by another functional group, or the absolute stereochemistry of one or more chiral centers of the reference compound. Accordingly, an analog is a compound that is similar or comparable in function and appearance but not in structure or origin to a reference compound.

[00140] As used herein, common organic and cell types abbreviations are defined as follows:

Ac	Acetyl
Ala	Alanine
Asn	Asparagine
aq.	Aqueous
β-Ala	beta-alanine
°C	Temperature in degrees Centigrade
Cit	Citrulline
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DIEA	Diisopropylethylamine
DMF	*N*,*N*'-Dimethylformamide
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
Et	Ethyl
Eq	Equivalents
g	Gram(s)
Gly	Glycine
h	Hour (hours)
HATU	2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

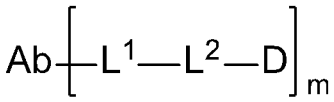
HOBt	N-Hydroxybenzotriazole
HPLC	High-performance liquid chromatography
LC/MS	Liquid chromatography-mass spectrometry
LYM	Lymphocytes
Lys	Lysine
Me	Methyl
mg	milligrams
MeOH	Methanol
mL	Milliliter(s)
μL / μL	Microliter(s)
MONO	Monocytes
mol	moles
mmol	millimoles
μmol/umol	micromoles
MS	mass spectrometry
NHS	N-Hydroxysuccinimide
NEUT	Neutrophils
PABC	p-aminobenzyloxycarbonyl
Phe	Phenylalanine
PLT	Platelets
RP-HPLC	reverse phase HPLC
rt	room temperature
Ser	Serine
t-Bu	tert-Butyl
Tert, t	tertiary
TFA	Trifluoracetic acid
Thr	Threonine
Val	Valine

Compositions

Antibody-Drug Conjugates

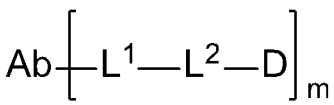
[00141] In one aspect, provided herein is an antibody-drug conjugate (ADC) comprising a monoclonal antibody (Ab), a drug moiety (D), and a linker moiety that covalently attaches the monoclonal antibody to the drug moiety.

[00142] In another aspect, provided herein is an antibody drug conjugate (ADC) of formula (I):



or a pharmaceutically acceptable salt thereof, wherein:
Ab is a monoclonal antibody;
m is an integer from 1 to 8;
L^1 is a linker bound to the monoclonal antibody;
L^2 comprises or is a bond, -C(O)-, -NH-, Amino Acid Unit, $-(CH_2CH_2O)_n-$, $-(CH_2)_n-$, –(4-aminobenzyloxycarbonyl)–, $-(C(O)CH_2CH_2NH)-$, or any combination thereof, where n is an integer from 1 to 24; and
D is a drug moiety.

[00143] In another aspect, provided herein is an antibody drug conjugate (ADC) of formula (I):



or a pharmaceutically acceptable salt thereof, wherein:
Ab is an anti-FOLR1 antibody;
m is an integer from 1 to 8;
L^1 is a linker bound to the anti-FOLR1 antibody;
L^2 comprises or is a bond, -C(O)-, -NH-, Amino Acid Unit, $-(CH_2CH_2O)_n-$, $-(CH_2)_n-$,

–(4-aminobenzyloxycarbonyl)–, –(C(O)CH_2CH_2NH)–, or any combination thereof; wherein n is an integer from 1 to 24; and
D is a drug moiety.

[00144] In embodiments, m is an integer from 1 to 8. In embodiments, m is an integer from 2 to 8. In embodiments, m is an integer from 2 to 4. In embodiments, m is 1. In embodiments, m is 2. In embodiments, m is 3. In embodiments, m is 4. In embodiments, m is 5. In embodiments, m is 6. In embodiments, m is 7. In embodiments, m is 8.

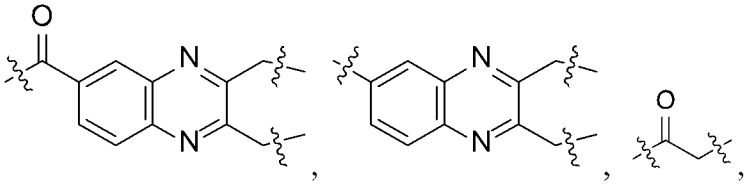
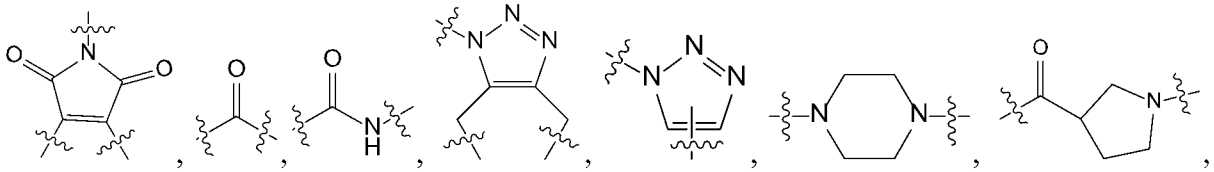
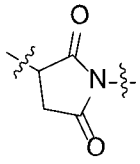
[00145] In embodiments, n is an integer from 1 to 24. In embodiments, n is 1. In embodiments, n is 2. In embodiments, n is 3. In embodiments, n is 4. In embodiments, n is 5. In embodiments, n is 6. In embodiments, n is 7. In embodiments, n is 8. In embodiments, n is 9. In embodiments, n is 10. In embodiments, n is 11. In embodiments, n is 12. In embodiments, n is 13. In embodiments, n is 14. In embodiments, n is 15. In embodiments, n is 16. In embodiments, n is 17. In embodiments, n is 18. In embodiments, n is 19. In embodiments, n is 20. In embodiments, n is 21. In embodiments, n is 22. In embodiments, n is 23. In embodiments, n is 24.

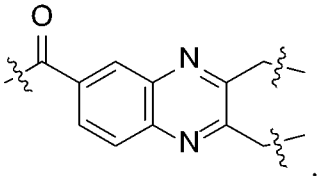
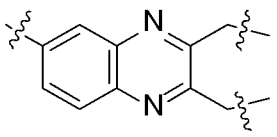
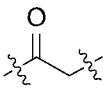
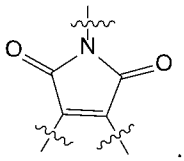
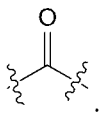
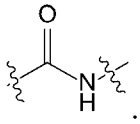
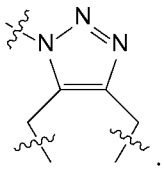
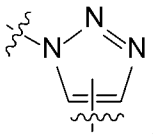
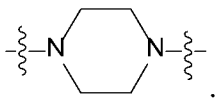
[00146] In embodiments, Ab is a monoclonal antibody. In embodiments, the monoclonal antibody is an anti-FOLR1 antibody.

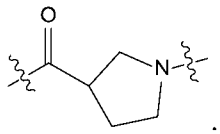
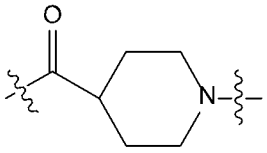
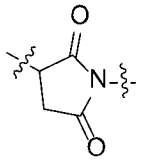
[00147] In embodiments, L^1 is a linker bound to the anti-FOLR1 antibody. In embodiments, L^1 is a linker bound to one or two sulfur or nitrogen atoms on the anti-FOLR1 antibody. In embodiments, L^1 is a linker bound to one sulfur atom on the anti-FOLR1 antibody. In embodiments, L^1 is a linker bound to two sulfur atoms on the anti-FOLR1 antibody. In embodiments, L^1 is a linker bound to one nitrogen atom on the anti-FOLR1 antibody. In embodiments, L^1 is a linker bound to two nitrogen atoms on the anti-FOLR1 antibody.

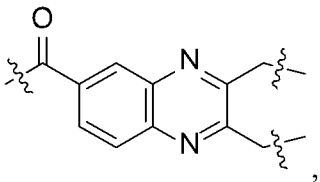
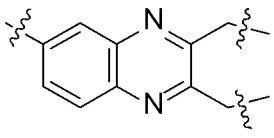
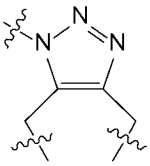
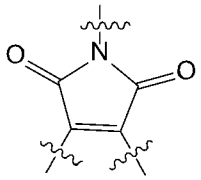
[00148] In embodiments, L^1 is a linker bound to one cysteine molecule on the anti-FOLR1 antibody. In embodiments, L^1 is a linker bound to two cysteine molecules on the anti-FOLR1 antibody. In embodiments, L^1 is a linker bound to one lysine molecule on the anti-FOLR1

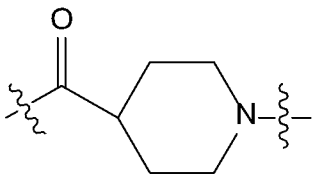
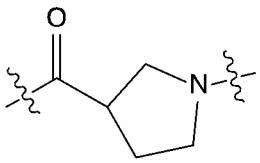
antibody. In embodiments, L^1 is a linker bound to two lysine molecules on the anti-FOLR1 antibody.

[00149] In embodiments, L^1 is , , or .

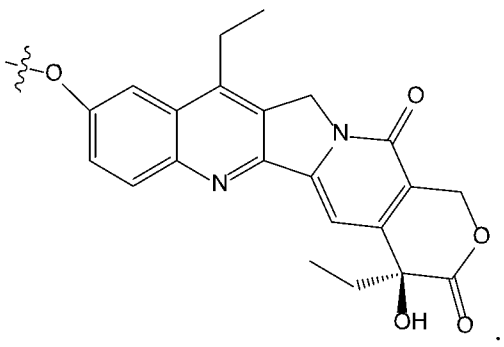
[00150] In embodiments, L^1 is . In embodiments, L^1 is . In embodiments, L^1 is . In embodiments, L^1 is . In embodiments, L^1 is . In embodiments, L^1 is . In embodiments, L^1 is . In embodiments, L^1 is . In embodiments, L^1 is . In embodiments, L^1

is  . In embodiments, L^1 is  . In embodiments, L^1 is  .

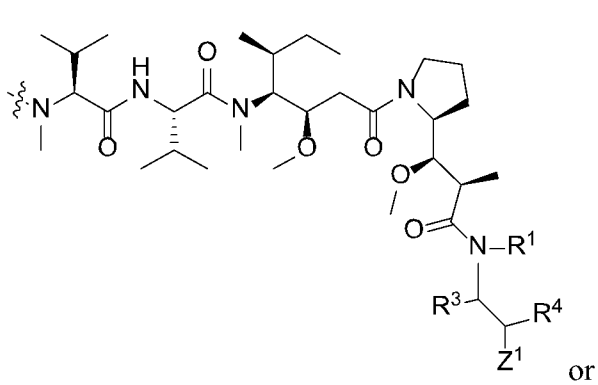
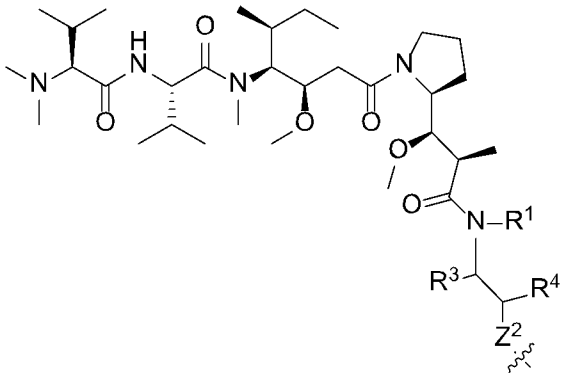
[00151] Where L^1 is  ,  , or  the two CH_2 moieties shown on the right side of the structure may each be bound to a separate sulfur of the anti-FOLR1 antibody. Where L^1 is  , the two alkene carbons shown on the bottom of the structure may each be bound to a separate sulfur of the anti-FOLR1 antibody.

[00152] Where L^1 is  or  the carbonyl is bound to an amine of a lysine of the anti-FOLR1 antibody.

[00153] In embodiments, D is:

 .

[00154] In embodiments, D is:

or

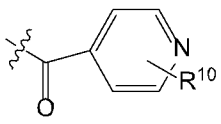
R^1 is H or $-C_1$-C_8 alkyl;

R^3 is H, halogen, $-CCl_3$, $-CBr_3$, $-CF_3$, $-CI_3$, $-CHCl_2$, $-CHBr_2$, $-CHF_2$, $-CHI_2$, $-CH_2Cl$, $-CH_2Br$, $-CH_2F$, $-CH_2I$, -CN, $-OR^{3A}$, $-NR^{3A}R^{3B}$, $-(CH_2)_vOR^6$, $-C(O)NHSO_2R^7$, substituted or unsubstituted alkyl, or substituted or unsubstituted heteroalkyl;

R^4 is H, halogen, $-OR^{4A}$, $-NR^{4A}R^{4B}$, substituted or unsubstituted alkyl, or substituted or unsubstituted heteroalkyl;

Z^1 is a substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, or substituted or unsubstituted heterocycloalkyl;

Z^2 is a substituted aryl, substituted heteroaryl, substituted cycloalkyl, or substituted heterocycloalkyl;

R^6 is H, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, $-CO(CH_2CH_2O)_wCH_2CH_2Y$, $-CONH(CH_2CH_2O)_wCH_2CH_2Y$,  a Charged Group, or a saccharide derivative, wherein

v is an integer from 1 to 24; w is an integer from 1 to 24; Y is $-NH_2$, -OH, -COOH, or $-OCH_3$;

R^7 is independently H, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl;

R^{10} is -OH, $-OCH_3$ or -COOH;

each R^{3A}, R^{3B}, R^{4A}, and R^{4B} is independently H or substituted or unsubstituted alkyl.

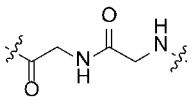
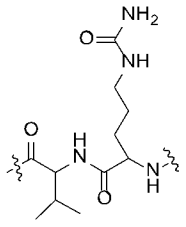
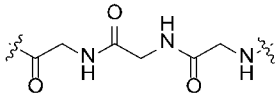
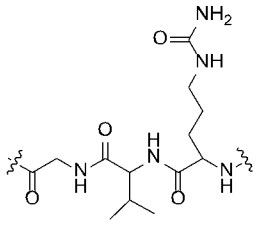
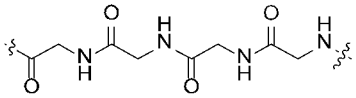
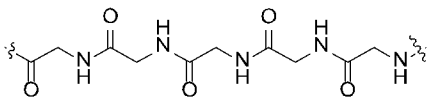
[00155] In embodiments, L^2 comprises or is a bond, -C(O)-, -NH-, -Val-, -Phe-, -Lys-, –(4-aminobenzyloxycarbonyl)–, -Gly-, -Ser-, -Thr-, -Ala-, -β-Ala-, -citrulline- (Cit), $–(CH_2)_n–$, $–(CH_2CH_2O)_n–$, or any combination thereof.

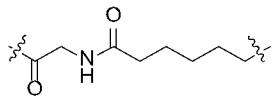
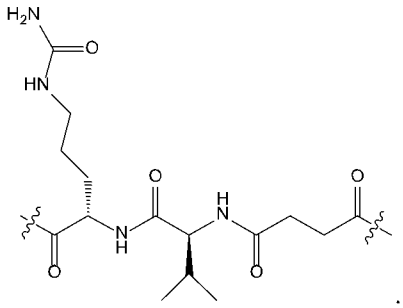
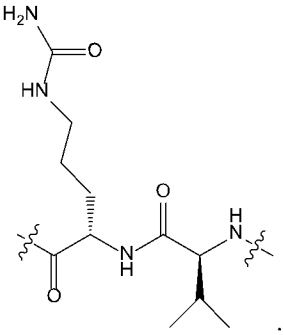
[00156] In embodiments, L^2 comprises or is a bond, -C(O)-, -NH-, -Val-, -Phe-, -Lys-, –(4-aminobenzyloxycarbonyl)–, $–(CH_2)_n–$, $–(CH_2CH_2O)_n–$, or any combination thereof.

[00157] In embodiments, L^2 comprises or is a bond, -C(O)-, -NH-, -Gly-, -Ser-, -Thr-, -Ala-, -β-Ala-, -Cit-, $–(CH_2)_n–$, $–(CH_2CH_2O)_n–$, or any combination thereof.

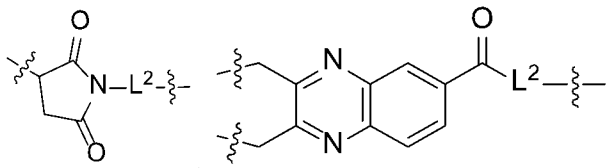
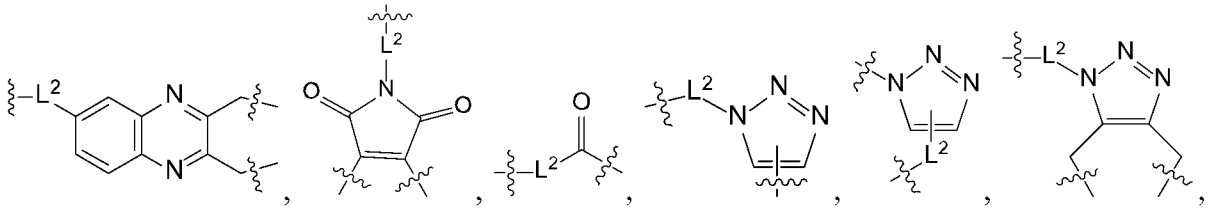
[00158] In embodiments, L^2 comprises or is a bond, -C(O)-, -NH-, Val, Gly, Lys, Cit, $–(CH_2)_n–$, $–(CH_2CH_2O)_n–$, or any combination thereof.

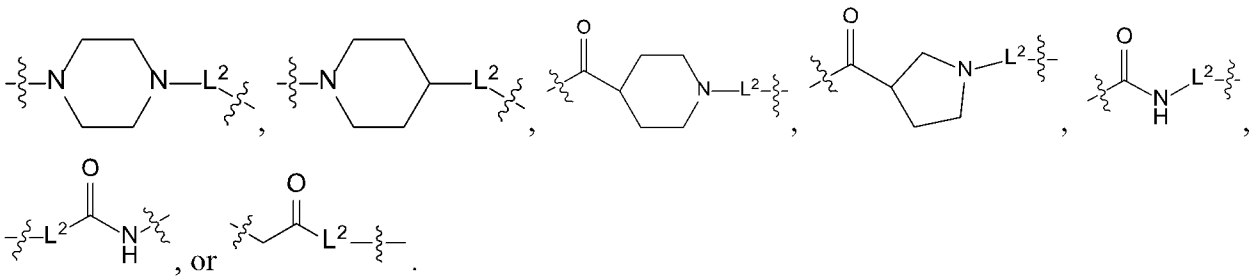
[00159] In embodiments, L^2 comprises or is a bond, -C(O)-, Val, Gly, Cit, $–(CH_2)_n–$, or any combination thereof.

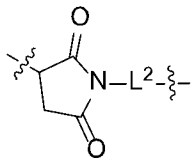
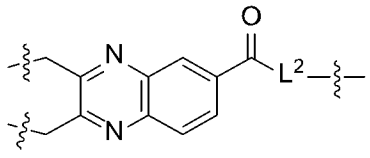
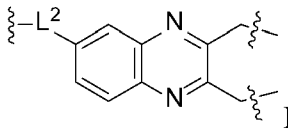
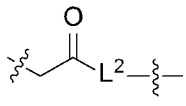
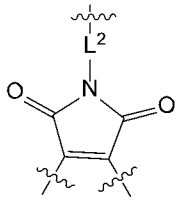
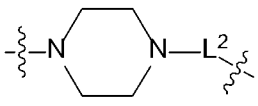
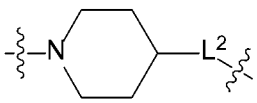
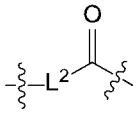
[00160] In embodiments, L^2 comprises or is . In embodiments, L^2 comprises or is . In embodiments, L^2 comprises or is . In embodiments, L^2 comprises or is . In embodiments, L^2 comprises or is . In embodiments, L^2 comprises or is . In embodiments, L^2 comprises or is $-C(O)-(CH_2)_5-$. In

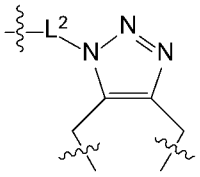
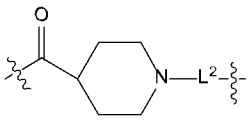
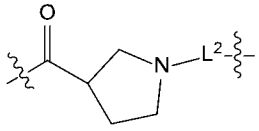
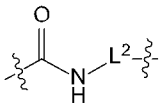
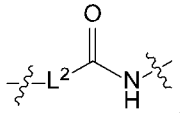
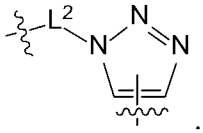
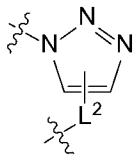
embodiments, L^2 comprises or is . In embodiments, L^2 comprises or is . In embodiments, L^2 comprises or is .

[00161] In embodiments, L^2 comprises or is a bond. In embodiments, L^2 comprises or is -C(O)-. In embodiments, L^2 comprises or is -NH-. In embodiments, L^2 comprises or is -Val-. In embodiments, L^2 comprises or is -Phe-. In embodiments, L^2 comprises or is -Lys-. In embodiments, L^2 comprises or is –(4-aminobenzyloxycarbonyl)–. In embodiments, L^2 comprises or is $-(CH_2)_n-$. In embodiments, L^2 comprises or is $-(CH_2CH_2O)_n-$. In embodiments, L^2 comprises or is -Gly-. In embodiments, L^2 comprises or is -Ser-. In embodiments, L^2 comprises or is -Thr-. In embodiments, L^2 comprises or is -Ala-. In embodiments, L^2 comprises or is -β-Ala-. In embodiments, L^2 comprises or is -Cit-.

[00162] In embodiments, $-L^1-L^2-$ is , .



[00163] In embodiments, -L^1-L^2- is . In embodiments, -L^1-L^2- is , where the two CH_2 moieties shown on the left side of the structure may each be bound to a separate sulfur of the anti-FOLR1 antibody. In embodiments, -L^1-L^2- is  In embodiments, where the two CH_2 moieties shown on the left side of the structure may each be bound to a separate sulfur of the anti-FOLR1 antibody. In embodiments, -L^1-L^2- is . In embodiments, -L^1-L^2- is , where the two alkene carbons shown on the bottom of the structure may each be bound to a separate sulfur of the anti-FOLR1 antibody. In embodiments, -L^1-L^2- is . In embodiments, -L^1-L^2- is . In embodiments, -L^1-L^2- is . In embodiments, -L^1-L^2- is

, where the two CH_2 moieties shown on the left side of the structure may each be bound to a separate sulfur of the anti-FOLR1 antibody. In embodiments, -L^1-L^2- is . In embodiments, -L^1-L^2- is . In embodiments, -L^1-L^2- is . In embodiments, -L^1-L^2- is . In embodiments, -L^1-L^2- is . In embodiments, -L^1-L^2- is .

[00164] In embodiments, R^1 is H. In embodiments, R^1 is $-C_1$-C_8 alkyl.

[00165] In embodiments, R^1 is methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, or hexyl. In embodiments, R^1 is methyl. In embodiments, R^1 is ethyl. In embodiments, R^1 is propyl. In embodiments, R^1 is isopropyl. In embodiments, R^1 is butyl. In embodiments, R^1 is isobutyl. In embodiments, R^1 is tert-butyl. In embodiments, R^1 is pentyl. In embodiments, R^1 is hexyl.

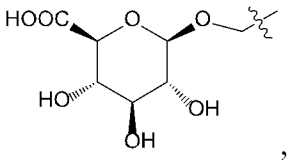
[00166] In embodiments, R^3 is H, halogen, $-CCl_3$, $-CBr_3$, $-CF_3$, $-CI_3$, $-CHCl_2$, $-CHBr_2$, $-CHF_2$, $-CHI_2$, $-CH_2Cl$, $-CH_2Br$, $-CH_2F$, $-CH_2I$, -CN, $-OR^{3A}$, $-NR^{3A}R^{3B}$, $-(CH_2)_vOR^6$, $-C(O)NHSO_2R^7$, substituted or unsubstituted alkyl (e.g., C_1-C_8 alkyl, C_1-C_6 alkyl, or C_1-C_4 alkyl), or substituted or unsubstituted heteroalkyl (e.g., 2 to 8 membered heteroalkyl, 2 to 6 membered heteroalkyl, or 2 to 4 membered heteroalkyl).

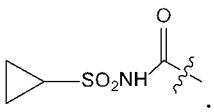
[00167] In embodiments, R^3 is H, $-OR^{3A}$, $-(CH_2)_vOR^6$, $-C(O)NHSO_2R^7$, substituted (e.g., substituted with at least one substituent group, size-limited substituent group, or lower substituent group) or unsubstituted alkyl (e.g., C_1-C_8 alkyl, C_1-C_6 alkyl, or C_1-C_4 alkyl), or substituted (e.g., substituted with at least one substituent group, size-limited substituent group, or

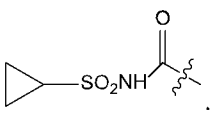
lower substituent group) or unsubstituted heteroalkyl (e.g., 2 to 8 membered heteroalkyl, 2 to 6 membered heteroalkyl, or 2 to 4 membered heteroalkyl).

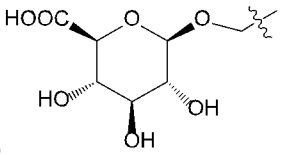
[00168] In embodiments, R^3 is H, $-OR^{3A}$, $-(CH_2)_vOR^6$, $-C(O)NHSO_2R^7$, substituted (e.g., substituted with at least one substituent group, size-limited substituent group, or lower substituent group) or unsubstituted C_1-C_6 alkyl.

[00169] In embodiments, R^3 is a substituted (e.g., substituted with at least one substituent group, size-limited substituent group, or lower substituent group) alkyl (e.g., C_1-C_8 alkyl, C_1-C_6 alkyl, or C_1-C_4 alkyl). In embodiments, R^3 is an unsubstituted alkyl (e.g., C_1-C_8 alkyl, C_1-C_6 alkyl, or C_1-C_4 alkyl). In embodiments, R^3 is a substituted (e.g., substituted with at least one substituent group, size-limited substituent group, or lower substituent group) heteroalkyl (e.g., 2 to 8 membered heteroalkyl, 2 to 6 membered heteroalkyl, or 2 to 4 membered heteroalkyl). In embodiments, R^3 is an unsubstituted heteroalkyl (e.g., 2 to 8 membered heteroalkyl, 2 to 6 membered heteroalkyl, or 2 to 4 membered heteroalkyl).

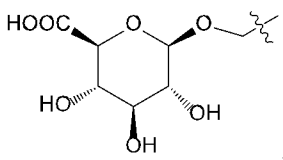
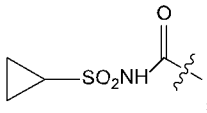
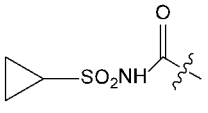
[00170] In embodiments, R^3 is methyl, ethyl, propyl, butyl, $-CH_2OH$, $-CH_2CH_2OH$, $-CH_2N_3$, $-CH_2CH_2N_3$, $-CH_2OCH_3$, $-CH_2OCH_2CH_3$, $-CH_2CH_2OCH_3$, $-CH_2CH_2OCH_2CH_3$, 

, or 

. In embodiments, R^3 is methyl. In embodiments, R^3 is ethyl. In embodiments, R^3 is propyl. In embodiments, R^3 is butyl. In embodiments, R^3 is $-CH_2OH$. In embodiments, R^3 is $-CH_2$ CH_2OH. In embodiments, R^3 is $-CH_2N_3$. In embodiments, R^3 is $-CH_2CH_2N_3$. In embodiments, R^3 is $-CH_2OCH_3$. In embodiments, R^3 is $-CH_2OCH_2CH_3$. In embodiments, R^3 is $-CH_2CH_2OCH_3$. In embodiments, R^3 is $-CH_2CH_2OCH_2CH_3$. In embodiments, R^3 is -OH. In embodiments, R^3 is H. In embodiments, R^3 is 

. In embodiments, R^3 is 

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[00171] In embodiments, R^3 is methyl, $-CH_2OH$, , , or $-CH_2N_3$. In embodiments, R^3 is  or $-CH_2N_3$.

[00172] In embodiments, v is an integer from 1 to 24. In embodiments, v is 1. In embodiments, v is 2. In embodiments, v is 3. In embodiments, v is 4. In embodiments, v is 5. In embodiments, v is 6. In embodiments, v is 7. In embodiments, v is 8. In embodiments, v is 9. In embodiments, v is 10. In embodiments, v is 11. In embodiments, v is 12. In embodiments, v is 13. In embodiments, v is 14. In embodiments, v is 15. In embodiments, v is 16. In embodiments, v is 17. In embodiments, v is 18. In embodiments, v is 19. In embodiments, v is 20. In embodiments, v is 21. In embodiments, v is 22. In embodiments, v is 23. In embodiments, v is 24.

[00173] In embodiments, R^4 is H, halogen, $-OR^{4A}$, $-NR^{4A}R^{4B}$, substituted or unsubstituted alkyl (e.g., C_1-C_8 alkyl, C_1-C_6 alkyl, or C_1-C_4 alkyl), or substituted or unsubstituted heteroalkyl (e.g., 2 to 8 membered heteroalkyl, 2 to 6 membered heteroalkyl, or 2 to 4 membered heteroalkyl).

[00174] In embodiments, R^4 is H, $-OR^{4A}$, substituted (e.g., substituted with at least one substituent group, size-limited substituent group, or lower substituent group) or unsubstituted alkyl (e.g., C_1-C_8 alkyl, C_1-C_6 alkyl, or C_1-C_4 alkyl), or substituted (e.g., substituted with at least one substituent group, size-limited substituent group, or lower substituent group) or unsubstituted heteroalkyl (e.g., 2 to 8 membered heteroalkyl, 2 to 6 membered heteroalkyl, or 2 to 4 membered heteroalkyl).

[00175] In embodiments, R^4 is a substituted (e.g., substituted with at least one substituent group, size-limited substituent group, or lower substituent group) alkyl (e.g., C_1-C_8 alkyl, C_1-C_6 alkyl, or C_1-C_4 alkyl). In embodiments, R^4 is an unsubstituted alkyl (e.g., C_1-C_8 alkyl, C_1-C_6 alkyl, or C_1-C_4 alkyl). In embodiments, R^4 is a substituted (e.g., substituted with at least one

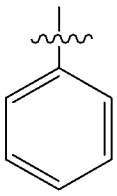
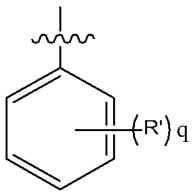
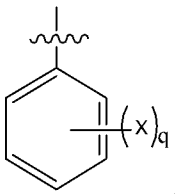
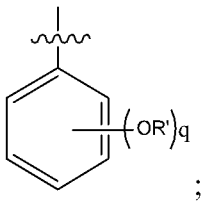
substituent group, size-limited substituent group, or lower substituent group) heteroalkyl (e.g., 2 to 8 membered heteroalkyl, 2 to 6 membered heteroalkyl, or 2 to 4 membered heteroalkyl). In embodiments, R^4 is an unsubstituted heteroalkyl (e.g., 2 to 8 membered heteroalkyl, 2 to 6 membered heteroalkyl, or 2 to 4 membered heteroalkyl).

[00176] In embodiments, R^4 is H, -OH, methyl, ethyl, propyl or butyl. In embodiments, R^4 is methyl. In embodiments, R^4 is ethyl. In embodiments, R^4 is propyl. In embodiments, R^4 is butyl. In embodiments, R^4 is H. In embodiments, R^4 is -OH.

[00177] In embodiments, R^4 is H or -OH.

[00178] In embodiments, Z^1 is a substituted (e.g. with a substituent group, a size-limited substituent group or a lower substituent group) or unsubstituted cycloalkyl (e.g., C_3-C_8 cycloalkyl, C_3-C_6 cycloalkyl, or C_5-C_6 cycloalkyl). In embodiments, Z^1 is a substituted (e.g. with a substituent group, a size-limited substituent group or a lower substituent group) cycloalkyl (e.g., C_3-C_8 cycloalkyl, C_3-C_6 cycloalkyl, or C_5-C_6 cycloalkyl). In embodiments, Z^1 is an unsubstituted cycloalkyl (e.g., C_3-C_8 cycloalkyl, C_3-C_6 cycloalkyl, or C_5-C_6 cycloalkyl). In embodiments, Z^1 is a substituted (e.g. with a substituent group, a size-limited substituent group or a lower substituent group) or unsubstituted heterocycloalkyl (e.g., 3 to 8 membered heterocycloalkyl, 3 to 6 membered heterocycloalkyl, or 5 to 6 membered heterocycloalkyl). In embodiments, Z^1 is a substituted (e.g. with a substituent group, a size-limited substituent group or a lower substituent group) heterocycloalkyl (e.g., 3 to 8 membered heterocycloalkyl, 3 to 6 membered heterocycloalkyl, or 5 to 6 membered heterocycloalkyl). In embodiments, Z^1 is an unsubstituted heterocycloalkyl (e.g., 3 to 8 membered heterocycloalkyl, 3 to 6 membered heterocycloalkyl, or 5 to 6 membered heterocycloalkyl). In embodiments, Z^1 is a substituted (e.g. with a substituent group, a size-limited substituent group or a lower substituent group) or unsubstituted aryl (e.g., C_6-C_{10} aryl, C_{10} aryl, or phenyl). In embodiments, Z^1 is a substituted (e.g. with a substituent group, a size-limited substituent group or a lower substituent group) aryl (e.g., C_6-C_{10} aryl, C_{10} aryl, or phenyl). In embodiments, Z^1 is an unsubstituted aryl (e.g., C_6-C_{10} aryl, C_{10} aryl, or phenyl). In embodiments, Z^1 is a substituted (e.g. with a substituent group, a size-limited substituent group or a lower substituent group) or unsubstituted heteroaryl (e.g., 5 to

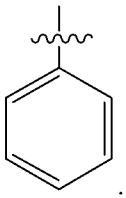
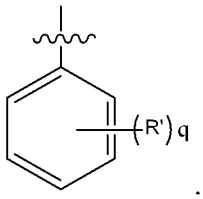
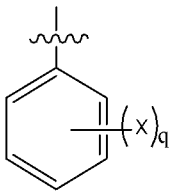
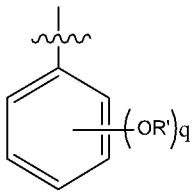
10 membered heteroaryl, 5 to 9 membered heteroaryl, or 5 to 6 membered heteroaryl). In embodiments, Z^1 is a substituted (e.g. with a substituent group, a size-limited substituent group or a lower substituent group) heteroaryl (e.g., 5 to 10 membered heteroaryl, 5 to 9 membered heteroaryl, or 5 to 6 membered heteroaryl). In embodiments, Z^1 is an unsubstituted heteroaryl (e.g., 5 to 10 membered heteroaryl, 5 to 9 membered heteroaryl, or 5 to 6 membered heteroaryl).

[00179] In embodiments, Z^1 is , , , or ; wherein each X is independently Cl, Br, I, or F; each R' is independently $-CH_3$, $-CH_2CH_3$ or $-CH_2CH_2CH_3$; and q is an integer from 1 to 5.

[00180] In embodiments, q is 1. In embodiments q is 2. In embodiments q is 3. In embodiments q is 4. In embodiments q is 5.

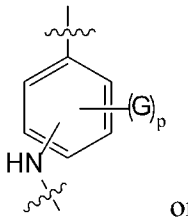
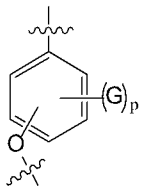
[00181] In embodiments, X is Cl. In embodiments, X is Br. In embodiments, X is I. In embodiments, X is F.

[00182] In embodiments, R' is $-CH_3$. In embodiments, R' is $-CH_2CH_3$. In embodiments, R' is $-CH_2CH_2CH_3$.

[00183] In embodiments, Z^1 is . In embodiments, Z^1 is . In embodiments, Z^1 is . In embodiments, Z^1 is .

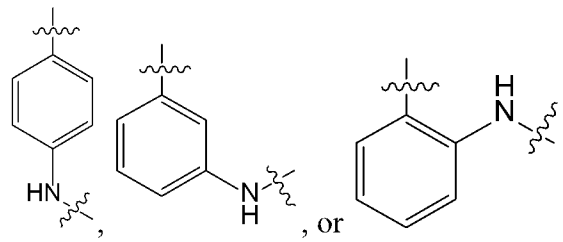
[00184] In embodiments, Z^2 is a substituted (e.g. with a substituent group, a size-limited substituent group or a lower substituent group) cycloalkyl (e.g., C_3-C_8 cycloalkyl, C_3-C_6

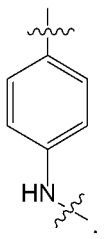
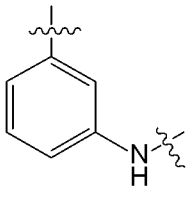
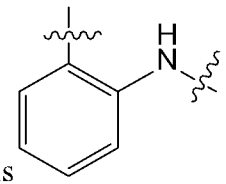
cycloalkyl, or C_5-C_6 cycloalkyl). In embodiments, Z^2 is a substituted (e.g. with a substituent group, a size-limited substituent group or a lower substituent group) heterocycloalkyl (e.g., 3 to 8 membered heterocycloalkyl, 3 to 6 membered heterocycloalkyl, or 5 to 6 membered heterocycloalkyl). In embodiments, Z^2 is a substituted (e.g. with a substituent group, a size-limited substituent group or a lower substituent group) aryl (e.g., C_6-C_{10} aryl, C_{10} aryl, or phenyl). In embodiments, Z^2 is a substituted (e.g. with a substituent group, a size-limited substituent group or a lower substituent group) heteroaryl (e.g., 5 to 10 membered heteroaryl, 5 to 9 membered heteroaryl, or 5 to 6 membered heteroaryl).

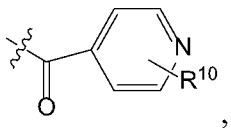
[00185] In embodiments, Z^2 is  or  ; wherein each G is independently Cl, Br, I, F, $-CH_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, $-OCH_3$, $-OCH_2CH_3$, -OH, or $-NH_2$; and p is an integer from 0-4.

[00186] In embodiments p is 0. In embodiments p is 1. In embodiments p is 2. In embodiments p is 3. In embodiments p is 4.

[00187] In embodiments, G is Cl. In embodiments, G is Br. In embodiments, G is I. In embodiments, G is F. In embodiments, G is $-CH_3$. In embodiments, G is $-CH_2CH_3$. In embodiments, G is $-CH_2CH_2CH_3$. In embodiments, G is $-OCH_3$. In embodiments, G is $-OCH_2CH_3$. In embodiments, G is -OH. In embodiments, G is $-NH_2$.

[00188] In embodiments, Z^2 is  . In embodiments,

Z^2 is . In embodiments, Z^2 is . In embodiments, Z^2 is .

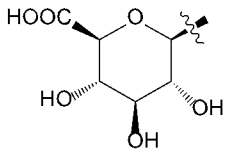
[00189] In embodiments, R^6 is H, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, $-CO(CH_2CH_2O)_wCH_2CH_2Y$, $-CONH(CH_2CH_2O)_wCH_2CH_2Y$, , a Charged Group, or a saccharide derivative, w is an integer from 1 to 24; Y is $-NH_2$, -OH, -COOH, or $-OCH_3$; R^{10} is -OH, $-OCH_3$ or -COOH.

[00190] In embodiments, R^6 is H or substituted (e.g., substituted with at least one substituent group, size-limited substituent group, or lower substituent group) or unsubstituted alkyl (e.g., C_1-C_8 alkyl, C_1-C_6 alkyl, or C_1-C_4 alkyl), substituted (e.g., substituted with at least one substituent group, size-limited substituent group, or lower substituent group) or unsubstituted cycloalkyl (e.g., C_3-C_8 cycloalkyl, C_3-C_6 cycloalkyl, or C_5-C_6 cycloalkyl), substituted (e.g., substituted with at least one substituent group, size-limited substituent group, or lower substituent group) or unsubstituted heterocycloalkyl (e.g., 3 to 8 membered heterocycloalkyl, 3 to 6 membered heterocycloalkyl, or 5 to 6 membered heterocycloalkyl), substituted (e.g., substituted with at least one substituent group, size-limited substituent group, or lower substituent group) or unsubstituted aryl (e.g., C_6-C_{10} aryl, C_{10} aryl, or phenyl), substituted (e.g., substituted with at least one substituent group, size-limited substituent group, or lower substituent group) or unsubstituted heteroaryl (e.g., 5 to 10 membered heteroaryl, 5 to 9 membered heteroaryl, or 5 to 6 membered heteroaryl), or a saccharide derivative.

[00191] In embodiments, R^6 is H, a substituted (e.g. with a substituent group, a size-limited substituent group or a lower substituent group) or unsubstituted heterocycloalkyl (e.g., 3 to 8 membered heterocycloalkyl, 3 to 6 membered heterocycloalkyl, or 5 to 6 membered heterocycloalkyl). In embodiments, R^6 is a substituted (e.g. with a substituent group, a size-

limited substituent group or a lower substituent group) heterocycloalkyl (e.g., 3 to 8 membered heterocycloalkyl, 3 to 6 membered heterocycloalkyl, or 5 to 6 membered heterocycloalkyl). In embodiments, R^6 is an unsubstituted heterocycloalkyl (e.g., 3 to 8 membered heterocycloalkyl, 3 to 6 membered heterocycloalkyl, or 5 to 6 membered heterocycloalkyl).

[00192] In embodiments, R^6 is H or substituted (e.g. with a substituent group, a size-limited substituent group or a lower substituent group) heterocycloalkyl (e.g., 3 to 8 membered heterocycloalkyl, 3 to 6 membered heterocycloalkyl, or 5 to 6 membered heterocycloalkyl).

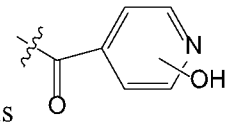
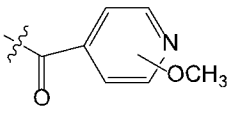
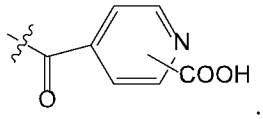
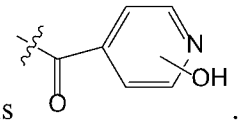
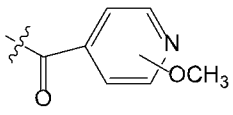
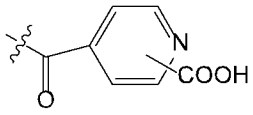
[00193] In embodiments, R^6 is H or 

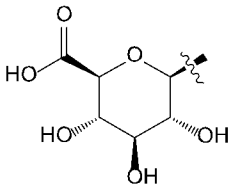
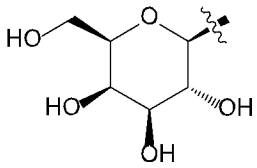
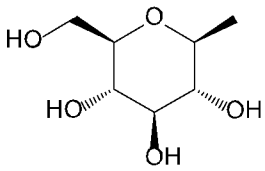
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[00194] In embodiments, R^6 is $-CO(CH_2CH_2O)_wCH_2CH_2Y$ or $-CONH(CH_2CH_2O)_wCH_2CH_2Y$, where w is an integer from 1 to 24 and Y is $-NH_2$, -OH, -COOH, or $-OCH_3$. In embodiments, R^6 is $-CO(CH_2CH_2O)_wCH_2CH_2NH_2$. In embodiments, R^6 is $-CO(CH_2CH_2O)_wCH_2CH_2OH$. In embodiments, R^6 is $-CO(CH_2CH_2O)_wCH_2CH_2COOH$. In embodiments, R^6 is $-CO(CH_2CH_2O)_wCH_2CH_2OCH_3$. In embodiments, R^6 is $-CONH(CH_2CH_2O)_wCH_2CH_2NH_2$. In embodiments, R^6 is $-CONH(CH_2CH_2O)_wCH_2CH_2OH$. In embodiments, R^6 is $-CONH(CH_2CH_2O)_wCH_2CH_2COOH$. In embodiments, R^6 is $-CONH(CH_2CH_2O)_wCH_2CH_2OCH_3$.

[00195] In embodiments, w is an integer from 1 to 24. In embodiments, w is 1. In embodiments, w is 2. In embodiments, w is 3. In embodiments, w is 4. In embodiments, w is 5. In embodiments, w is 6. In embodiments, w is 7. In embodiments, w is 8. In embodiments, w is 9. In embodiments, w is 10. In embodiments, w is 11. In embodiments, w is 12. In embodiments, w is 13. In embodiments, w is 14. In embodiments, w is 15. In embodiments, w is 16. In embodiments, w is 17. In embodiments, w is 18. In embodiments, w is 19. In embodiments, w is 20. In embodiments, w is 21. In embodiments, w is 22. In embodiments, w is 23. In embodiments, w is 24.

[00196] In embodiments, Y is $-NH_2$, -OH, -COOH, or $-OCH_3$. In embodiments, Y is $-NH_2$. In embodiments, Y is -OH. In embodiments, Y is -COOH. In embodiments, Y is $-OCH_3$.

[00197] In embodiments, R^6 is , , or . In embodiments, R^6 is . In embodiments, R^6 is . In embodiments, R^6 is .

[00198] In embodiments, R^6 is a saccharide derivative. In embodiments, R^6 is . In embodiments, R^6 is . In embodiments, R^6 is .

[00199] In embodiments, R^7 is H, substituted or unsubstituted alkyl, or substituted or unsubstituted cycloalkyl.

[00200] In embodiments, R^7 is H or substituted (e.g., substituted with at least one substituent group, size-limited substituent group, or lower substituent group) or unsubstituted alkyl (e.g., C_1-C_8 alkyl, C_1-C_6 alkyl, or C_1-C_4 alkyl), or substituted (e.g., substituted with at least one substituent group, size-limited substituent group, or lower substituent group) or unsubstituted cycloalkyl (e.g., C_3-C_8 cycloalkyl, C_3-C_6 cycloalkyl, or C_5-C_6 cycloalkyl).

[00201] In embodiments, R^7 is H, a substituted (e.g. with a substituent group, a size-limited substituent group or a lower substituent group) or unsubstituted cycloalkyl (e.g., 3 to 8

membered cycloalkyl, 3 to 6 membered cycloalkyl, or 5 to 6 membered cycloalkyl). In embodiments, R^7 is a substituted (e.g. with a substituent group, a size-limited substituent group or a lower substituent group) cycloalkyl (e.g., 3 to 8 membered cycloalkyl, 3 to 6 membered cycloalkyl, or 5 to 6 membered cycloalkyl). In embodiments, R^7 is an unsubstituted cycloalkyl (e.g., 3 to 8 membered cycloalkyl, 3 to 6 membered cycloalkyl, or 5 to 6 membered cycloalkyl).

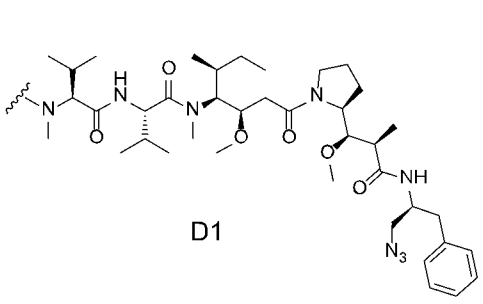
[00202] In embodiments, R^7 is cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, or cycloheptyl. In embodiments, R^7 is cyclopropyl. In embodiments, R^7 is cyclobutyl. In embodiments, R^7 is cyclopentyl. In embodiments, R^7 is cyclohexyl. In embodiments, R^7 is cycloheptyl.

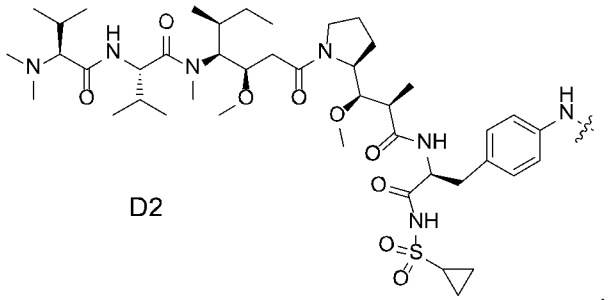
[00203] In embodiments, each R^{3A}, R^{3B}, R^{4A}, and R^{4B} is independently H or substituted or unsubstituted alkyl (e.g., C_1-C_8 alkyl, C_1-C_6 alkyl, or C_1-C_4 alkyl).

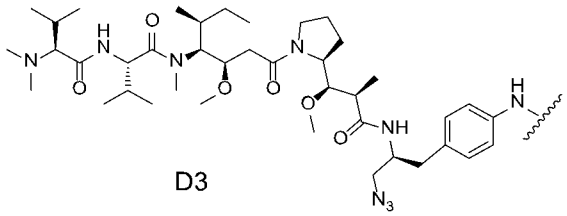
[00204] In embodiments, each R^{3A}, R^{3B}, R^{4A}, and R^{4B} is independently H or substituted (e.g., substituted with at least one substituent group, size-limited substituent group, or lower substituent group) or unsubstituted alkyl (e.g., C_1-C_8 alkyl, C_1-C_6 alkyl, or C_1-C_4 alkyl). In embodiments, each R^{3A}, R^{3B}, R^{4A}, and R^{4B} is independently H. In embodiments, each R^{3A}, R^{3B}, R^{4A}, and R^{4B} is independently substituted (e.g., substituted with at least one substituent group, size-limited substituent group, or lower substituent group) alkyl (e.g., C_1-C_8 alkyl, C_1-C_6 alkyl, or C_1-C_4 alkyl). In embodiments, each R^{3A}, R^{3B}, R^{4A}, and R^{4B} is independently unsubstituted alkyl (e.g., C_1-C_8 alkyl, C_1-C_6 alkyl, or C_1-C_4 alkyl).

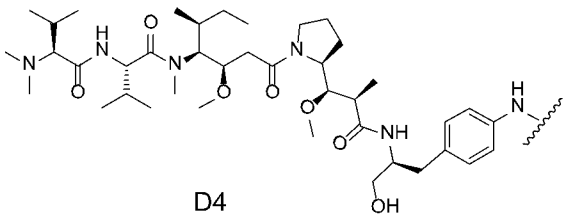
[00205] In embodiments, each R^{3A}, R^{3B}, R^{4A}, and R^{4B} is independently H, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, or pentyl. In embodiments, each R^{3A}, R^{3B}, R^{4A}, and R^{4B} is independently H. In embodiments, each R^{3A}, R^{3B}, R^{4A}, and R^{4B} is independently methyl. In embodiments, each R^{3A}, R^{3B}, R^{4A}, and R^{4B} is independently ethyl. In embodiments, each R^{3A}, R^{3B}, R^{4A}, and R^{4B} is independently propyl. In embodiments, each R^{3A}, R^{3B}, R^{4A}, and R^{4B} is independently isopropyl. In embodiments, each R^{3A}, R^{3B}, R^{4A}, and R^{4B} is independently butyl. In embodiments, each R^{3A}, R^{3B}, R^{4A}, and R^{4B} is independently isobutyl. In embodiments, each R^{3A}, R^{3B}, R^{4A}, and R^{4B} is independently tert-butyl. In embodiments, each R^{3A}, R^{3B}, R^{4A}, and R^{4B} is independently pentyl.

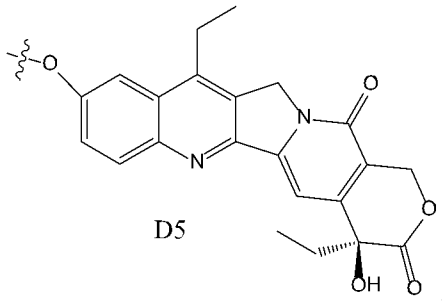
[00206] In embodiments, D is:

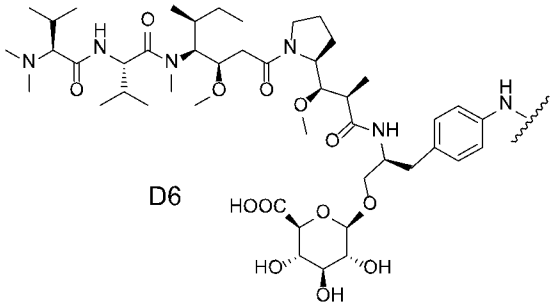

D1


D2

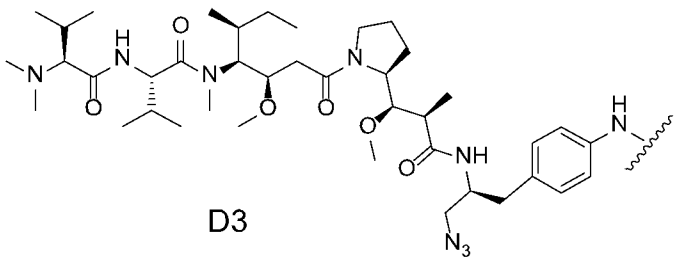

D3


D4

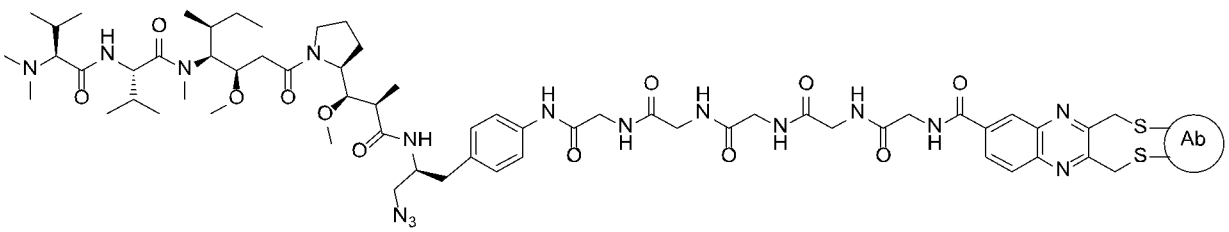

D5


D6

[00207] In embodiments, D is:

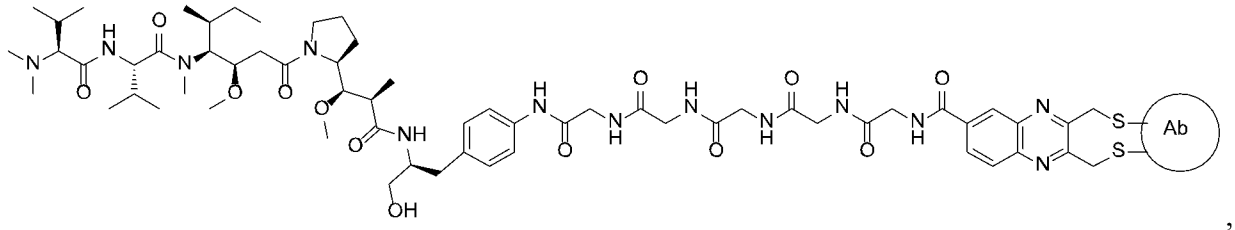

D3

[00208] In embodiments, the ADC (e.g., anti-FOLR1 ADC) is:



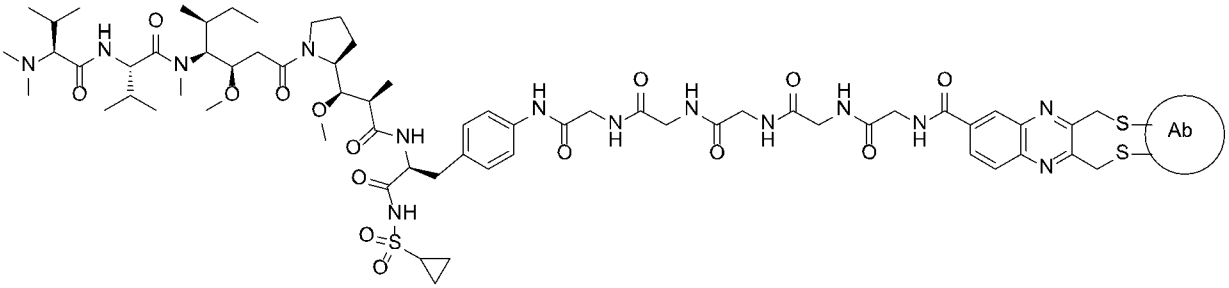
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ADC-1



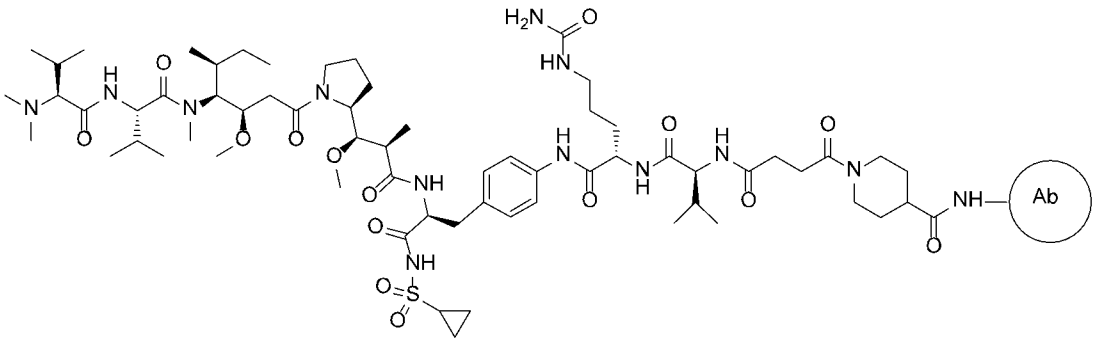
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ADC-2



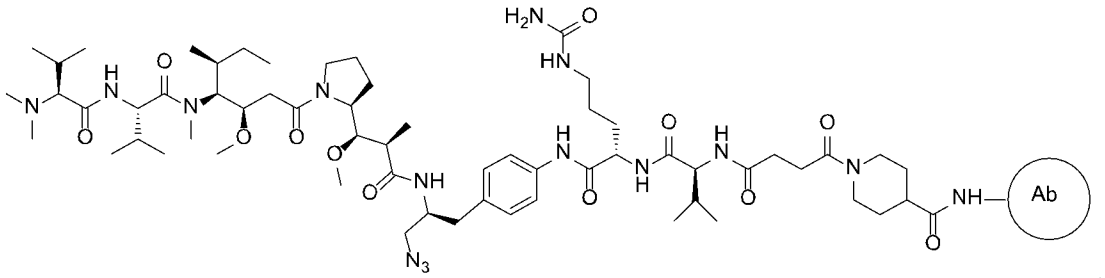
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ADC-3



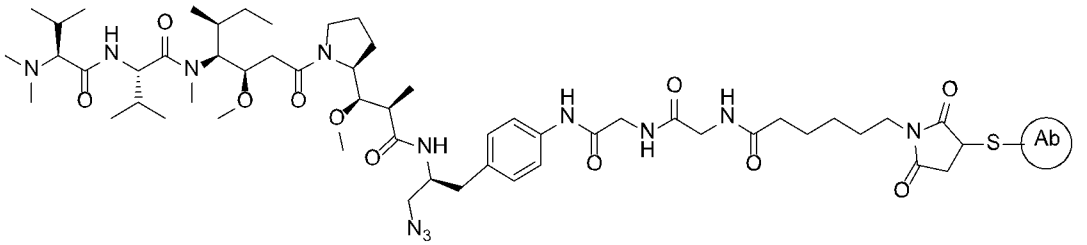
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ADC-4



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ADC-5



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ADC-6

or a pharmaceutically acceptable salt thereof, wherein m is an integer from 1 to 8.

Drug Loading

[00209] Drug loading is represented by m, the average number of drug moieties (i.e., D) per monoclonal antibody in an antibody drug conjugate (ADC) of formula (I) and variations thereof. Drug loading may range from 1 to 20 drug moieties per antibody. The ADCs of formula (I), and any embodiment, variation, or aspect thereof, include collections of antibodies conjugated with a range of drug moieties, from 1 to 20, such as from 1 to 8. The average number of drug moieties per antibody in preparations of ADCs from conjugation reactions may be characterized by conventional means such as mass spectroscopy, ELISA assay, and HPLC. The quantitative distribution of ADCs in terms of m may also be determined. In some instances, separation, purification, and characterization of homogeneous ADCs where m is a certain value from ADCs with other drug loadings may be achieved by means such as reverse phase HPLC or electrophoresis. In embodiments, the monoclonal antibody is an anti-FOLR1 antibody. In

embodiments, the average number of drug moieties (i.e. D) per anti-FOLR1 antibody may range from 1 to 20 drug moieties per antibody, such as from 1 to 8.

[00210] For some ADCs, m may be limited by the number of attachment sites on the antibody. For example, where the attachment is a cysteine thiol, as in some of the exemplary embodiments described herein, an antibody may have only one or several cysteine thiol groups, or may have only one or several sufficiently reactive thiol groups through which a linker may be attached. In embodiments, the average drug loading for an ADC ranges from 1 to about 8, or from about 2 to about 8. In embodiments, L^1 is capable of forming a covalent bond with the thiol groups of the free cysteine(s) in the IgG antibody. In embodiments, L^1 is capable of forming a covalent bond with the amine groups of the lysine(s) in the IgG antibody.

[00211] In embodiments, fewer than the theoretical maximum of drug moieties are conjugated to an antibody during a conjugation reaction. Generally, antibodies do not contain many free and reactive cysteine thiol groups which may be linked to a drug moiety; indeed, most cysteine thiol residues in antibodies exist as disulfide bridges. In embodiments, an antibody may be reduced with a reducing agent such as dithiothreitol (DTT) or tricarbonylethylphosphine (TCEP), under partial or total reducing conditions, to generate reactive cysteine thiol groups. In embodiments, an antibody is subjected to denaturing conditions to reveal reactive nucleophilic groups such as lysine or cysteine.

[00212] The loading (drug/antibody ratio or "dar") of an ADC may be controlled in different ways, and for example, by: (i) limiting the molar excess of drug-linker intermediate or linker reagent relative to antibody, (ii) limiting the conjugation reaction time or temperature, and (iii) limiting reductive conditions for cysteine thiol modification.

[00213] In embodiments, provided herein are antibody drug conjugates, wherein a linker moiety reacts with either cysteine or lysine on an antibody in a controlled fashion. In embodiments, the drug antibody ratio (DAR) for conjugation with antibody lysines is 2, for the majority of conjugates. In embodiments, the drug antibody ratio (DAR) for conjugation with antibody cysteines is 4, for the majority of conjugates.

[00214] It is to be understood that where more than one nucleophilic group reacts with a drug-linker intermediate or linker reagent, then the resulting product is a mixture of ADC compounds

with a distribution of one or more drug moieties attached to an antibody. The average number of drugs per antibody may be calculated from the mixture by a dual ELISA antibody assay, which is specific for antibody and specific for the drug. Individual ADC molecules may be identified in the mixture by mass spectroscopy and separated by HPLC, e.g. hydrophobic interaction chromatography (*see, e.g.*, McDonagh et al (2006) Prot. Engr. Design & Selection 19(7):299-307; Hamblett et al (2004) Clin. Cancer Res. 10:7063-7070; Hamblett, K.J., et al. "Effect of drug loading on the pharmacology, pharmacokinetics, and toxicity of an anti-CD30 antibody-drug conjugate," Abstract No. 624, American Association for Cancer Research, 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004; Alley, S.C., et al. "Controlling the location of drug attachment in antibody-drug conjugates," Abstract No. 627, American Association for Cancer Research, 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004). In embodiments, a homogeneous ADC with a single loading value may be isolated from the conjugation mixture by electrophoresis or chromatography. In embodiments enhanced selectivity of the lysine can result in a less heterogeneous mixture (U.S. Patent No. 9,981,046).

Anti-FOLR1 Antibodies

The disclosure of WO2020/016661 is incorporated herein in its entirety by reference.

i. Exemplary Antibodies and Antibody Sequences

[00215] In embodiments, the ADC comprises an antibody that binds to FOLR1. FOLR1 has been reported to be overexpressed in epithelial-derived tumors including ovarian, uterine, breast, endometrial, pancreatic, renal, lung, colorectal, and brain tumors.

[00216] In embodiments, the anti-FOLR1 antibody provided herein comprises a cysteine. In embodiments, the anti-FOLR1 antibody is bound to a drug through the sulfur of a cysteine residue. In embodiments, the anti-FOLR1 antibody is bound to a drug through the sulfurs of two cysteine residues.

[00217] In embodiments, the anti-FOLR1 antibody provided herein comprises a lysine. In embodiments, the anti-FOLR1 antibody is bound to a drug through the amine of a lysine residue.

In embodiments, the anti-FOLR1 antibody is bound to a drug through the amines of two lysine residues.

[00218] In embodiments, the ADC provided herein comprises an anti-FOLR1 antibody comprising a light chain variable region and a heavy chain variable region, wherein the light chain variable region comprises a light chain complementarity determining region 1 (CDR1) a light chain CDR2 and a light chain CDR3, and the heavy chain variable region comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3.

[00219] In embodiments, the ADC provided herein comprises an anti-FOLR1 antibody comprising at least one, two, three, four, five, or six CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 1; (b) VL CDR2 comprising the sequence of SEQ ID NO: 2; (c) VL CDR3 comprising the sequence of SEQ ID NO: 3; (d) VH CDR1 comprising the sequence of SEQ ID NO: 4; (e) VH CDR2 comprising the sequence of SEQ ID NO: 5; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 6. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising at least one CDR selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 1; (b) VL CDR2 comprising the sequence of SEQ ID NO: 2; (c) VL CDR3 comprising the sequence of SEQ ID NO: 3; (d) VH CDR1 comprising the sequence of SEQ ID NO: 4; (e) VH CDR2 comprising the sequence of SEQ ID NO: 5; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 6. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising at least two CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 1; (b) VL CDR2 comprising the sequence of SEQ ID NO: 2; (c) VL CDR3 comprising the sequence of SEQ ID NO: 3; (d) VH CDR1 comprising the sequence of SEQ ID NO: 4; (e) VH CDR2 comprising the sequence of SEQ ID NO: 5; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 6. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising at least three CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 1; (b) VL CDR2 comprising the sequence of SEQ ID NO: 2; (c) VL CDR3 comprising the sequence of SEQ ID NO: 3; (d) VH CDR1 comprising the sequence of SEQ ID NO: 4; (e) VH CDR2 comprising the sequence of SEQ ID NO: 5; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 6. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising at least four CDRs selected from (a) VL CDR1 comprising the

sequence of SEQ ID NO: 1; (b) VL CDR2 comprising the sequence of SEQ ID NO: 2; (c) VL CDR3 comprising the sequence of SEQ ID NO: 3; (d) VH CDR1 comprising the sequence of SEQ ID NO: 4; (e) VH CDR2 comprising the sequence of SEQ ID NO: 5; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 6. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising at least five CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 1; (b) VL CDR2 comprising the sequence of SEQ ID NO: 2; (c) VL CDR3 comprising the sequence of SEQ ID NO: 3; (d) VH CDR1 comprising the sequence of SEQ ID NO: 4; (e) VH CDR2 comprising the sequence of SEQ ID NO: 5; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 6. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising at least six CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 1; (b) VL CDR2 comprising the sequence of SEQ ID NO: 2; (c) VL CDR3 comprising the sequence of SEQ ID NO: 3; (d) VH CDR1 comprising the sequence of SEQ ID NO: 4; (e) VH CDR2 comprising the sequence of SEQ ID NO: 5; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 6.

[00220] In embodiments, the ADC provided herein comprises an anti-FOLR1 antibody comprising at least one, two, three, four, five, or six CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 13; (c) VL CDR3 comprising the sequence of SEQ ID NO: 14; (d) VH CDR1 comprising the sequence of SEQ ID NO: 15; (e) VH CDR2 comprising the sequence of SEQ ID NO: 16; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 17. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising at least one CDR selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 13; (c) VL CDR3 comprising the sequence of SEQ ID NO: 14; (d) VH CDR1 comprising the sequence of SEQ ID NO: 15; (e) VH CDR2 comprising the sequence of SEQ ID NO: 16; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 17. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising at least two CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 13; (c) VL CDR3 comprising the sequence of SEQ ID NO: 14; (d) VH CDR1 comprising the sequence of SEQ ID NO: 15; (e) VH CDR2 comprising the sequence of SEQ ID NO: 16; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 17. In embodiments, the ADC comprises an anti-

FOLR1 antibody comprising at least three CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 13; (c) VL CDR3 comprising the sequence of SEQ ID NO: 14; (d) VH CDR1 comprising the sequence of SEQ ID NO: 15; (e) VH CDR2 comprising the sequence of SEQ ID NO: 16; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 17. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising at least four CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 13; (c) VL CDR3 comprising the sequence of SEQ ID NO: 14; (d) VH CDR1 comprising the sequence of SEQ ID NO: 15; (e) VH CDR2 comprising the sequence of SEQ ID NO: 16; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 17. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising at least five CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 13; (c) VL CDR3 comprising the sequence of SEQ ID NO: 14; (d) VH CDR1 comprising the sequence of SEQ ID NO: 15; (e) VH CDR2 comprising the sequence of SEQ ID NO: 16; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 17. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising at least six CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 13; (c) VL CDR3 comprising the sequence of SEQ ID NO: 14; (d) VH CDR1 comprising the sequence of SEQ ID NO: 15; (e) VH CDR2 comprising the sequence of SEQ ID NO: 16; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 17.

[00221] In embodiments, the ADC provided herein comprises an anti-FOLR1 antibody comprising at least one, two, three, four, five, or six CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 18; (c) VL CDR3 comprising the sequence of SEQ ID NO: 19; (d) VH CDR1 comprising the sequence of SEQ ID NO: 20; (e) VH CDR2 comprising the sequence of SEQ ID NO: 21; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 22. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising at least one CDR selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 18; (c) VL CDR3 comprising the sequence of SEQ ID NO: 19; (d) VH CDR1 comprising the sequence of SEQ ID NO: 20; (e) VH CDR2 comprising the sequence of SEQ ID NO: 21; and (f) VH

CDR3 comprising the sequence of SEQ ID NO: 22. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising at least two CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 18; (c) VL CDR3 comprising the sequence of SEQ ID NO: 19; (d) VH CDR1 comprising the sequence of SEQ ID NO: 20; (e) VH CDR2 comprising the sequence of SEQ ID NO: 21; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 22. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising at least three CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 18; (c) VL CDR3 comprising the sequence of SEQ ID NO: 19; (d) VH CDR1 comprising the sequence of SEQ ID NO: 20; (e) VH CDR2 comprising the sequence of SEQ ID NO: 21; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 22. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising at least four CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 18; (c) VL CDR3 comprising the sequence of SEQ ID NO: 19; (d) VH CDR1 comprising the sequence of SEQ ID NO: 20; (e) VH CDR2 comprising the sequence of SEQ ID NO: 21; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 22. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising at least five CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 18; (c) VL CDR3 comprising the sequence of SEQ ID NO: 19; (d) VH CDR1 comprising the sequence of SEQ ID NO: 20; (e) VH CDR2 comprising the sequence of SEQ ID NO: 21; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 22. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising at least six CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 18; (c) VL CDR3 comprising the sequence of SEQ ID NO: 19; (d) VH CDR1 comprising the sequence of SEQ ID NO: 20; (e) VH CDR2 comprising the sequence of SEQ ID NO: 21; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 22.

[00222] In embodiments, the ADC comprises an anti-FOLR1 antibody comprising one CDR selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 1; (b) VL CDR2 comprising the sequence of SEQ ID NO: 2; (c) VL CDR3 comprising the sequence of SEQ ID

NO: 3; (d) VH CDR1 comprising the sequence of SEQ ID NO: 4; (e) VH CDR2 comprising the sequence of SEQ ID NO: 5; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 6. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising two CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 1; (b) VL CDR2 comprising the sequence of SEQ ID NO: 2; (c) VL CDR3 comprising the sequence of SEQ ID NO: 3; (d) VH CDR1 comprising the sequence of SEQ ID NO: 4; (e) VH CDR2 comprising the sequence of SEQ ID NO: 5; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 6. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising three CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 1; (b) VL CDR2 comprising the sequence of SEQ ID NO: 2; (c) VL CDR3 comprising the sequence of SEQ ID NO: 3; (d) VH CDR1 comprising the sequence of SEQ ID NO: 4; (e) VH CDR2 comprising the sequence of SEQ ID NO: 5; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 6. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising four CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 1; (b) VL CDR2 comprising the sequence of SEQ ID NO: 2; (c) VL CDR3 comprising the sequence of SEQ ID NO: 3; (d) VH CDR1 comprising the sequence of SEQ ID NO: 4; (e) VH CDR2 comprising the sequence of SEQ ID NO: 5; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 6. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising five CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 1; (b) VL CDR2 comprising the sequence of SEQ ID NO: 2; (c) VL CDR3 comprising the sequence of SEQ ID NO: 3; (d) VH CDR1 comprising the sequence of SEQ ID NO: 4; (e) VH CDR2 comprising the sequence of SEQ ID NO: 5; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 6. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising six CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 1; (b) VL CDR2 comprising the sequence of SEQ ID NO: 2; (c) VL CDR3 comprising the sequence of SEQ ID NO: 3; (d) VH CDR1 comprising the sequence of SEQ ID NO: 4; (e) VH CDR2 comprising the sequence of SEQ ID NO: 5; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 6.

[00223] In embodiments, the ADC comprises an anti-FOLR1 antibody comprising one CDR selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 13; (c) VL CDR3 comprising the sequence of SEQ ID

NO: 14; (d) VH CDR1 comprising the sequence of SEQ ID NO: 15; (e) VH CDR2 comprising the sequence of SEQ ID NO: 16; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 17. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising two CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 13; (c) VL CDR3 comprising the sequence of SEQ ID NO: 14; (d) VH CDR1 comprising the sequence of SEQ ID NO: 15; (e) VH CDR2 comprising the sequence of SEQ ID NO: 16; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 17. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising three CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 13; (c) VL CDR3 comprising the sequence of SEQ ID NO: 14; (d) VH CDR1 comprising the sequence of SEQ ID NO: 15; (e) VH CDR2 comprising the sequence of SEQ ID NO: 16; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 17. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising four CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 13; (c) VL CDR3 comprising the sequence of SEQ ID NO: 14; (d) VH CDR1 comprising the sequence of SEQ ID NO: 15; (e) VH CDR2 comprising the sequence of SEQ ID NO: 16; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 17. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising five CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 13; (c) VL CDR3 comprising the sequence of SEQ ID NO: 14; (d) VH CDR1 comprising the sequence of SEQ ID NO: 15; (e) VH CDR2 comprising the sequence of SEQ ID NO: 16; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 17. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising six CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 13; (c) VL CDR3 comprising the sequence of SEQ ID NO: 14; (d) VH CDR1 comprising the sequence of SEQ ID NO: 15; (e) VH CDR2 comprising the sequence of SEQ ID NO: 16; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 17.

[00224] In embodiments, the ADC comprises an anti-FOLR1 antibody comprising one CDR selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 18; (c) VL CDR3 comprising the sequence of SEQ ID

NO: 19; (d) VH CDR1 comprising the sequence of SEQ ID NO: 20; (e) VH CDR2 comprising the sequence of SEQ ID NO: 21; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 22. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising two CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 18; (c) VL CDR3 comprising the sequence of SEQ ID NO: 19; (d) VH CDR1 comprising the sequence of SEQ ID NO: 20; (e) VH CDR2 comprising the sequence of SEQ ID NO: 21; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 22. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising three CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 18; (c) VL CDR3 comprising the sequence of SEQ ID NO: 19; (d) VH CDR1 comprising the sequence of SEQ ID NO: 20; (e) VH CDR2 comprising the sequence of SEQ ID NO: 21; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 22. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising four CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 18; (c) VL CDR3 comprising the sequence of SEQ ID NO: 19; (d) VH CDR1 comprising the sequence of SEQ ID NO: 20; (e) VH CDR2 comprising the sequence of SEQ ID NO: 21; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 22. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising five CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 18; (c) VL CDR3 comprising the sequence of SEQ ID NO: 19; (d) VH CDR1 comprising the sequence of SEQ ID NO: 20; (e) VH CDR2 comprising the sequence of SEQ ID NO: 21; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 22. In embodiments, the ADC comprises an anti-FOLR1 antibody comprising six CDRs selected from (a) VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) VL CDR2 comprising the sequence of SEQ ID NO: 18; (c) VL CDR3 comprising the sequence of SEQ ID NO: 19; (d) VH CDR1 comprising the sequence of SEQ ID NO: 20; (e) VH CDR2 comprising the sequence of SEQ ID NO: 21; and (f) VH CDR3 comprising the sequence of SEQ ID NO: 22.

[00225] In embodiments, the anti-FOLR1 antibody comprises a VL CDR1 comprising the sequence of SEQ ID NO: 1, a VL CDR2 comprising the sequence of SEQ ID NO: 2, a VL CDR3 comprising the sequence of SEQ ID NO: 3, a VH CDR1 comprising the sequence of SEQ ID

NO: 4, a VH CDR2 comprising the sequence of SEQ ID NO: 5, and a VH CDR3 comprising the sequence of SEQ ID NO: 6. In embodiments, the anti-FOLR1 antibody comprises a VL CDR1 comprising the sequence of SEQ ID NO: 1. In embodiments, the anti-FOLR1 antibody comprises a VL CDR2 comprising the sequence of SEQ ID NO: 2. In embodiments, the anti-FOLR1 antibody comprises a VL CDR3 comprising the sequence of SEQ ID NO: 3. In embodiments, the anti-FOLR1 antibody comprises a VH CDR1 comprising the sequence of SEQ ID NO: 4. In embodiments, the anti-FOLR1 antibody comprises a VH CDR2 comprising the sequence of SEQ ID NO: 5. In embodiments, the anti-FOLR1 antibody comprises a VH CDR3 comprising the sequence of SEQ ID NO: 6.

[00226] In embodiments, the anti-FOLR1 antibody comprises a VL CDR1 comprising the sequence of SEQ ID NO: 12, a VL CDR2 comprising the sequence of SEQ ID NO: 13, a VL CDR3 comprising the sequence of SEQ ID NO: 14, a VH CDR1 comprising the sequence of SEQ ID NO: 15, a VH CDR2 comprising the sequence of SEQ ID NO: 16, and a VH CDR3 comprising the sequence of SEQ ID NO: 17. In embodiments, the anti-FOLR1 antibody comprises a VL CDR1 comprising the sequence of SEQ ID NO: 12. In embodiments, the anti-FOLR1 antibody comprises a VL CDR2 comprising the sequence of SEQ ID NO: 13. In embodiments, the anti-FOLR1 antibody comprises a VL CDR3 comprising the sequence of SEQ ID NO: 14. In embodiments, the anti-FOLR1 antibody comprises a VH CDR1 comprising the sequence of SEQ ID NO: 15. In embodiments, the anti-FOLR1 antibody comprises a VH CDR2 comprising the sequence of SEQ ID NO: 16. In embodiments, the anti-FOLR1 antibody comprises a VH CDR3 comprising the sequence of SEQ ID NO: 17.

[00227] In embodiments, the anti-FOLR1 antibody comprises a VL CDR1 comprising the sequence of SEQ ID NO: 12, a VL CDR2 comprising the sequence of SEQ ID NO: 18, a VL CDR3 comprising the sequence of SEQ ID NO: 19, a VH CDR1 comprising the sequence of SEQ ID NO: 20, a VH CDR2 comprising the sequence of SEQ ID NO: 21, and a VH CDR3 comprising the sequence of SEQ ID NO: 22. In embodiments, the anti-FOLR1 antibody comprises a VL CDR1 comprising the sequence of SEQ ID NO: 12. In embodiments, the anti-FOLR1 antibody comprises a VL CDR2 comprising the sequence of SEQ ID NO: 18. In embodiments, the anti-FOLR1 antibody comprises a VL CDR3 comprising the sequence of SEQ

ID NO: 19. In embodiments, the anti-FOLR1 antibody comprises a VH CDR1 comprising the sequence of SEQ ID NO: 20. In embodiments, the anti-FOLR1 antibody comprises a VH CDR2 comprising the sequence of SEQ ID NO: 21. In embodiments, the anti-FOLR1 antibody comprises a VH CDR3 comprising the sequence of SEQ ID NO: 22.

[00228] In embodiments, the ADC comprises an anti-FOLR1 antibody comprising the light chain CDR1 having the amino acid sequence of SEQ ID NO:1, the light chain CDR2 having the amino acid sequence of SEQ ID NO:2, the light chain CDR3 having the amino acid sequence of SEQ ID NO:3, the heavy chain CDR1 having the amino acid sequence of SEQ ID NO:4, the heavy chain CDR2 having the amino acid sequence of SEQ ID NO:5, and the heavy chain CDR3 having the amino acid sequence of SEQ ID NO:6.

[00229] In embodiments, the ADC comprises an anti-FOLR1 antibody comprising the light chain CDR1 having the amino acid sequence of SEQ ID NO:12, the light chain CDR2 having the amino acid sequence of SEQ ID NO:13, the light chain CDR3 having the amino acid sequence of SEQ ID NO:14, the heavy chain CDR1 having the amino acid sequence of SEQ ID NO:15, the heavy chain CDR2 having the amino acid sequence of SEQ ID NO:16, and the heavy chain CDR3 having the amino acid sequence of SEQ ID NO:17.

[00230] In embodiments, the ADC comprises an anti-FOLR1 antibody comprising the light chain CDR1 having the amino acid sequence of SEQ ID NO:12, the light chain CDR2 having the amino acid sequence of SEQ ID NO:18, the light chain CDR3 having the amino acid sequence of SEQ ID NO:19, the heavy chain CDR1 having the amino acid sequence of SEQ ID NO:20, the heavy chain CDR2 having the amino acid sequence of SEQ ID NO:21, and the heavy chain CDR3 having the amino acid sequence of SEQ ID NO:22.

[00231] In embodiments, the anti-FOLR1 antibody comprises a VL having a sequence with at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 7, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29 or SEQ ID NO: 30. In embodiments, the anti-FOLR1 antibody comprises a VL having the sequence of SEQ ID NO: 7. In embodiments, a VL sequence having at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 7, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29 or SEQ ID NO: 30 contains substitutions (e.g.,

conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-FOLR1 antibody comprising that sequence retains the ability to bind to FOLR1. In embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 7, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29 or SEQ ID NO: 30. In embodiments, a total of 1 to 5 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 7, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29 or SEQ ID NO: 30. In embodiments, substitutions, insertions, or deletions occur in regions outside the CDRs (i.e., in the FRs). In embodiments, the anti-FOLR1 antibody comprises the VL sequence of SEQ ID NO: 7, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29 or SEQ ID NO: 30, and includes post-translational modifications of that sequence.

[00232] In embodiments, the anti-FOLR1 antibody comprises a VH having a sequence with at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 8, SEQ ID NO: 24, SEQ ID NO: 26 or SEQ ID NO: 28. In embodiments, the anti-FOLR1 antibody comprises a VH having the sequence of SEQ ID NO: 8, SEQ ID NO: 24, SEQ ID NO: 26 or SEQ ID NO: 28. In embodiments, a VH sequence having at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 8, SEQ ID NO: 24, SEQ ID NO: 26 or SEQ ID NO: 28 contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-FOLR1 antibody comprising that sequence retains the ability to bind to FOLR1. In embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 8, SEQ ID NO: 24, SEQ ID NO: 26 or SEQ ID NO: 28. In embodiments, a total of 1 to 5 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 8, SEQ ID NO: 24, SEQ ID NO: 26 or SEQ ID NO: 28. In embodiments, substitutions, insertions, or deletions occur in regions outside the CDRs (i.e., in the FRs). In embodiments, the anti-FOLR1 antibody comprises the VH sequence of SEQ ID NO: 8, SEQ ID NO: 24, SEQ ID NO: 26 or SEQ ID NO: 28, and includes post-translational modifications of that sequence.

[00233] In embodiments, the anti-FOLR1 antibody is an IgG antibody. In embodiments, the anti-FOLR1 antibody is an IgG1, IgG2, IgG3 or IgG4 antibody. In embodiments, the anti-FOLR1 antibody is an IgG1 or IgG4 antibody. In embodiments, the anti-FOLR1 antibody is an IgG1 antibody.

[00234] In embodiments, an anti-FOLR1 antibody binds a human FOLR1. In embodiments, the human FOLR1 has the amino acid sequence of SEQ ID NO: 9.

[00235] In any of the above embodiments, an anti-FOLR1 antibody is humanized. In embodiment, an anti-FOLR1 antibody comprises CDRs as in any of the above embodiments, and further comprises a human acceptor framework, e.g. a human immunoglobulin framework or a human consensus framework. In embodiments, a humanized anti-FOLR1 antibody comprises (a) a VL CDR1 comprising the sequence of SEQ ID NO: 1; (b) a VL CDR2 comprising the sequence of SEQ ID NO: 2; (c) a VL CDR3 comprising the sequence of SEQ ID NO: 3; (d) a VH CDR1 comprising the sequence of SEQ ID NO: 4; (e) a VH CDR2 comprising the sequence of SEQ ID NO: 5; and (f) a VH CDR3 comprising the sequence of SEQ ID NO: 6.

[00236] In any of the above embodiments, an anti-FOLR1 antibody is humanized. In embodiment, an anti-FOLR1 antibody comprises CDRs as in any of the above embodiments, and further comprises a human acceptor framework, e.g. a human immunoglobulin framework or a human consensus framework. In embodiments, a humanized anti-FOLR1 antibody comprises (a) a VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) a VL CDR2 comprising the sequence of SEQ ID NO: 13; (c) a VL CDR3 comprising the sequence of SEQ ID NO: 14; (d) a VH CDR1 comprising the sequence of SEQ ID NO: 15; (e) a VH CDR2 comprising the sequence of SEQ ID NO: 16; and (f) a VH CDR3 comprising the sequence of SEQ ID NO: 17.

[00237] In any of the above embodiments, an anti-FOLR1 antibody is humanized. In embodiment, an anti-FOLR1 antibody comprises CDRs as in any of the above embodiments, and further comprises a human acceptor framework, e.g. a human immunoglobulin framework or a human consensus framework. In embodiments, a humanized anti-FOLR1 antibody comprises (a) a VL CDR1 comprising the sequence of SEQ ID NO: 12; (b) a VL CDR2 comprising the sequence of SEQ ID NO: 18; (c) a VL CDR3 comprising the sequence of SEQ ID NO: 19; (d) a VH CDR1 comprising the sequence of SEQ ID NO: 20; (e) a VH CDR2 comprising the sequence of SEQ ID NO: 21; and (f) a VH CDR3 comprising the sequence of SEQ ID NO: 22.

[00238] In embodiments, the anti-FOLR1 antibody comprises a VL having a sequence of SEQ ID NO: 7□ a VH having a sequence of SEQ ID NO: 8, a light chain constant region having

a sequence of SEQ ID NO: 10, and a heavy chain constant region having a sequence of SEQ ID NO: 11.

[00239] In embodiments, the anti-FOLR1 antibody is a monoclonal antibody, including a chimeric, humanized, or human antibody. In one embodiment, an anti-FOLR1 antibody is an antibody fragment, e.g., a Fv, Fab, Fab', scFv, diabody, or $F(ab')_2$ fragment. In another embodiment, the antibody is a substantially full-length antibody, e.g., an IgG1 antibody or other antibody class or isotype as defined herein.

ii. Antibody Affinity

[00240] In embodiments, an anti-FOLR1 antibody provided herein binds a human FOLR1 with an affinity of $\leq$ 10 nM, or $\leq$ 5 nM, or $\leq$ 4 nM, or $\leq$ 3 nM, or $\leq$ 2 nM. In embodiments, an anti-FOLR1 antibody binds a human FOLR1 with an affinity of $\geq$ 0.0001 nM, or $\geq$ 0.001 nM, or $\geq$ 0.01 nM. Standard assays known to the skilled artisan can be used to determine binding affinity. For example, whether an anti-FOLR1 antibody "binds with an affinity of" $\leq$ 10 nM, or $\leq$ 5 nM, or $\leq$ 4 nM, or $\leq$ 3 nM, or $\leq$ 2 nM, can be determined using standard Scatchard analysis utilizing a non-linear curve fitting program (see, for example, Munson et al., Anal Biochem, 107: 220-239, 1980).

[00241] In embodiments, the anti-FOLR1 antibody provided herein has a dissociation constant (Kd) of $\leq$ 1μM, $\leq$ 100 nM, $\leq$ 10 nM, $\leq$ 1 nM, $\leq$ 0.1 nM, $\leq$ 0.01 nM, or $\leq$ 0.001 nM, and optionally is $\geq 10^{-13}$ M. (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M).

[00242] In embodiments, Kd is measured by a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen as described by the following assay. Solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, e.g., Chen et al., J. Mol. Biol. 293:865-881(1999)). To establish conditions for the assay, MICROTITER® multi-well plates (Thermo Scientific) are coated overnight with 5 μg/ml of a

capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (e.g., consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., Cancer Res. 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (e.g., up to about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, 150 μL/well of scintillant (MICROSCINT-20 TM; Packard) is added, and the plates are counted on a TOPCOUNT TM gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

[00243] According to another embodiment, Kd is measured using surface plasmon resonance assays using a BIACORE®-2000 or a BIACORE ®-3000 (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with N-ethyl-N'- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 μg/ml (~0.2 μM) before injection at a flow rate of 5 μL/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20TM) surfactant (PBST) at 25°C at a flow rate of approximately 25 μL/min. Association rates (kon) and dissociation rates (koff) are calculated using a simple one-to-one Langmuir binding model (BIACORE ® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio koff/kon. See, e.g., Chen et al., J. Mol. Biol. 293:865-881 (1999). If the on-rate exceeds 106 M^{-1} s^{-1} by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease

in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO TM spectrophotometer (ThermoSpectronic) with a stirred cuvette.

iii. Antibody Fragments

[00244] In embodiments, the anti-FOLR1 antibody provided herein is an antibody fragment. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, $F(ab')_2$, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, see Hudson et al. *Nat. Med.* 9:129-134 (2003). For a review of scFv fragments, see, e.g., Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and $F(ab')_2$ fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046.

[00245] Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., *Nat. Med.* 9:129-134 (2003); and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., *Nat. Med.* 9:129-134 (2003).

[00246] Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; *see,* e.g., U.S. Patent No. 6,248,516 B1).

[00247] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. *E. coli* or phage), as described herein.

iv. Chimeric and Humanized Antibodies

[00248] In embodiments, the anti-FOLR1 antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, e.g., in U.S. Patent No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984). In one example, a chimeric antibody comprises a non-human variable region (e.g., a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

[00249] In embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, e.g., CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the HVR residues are derived), e.g., to restore or improve antibody specificity or affinity.

[00250] Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, e.g., in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, *Methods* 36:25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer*, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

[00251] Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, e.g., Sims et al. *J. Immunol.* 151:2296 (1993)); framework regions derived from the consensus sequence of human

antibodies of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter et al. *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); and Presta et al. *J. Immunol.*, 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (see, e.g., Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, e.g., Baca et al., *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok et al., *J. Biol. Chem.* 271:22611-22618 (1996)).

v. Human Antibodies

[00252] In embodiments, the anti-FOLR1 antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008).

[00253] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat. Biotech.* 23:1117-1125 (2005). See also, e.g., U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HUMAB® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE® technology). Human variable regions from intact antibodies generated by such animals may be further modified, e.g., by combining with a different human constant region.

[00254] Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, e.g., Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc.,

New York, 1987); and Boerner et al., *J. Immunol.*, 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, *Xiandai Mianyixue*, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

[00255] Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

vi. Library-Derived Antibodies

[00256] In embodiments, the anti-FOLR1 antibody provided herein is derived from an antibody library. Antibodies may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, e.g., in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132(2004).

[00257] In phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be

screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al., *EMBO J,* 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[00258] Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

vii. Multispecific Antibodies

[00259] In embodiments, the anti-FOLR1 antibody provided herein is a multispecific antibody, e.g. a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In embodiments, one of the binding specificities is for FOLR1 and the other is for any other antigen. In embodiments, bispecific antibodies may bind to two different epitopes of FOLR1. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express FOLR1. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

[00260] Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, *Nature* 305: 537 (1983)), WO 93/08829, and Traunecker et al., *EMBO J.* 10: 3655 (1991)), and "knob-in-hole" engineering (see, e.g., U.S.

Patent No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (see, e.g., US Patent No. 4,676,980, and Brennan et al., *Science*, 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (see, e.g., Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992)); using "diabody" technology for making bispecific antibody fragments (see, e.g., Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)); and using single-chain Fv (scFv) dimers (see,e.g. Gruber et al., *J. Immunol.*, 152:5368 (1994)); and preparing trispecific antibodies as described, e.g., in Tutt et al. *J. Immunol.* 147: 60 (1991).

[00261] Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (see, e.g. US 2006/0025576A1).

[00262] The antibody or fragment herein also includes a "Dual Acting FAb" or "DAF" comprising an antigen binding site that binds to FOLR1 as well as another, different antigen.

viii. Antibody Variants

[00263] In embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding.

a) Substitution, Insertion, and Deletion Variants

[00264] In embodiments, the anti-FOLR1 antibody provided herein has one or more amino acid substitutions. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table 1 under the heading of "preferred substitutions."

More substantial changes are provided in Table 1 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products are screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

Table 1. Exemplary Amino acid substitutions.

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

Amino acids may be grouped according to common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

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

[00265] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.* a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g. binding affinity).

[00266] Alterations (e.g., substitutions) may be made in HVRs, e.g., to improve antibody affinity. Such alterations may be made in HVR "hotspots," i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001).) In embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or

oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (e.g., 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[00267] In embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR "hotspots" or SDRs. In embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[00268] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex is used to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[00269] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants

of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g. for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

b) Glycosylation Variants

[00270] In embodiments, an anti-FOLR1 antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[00271] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In embodiments, modifications of the oligosaccharide in an antibody may be made in order to create antibody variants with certain improved properties.

[00272] In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, e.g., US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of

publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8,* knockout CHO cells (see, e.g., Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

[00273] Antibody variants are further provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, e.g., in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana *et al.*). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

c) Fc Region Variants

[00274] In embodiments, one or more amino acid modifications may be introduced into the Fc region of an anti-FLOR1 antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (*e.g.*, a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (*e.g.* a substitution) at one or more amino acid positions.

[00275] In embodiments, an antibody variant that possesses some but not all effector functions is contemplated, which make it a desirable candidate for applications in which the half life of the antibody *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (see, e.g. Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (see Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in a animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996); Cragg, M.S. et al., *Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half life determinations can also be performed using methods known in the art (see, e.g., Petkova, S.B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006)).

[00276] Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

[00277] Certain antibody variants with improved or diminished binding to FcRs are described. (See, e.g., U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.* 9(2): 6591-6604 (2001).)

[00278] Antibodies with increased half-lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (US Patent No. 7,371,826).

[00279] See also Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

ix. Antibody Derivatives

[00280] In embodiments, an anti-FOLR1 antibody provided herein may be further modified to contain additional non-proteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene

glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

x. Recombinant Methods and Compositions

[00281] Antibodies may be produced using recombinant methods and compositions, e.g., as described in U.S. Patent No. 4,816,567. One skilled in the art will be familiar with suitable host cells for antibody expression. Exemplary host cells include eukaryotic cells, e.g. a Chinese Hamster Ovary (CHO) cell or lymphoid cell (e.g., Y0, NS0, Sp20 cell).

[00282] For recombinant production of an anti-FOLR1 antibody, nucleic acid encoding an antibody, e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

[00283] Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, e.g., U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523. (See also Charlton, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody fragments in *E. coli*.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

[00284] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. See Gerngross, *Nat. Biotech.* 22:1409-1414 (2004), and Li et al., *Nat. Biotech.* 24:210-215 (2006).

[00285] Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells.

[00286] Plant cell cultures can also be utilized as hosts. *See,* e.g., US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES^{TM} technology for producing antibodies in transgenic plants).

[00287] Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, e.g., in Graham et al., *J. Gen Virol.* 36:59 (1977); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK); buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR^{-} CHO cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki and Wu, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003); Dhara, V.G. et al., *BioDrugs* 32**:** 571–584 (2018); Kunert, R. and Reinhart, D. *Applied microbiology and biotechnology*, 100(8): 3451–3461 (2016).

xi. Assays

[00288] Anti-FOLR1 antibodies described herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

[00289] In embodiment, an antibody is tested for its antigen binding activity, e.g., by known methods such as ELISA, BIACore®, FACS, or Western blot.

[00290] In another embodiment, competition assays may be used to identify an antibody that competes with any of the antibodies described herein for binding to FOLR1. In embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by an antibody described herein. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, NJ).

[00291] In an exemplary competition assay, immobilized FOLR1 is incubated in a solution comprising a first labeled antibody that binds to FOLR1 and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to FOLR1. The second antibody may be present in a hybridoma supernatant. As a control, immobilized FOLR1 is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to FOLR1, excess unbound antibody is removed, and the amount of label associated with immobilized FOLR1 is measured. If the amount of label associated with immobilized FOLR1 is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to FOLR1. In embodiments, immobilized FOLR1 is present on the surface of a cell or in a membrane preparation obtained from a cell expressing FLOR1 on its surface. *See* Harlow and Lane (1988) *Antibodies: A Laboratory Manual* ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

Methods of Preparing Antibody-Drug Conjugates

[00292] An ADC of formula (I) may be prepared by several routes employing organic chemistry reactions, conditions, and reagents known to those skilled in the art, including: (1) reaction of a nucleophilic group of an antibody with a linker reagent (B, B', or B'') to form Ab-B, Ab-B', or Ab-B'' via a covalent bond, followed by reaction with a drug moiety D or drug-linker molecule D-L^2; and (2) reaction of a nucleophilic group of a drug moiety D with a bivalent linker reagent (L^2 and/or B, B', or B'') to form D-L^2, D-L^2-B, D-L^2-B', or D-L^2-B'' via a covalent bond, followed by reaction with a nucleophilic group of an antibody or a reduced antibody. Several such methods are described by Agarwal et al., (2015), *Bioconjugate Chem.,* 26: 176-192.

[00293] Nucleophilic groups on antibodies include, but are not limited to: (i) N-terminal amine groups, (ii) side chain amine groups, e.g. lysine, (iii) side chain thiol groups, e.g. cysteine, and (iv) sugar hydroxyl or amino groups where the antibody is glycosylated. Amine, thiol, and hydroxyl groups are nucleophilic and capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; and (iii) aldehydes, ketones, carboxyl, and maleimide groups. In addition to NHS esters, functional groups used for conjugation to antibody lysines can include, as non-limiting examples, pentafluorophenyl, tetrafluorophenyl, tetrafluorobenzenesulfonate, nitrophenyl, isocyanate, isothiocyanate, and sulfonylchloride.

[00294] In yet another embodiment, an antibody may be conjugated to a "receptor" (such as streptavidin) for utilization in tumor pre-targeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g., avidin) which is conjugated to a cytotoxic agent (e.g., a drug or radionucleotide).

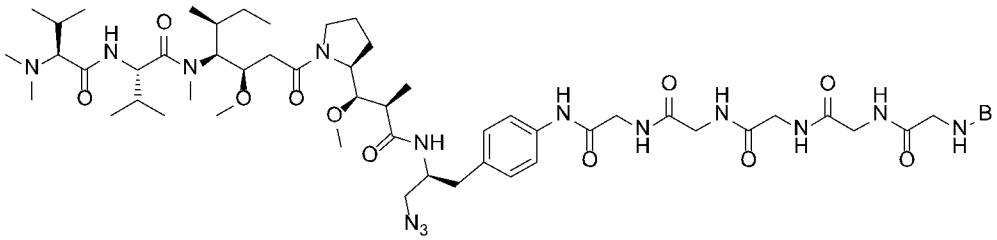
[00295] Certain antibodies have reducible interchain disulfides, i.e. cysteine bridges. Antibodies may be made reactive for conjugation with linker reagents by treatment with a reducing agent such as DTT (dithiothreitol) or tricarbonylethylphosphine (TCEP), such that the antibody is fully or partially reduced. Each cysteine bridge will thus form, theoretically, two reactive thiol nucleophiles. Additional nucleophilic groups can be introduced into antibodies

through modification of lysine residues, e.g., by reacting lysine residues with 2-iminothiolane (Traut's reagent), resulting in conversion of an amine into a thiol. Reactive thiol groups may also be introduced into an antibody by introducing one, two, three, four, or more cysteine residues (e.g., by preparing variant antibodies comprising one or more non-native cysteine amino acid residues). Nonlimiting examples of functional groups that can react with reactive thiols include, without limitation, maleimide, pyridyldithio, bromoacetyl, iodoacetyl, bromobenzyl, iodobenzyl, and 4-(cyanoethynyl)benzoyl.

[00296] In embodiments, an antibody may be reduced with a reducing agent such as dithiothreitol (DTT) or tricarbonylethylphosphine (TCEP), under partial or total reducing conditions, to generate reactive cysteine thiol groups. The inter-chain cysteine residues can then be alkylated for example using maleimide. Alternatively, the inter-chain cysteine residues can undergo bridging alkylation for example using bis sulfone linkers or propargyldibromomaleimide followed by Cu-click ligation. In embodiments, the antibody can be conjugated through lysine amino acid. Such conjugation can be a one-step conjugation or a two-step conjugation. In embodiments, the one-step conjugation entails conjugation of the ε-amino group of lysine residue to the drug-linker molecule (D-L^2, D-L^2-B, D-L^2-B', or D-L^2-B'') containing an amine-reactive group via amide bonds. In embodiments the amine-reactive group is an activated ester. In embodiments, the antibody can be conjugated via a two-step conjugation. The two-step conjugation entails a first step, where a bi-functional reagent containing both an amine and a thiol reactive functional groups is reacted with the lysine ε-amino group(s). In the second step, the drug-linker molecule (D-L^2, D-L^2-B, D-L^2-B', or D-L^2-B'') is conjugated to the thiol reactive group of the bifunctional reagent. Several examples are provided by Jain et al., (2015), *Pharm. Res.,* 32:3526-3540. In embodiments, the first step may involve the functionalization of the antibody with azide followed by a click chemistry reaction with an alkyne modified linker or drug-linker molecule (D-L^2, D-L^2-B, D-L^2-B', or D-L^2-B''). In embodiments, the first step may involve the functionalization of the antibody with an alkyne followed by a click chemistry reaction with an azide modified linker or drug-linker molecule (D-L^2, D-L^2-B, D-L^2-B', or D-L^2-B''). In embodiments, the first step may involve the functionalization of the antibody with an aldehyde followed by a click chemistry reaction with a

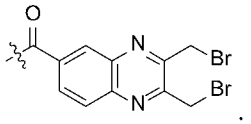
alkoxyamine or hydrazine modified linker or drug-linker molecule (D-L^2, D-L^2-B, D-L^2-B', or D-L^2-B''). In embodiments, the first step may involve the functionalization of the antibody with a tetrazine followed by a click chemistry reaction with a trans-cyclooctene or cyclopropene modified linker or drug-linker molecule (D-L^2, D-L^2-B, D-L^2-B', or D-L^2-B''). In embodiments, the first step may involve the functionalization of the antibody with a trans-cyclooctene or cyclopropene followed by a click chemistry reaction with a tetrazine modified linker or drug-linker molecule (D-L^2, D-L^2-B, D-L^2-B', or D-L^2-B''). Some examples are described by Pickens et al., (2018), *Bioconjug. Chem.*, 29:686-701; Li et al., (2018), *MAbs*, 10:712-719; and Chio et al., (2020), *Methods Mol. Biol.*, 2078:83-97.

[00297] In embodiments, an ADC of formula (I) can be prepared by reacting an anti-FOLR1 antibody (Ab) with a molecule of formula (P-I):

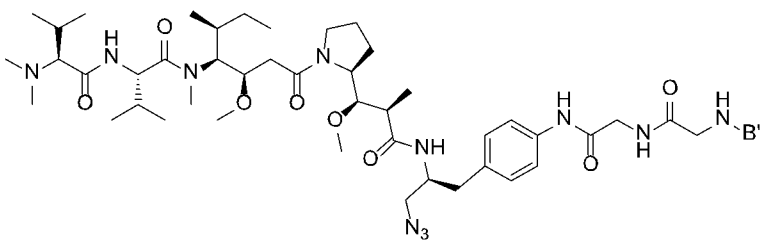


(P-I)

or a pharmaceutically acceptable salt thereof, wherein:
B is a reactive moiety capable of forming a bond with the anti-FOLR1 antibody.

[00298] In embodiments, B is a reactive moiety capable of forming a bond with two sulfhydryl groups of the anti-FOLR1 antibody. In embodiments, B is .

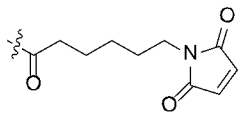
[00299] In embodiments, an ADC of formula (I) can be prepared by reacting an anti-FOLR1 antibody (Ab) with a molecule of formula (P-II):



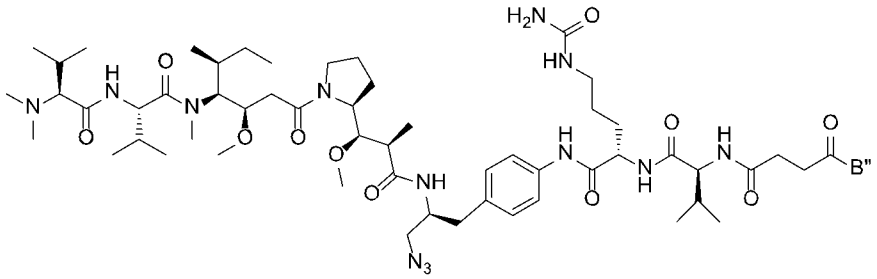
(P-II)

or a pharmaceutically acceptable salt thereof, wherein:

B' is a reactive moiety capable of forming a bond with the anti-FOLR1 antibody.

[00300] In embodiments, B' is a reactive moiety capable of forming a bond with a sulfhydryl group of the anti-FOLR1 antibody. In embodiments, B' is .

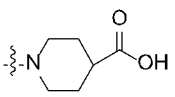
[00301] In embodiments, an ADC of formula (I) can be prepared by reacting an anti-FOLR1 antibody (Ab) with a molecule of formula (P-III):



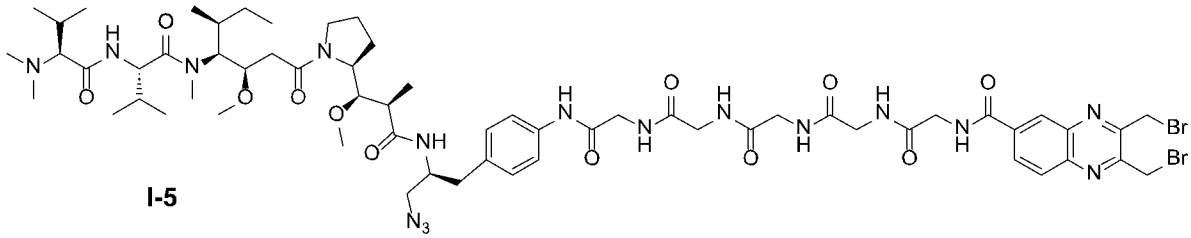
(P-III)

or a pharmaceutically acceptable salt thereof, wherein:

B" is a reactive moiety capable of forming a bond with the anti-FOLR1 antibody (the reaction is described in Example 3 of US patent No. 10,590,165).

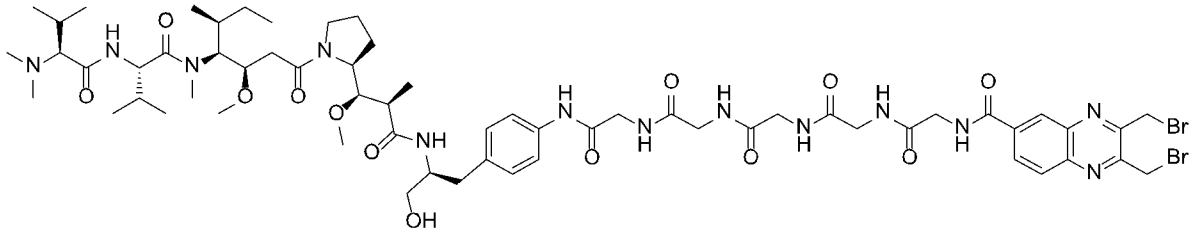
[00302] In embodiments, B" is a reactive moiety capable of forming a bond with an amine group of the anti-FOLR1 antibody. In embodiments, B" is .

[00303] In embodiments, P-I, P-II, or P-III is a compound selected from:

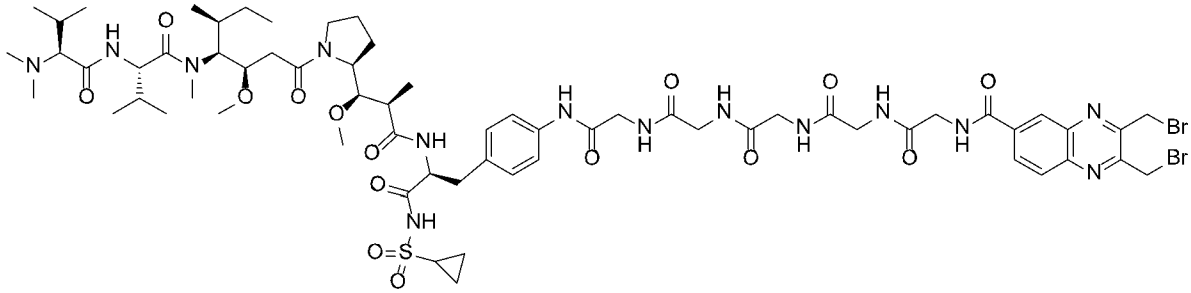


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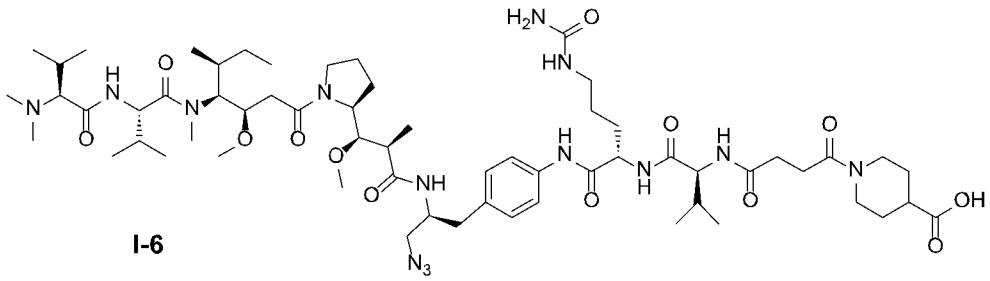
[00304] ,



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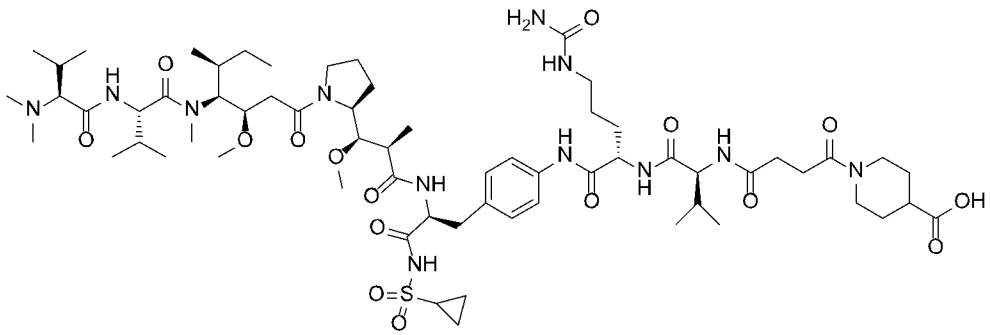


[00306] ,

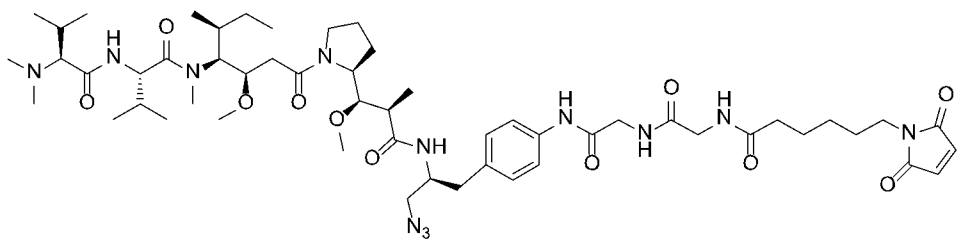


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[00307] ,



[00308] , and

[00309] ,

[00310] or a pharmaceutically acceptable salt thereof.

Pharmaceutical compositions

[00311] In an aspect, provided herein is a pharmaceutical composition including an ADC as described herein, including embodiments, and a pharmaceutically acceptable carrier. In embodiments, the ADC as described herein is included in a therapeutically effective amount.

[00312] In embodiments, the pharmaceutical composition is formulated as a tablet, a powder, a capsule, a pill, a cachet, or a lozenge as described herein. The pharmaceutical composition may be formulated as a tablet, capsule, pill, cachet, or lozenge for oral administration. The pharmaceutical composition may be formulated for dissolution into a solution for administration by such techniques as, for example, intravenous administration. The pharmaceutical composition may be formulated for oral administration, suppository administration, topical administration, intravenous administration, intraperitoneal administration, intramuscular administration, intralesional administration, intrathecal administration, intranasal administration, subcutaneous administration, implantation, transdermal administration, or transmucosal administration as described herein.

[00313] The ADCs and pharmaceutical compositions thereof are particularly useful for parenteral administration, i.e., subcutaneously (s.c.), intrathecally, intraperitoneally, intramuscularly (i.m.) or intravenously (i.v.). In embodiment, the ADCs and pharmaceutical compositions thereof are administered intravenously or subcutaneously.

[00314] The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the antigen binding protein of the invention in such pharmaceutical

formulation can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as about 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

[00315] Actual methods for preparing parenterally administrable compositions are well known or will be apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pa. For the preparation of intravenously administrable antigen binding protein formulations of the invention see Lasmar U and Parkins D "The formulation of Biopharmaceutical products", Pharma. Sci. Tech. today, page 129-137, Vol. 3 (3 Apr. 2000); Wang, W "Instability, stabilisation and formulation of liquid protein pharmaceuticals", Int. J. Pharm 185 (1999) 129-188; Stability of Protein Pharmaceuticals Part A and B ed Ahern T. J., Manning M. C., New York, N.Y.: Plenum Press (1992); Akers, M. J. "Excipient-Drug interactions in Parenteral Formulations", J. Pharm Sci 91 (2002) 2283-2300; Imamura, K et al "Effects of types of sugar on stabilization of Protein in the dried state", J Pharm Sci 92 (2003) 266-274; Izutsu, Kkojima, S. "Excipient crystallinity and its protein-structure-stabilizing effect during freeze-drying", J. Pharm. Pharmacol, 54 (2002) 1033-1039; Johnson, R, "Mannitol-sucrose mixtures-versatile formulations for protein peroxidise19g19n", J. Pharm. Sci, 91 (2002) 914-922; and Ha, E Wang W, Wang Y. j. "Peroxide formation in polysorbate 80 and protein stability", J. Pharm Sci, 91, 2252-2264, (2002) the entire contents of which are incorporated herein by reference and to which the reader is specifically referred.

[00316] In embodiments, the pharmaceutical composition may include optical isomers, diastereomers, enantiomers, isoforms, polymorphs, hydrates, solvates or products, or pharmaceutically acceptable salts of the compound described herein. The compound described herein (including pharmaceutically acceptable salts thereof) included in the pharmaceutical composition may be covalently attached to a carrier moiety, as described above. In embodiments, the compound described herein (including pharmaceutically acceptable salts thereof) included in the pharmaceutical composition is not covalently linked to a carrier moiety. A combination of covalently and not covalently linked compound described herein may be in a pharmaceutical composition herein.

Methods of use

[00317] In an aspect, provided herein is a method of treating a disease in a subject in need thereof, said method including administering an effective amount of an antibody drug conjugate (ADC) comprising an IgG antibody, a conjugation linker moiety (L^1) that binds to the thiol of cysteine residue(s) or to the amine of lysine residue(s) - of the IgG antibody, and a drug moiety covalently bound to either L^1, or optionally another linker L^2. In embodiments, the IgG antibody binds to FOLR1.

[00318] In one aspect, an ADC provided herein is used in a method of inhibiting proliferation of a FOLR1-expressing cell, the method comprising contacting the cell with the ADC, e.g., exposing the cell to the ADC under conditions permissive for binding of the anti-FOLR1 antibody of the ADC on the surface of the cell, thereby inhibiting the proliferation of the cell. In embodiments, the method is an *in vitro* or an *in vivo* method In embodiments, the cell is a B cell. In embodiments, the cell is a cancer cell. In embodiments, the cell is a multiple myeloma cell. In any of these embodiments, the cell may be a mammalian cell, such as a human cell.

[00319] Inhibition of cell proliferation in vitro may be assayed using the CellTiter-Glo™ Luminescent Cell Viability Assay, which is commercially available from Promega (Madison, WI). That assay determines the number of viable cells in culture based on quantitation of ATP present, which is an indication of metabolically active cells. *See* Crouch et al. (1993) *J. Immunol. Meth.* 160:81-88, US Pat. No. 6602677. The assay may be conducted in 96- or 384-well format, making it amenable to automated high-throughput screening (HTS). *See* Cree et al. (1995) *AntiCancer Drugs* 6:398-404. The assay procedure involves adding a single reagent (CellTiter-Glo® Reagent) directly to cultured cells. This results in cell lysis and generation of a luminescent signal produced by a luciferase reaction. The luminescent signal is proportional to the amount of ATP present, which is directly proportional to the number of viable cells present in culture. Data can be recorded by luminometer or CCD camera imaging device. The luminescence output is expressed as relative light units (RLU).

[00320] In another aspect, an ADC for use as a medicament is provided. In further aspects, an ADC for use in a method of treatment is provided. In another aspect, provided herein is a

method of treating a disease in a subject in need thereof, said method including administering an effective amount of a pharmaceutical composition of the ADC as described herein.

[00321] In embodiments, the disease is cancer. In embodiments, the cancer is associated with overexpression of FOLR1. In embodiments, provided herein is an ADC for use in a method of treating an individual having a FOLR1-expressing cancer, the method comprising administering to the individual an effective amount of the ADC. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent. In embodiments, the additional therapeutic agent is a VEGF inhibitor. In embodiments, the VEGF inhibitor is an anti-VEGF antibody or a small molecule VEGF inhibitor. In embodiments, the VEGF inhibitor is an anti-VEGF antibody. In embodiments, the VEGF inhibitor is a small molecule VEGF inhibitor. In embodiments, the anti-VEGF antibody is bevacizumab or ramucirumab . In embodiments, the anti-VEGF antibody is bevacizumab. In embodiments, the anti-VEGF antibody is ramucirumab. In embodiments, the small molecule VEGF inhibitor is sunitinib, sorafenib, axitinib, pazopanib or regorafenib. In embodiments, the small molecule VEGF inhibitor is sunitinib. In embodiments, the small molecule VEGF inhibitor is sorafenib. In embodiments, the small molecule VEGF inhibitor is axitinib. In embodiments, the small molecule VEGF inhibitor is pazopanib. In embodiments, the small molecule VEGF inhibitor is regorafenib.

[00322] In a further aspect, the present disclosure provides for the use of an ADC in the manufacture or preparation of a medicament. In embodiment, the medicament is for treatment of FOLR1-expressing cancer. In a further embodiment, the medicament is for use in a method of treating FOLR1-expressing cancer, the method comprising administering to an individual having FOLR1-expressing cancer an effective amount of the medicament. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent.

[00323] In embodiments, the present disclosure provides for the use of an ADC, as described herein including in embodiments, in combination with an additional therapeutic agent, in the manufacture or preparation of a medicament for treatment of FOLR1-expressing cancer. In embodiments, the present disclosure provides for the use of an ADC, as described herein

including in embodiments, in combination with a VEGF inhibitor, in the manufacture or preparation of a medicament for treatment of FOLR1-expressing cancer. In embodiments, the VEGF inhibitor is an anti-VEGF antibody or a small molecule VEGF inhibitor. In embodiments, the VEGF inhibitor is an anti-VEGF antibody. In embodiments, the VEGF inhibitor is a small molecule VEGF inhibitor. In embodiments, the anti-VEGF antibody is bevacizumab or ramucirumab. In embodiments, the anti-VEGF antibody is bevacizumab. In embodiments, the anti-VEGF antibody is ramucirumab. In embodiments, the small molecule VEGF inhibitor is sunitinib, sorafenib, axitinib, pazopanib or regorafenib. In embodiments, the small molecule VEGF inhibitor is sunitinib. In embodiments, the small molecule VEGF inhibitor is sorafenib. In embodiments, the small molecule VEGF inhibitor is axitinib. In embodiments, the small molecule VEGF inhibitor is pazopanib. In embodiments, the small molecule VEGF inhibitor is regorafenib. In embodiments, the ADC described herein is ADC-1, ADC-2, ADC-3, ADC-4, ADC-5, or ADC-6. In embodiments, the ADC is ADC-1, ADC-2, ADC-3, ADC-4, ADC-5, or ADC-6. In embodiments, the ADC is ADC-1. In embodiments, the ADC is ADC-2. In embodiments, the ADC is ADC-3. In embodiments, the ADC is ADC-4. In embodiments, the ADC is ADC-5. In embodiments, the ADC is ADC-6.

[00324] In embodiments, the methods provided herein are for treating cancer in a mammal. In embodiments, the methods provided herein are for treating cancer in a human.

[00325] In embodiments, the cancers that may be treated with an immunoconjugate or method provided herein include epithelial-derived tumors including ovarian, uterine, breast, endometrial, pancreatic, renal, lung, colorectal, and brain tumors. In embodiments, the cancers that may be treated with an immunoconjugate or a method provided herein include serous and endometrioid epithelial ovarian cancer, endometrial adenocarcinoma, non-small cell lung carcinoma (NSCLC) of the adenocarcinoma subtype, squamous lung cancer, and triple-negative breast cancer (TNBC). In embodiments, the cancers that may be treated with an immunoconjugate or method provided herein include hematopoietic cancers which include, but are not limited to, multiple myeloma (MM) including smoldering MM, monoclonal gammopathy of undetermined (or unknown or unclear) significance (MGUS), plasmacytoma (bone, extramedullary),

lymphoplasmacytic lymphoma (LPL), Waldenstrom's Macroglobulinemia, plasma cell leukemia, and primary amyloidosis (AL). In embodiments, the hematopoietic cancer is multiple myeloma.

[00326] In embodiments, the cancer is ovarian cancer. In embodiments, the cancer is breast cancer. In embodiments, the cancer is lung cancer. In embodiments, the cancer is triple-negative breast cancer.

[00327] In embodiments, a therapeutically effective amount of the antibody drug conjugate (ADC) provided herein is from about 0.5 to about 3,000 mg per day, from about 1 to about 2,000 mg per day, from about 1 to about 1,500 mg per day, from about 1 to about 1,000 mg per day, from about 10 to about 1,000 mg per day, from about 50 to about 1,000 mg per day, from about 50 to about 800 mg per day, from about 50 to about 700 mg per day, or from about 100 to about 500 mg per day.

[00328] In embodiments, the therapeutically effective amount is about 1, about 20, about 50, about 100, about 150, about 200, about 250, about 300, about 350, about 400, about 450, about 500, about 550, about 600, about 650, about 700, about 750, about 800, about 850, or about 1,000 mg per day.

[00329] In embodiments, the therapeutically effective amount is from about 0.1 to about 100 mg/kg/day, from about 0.1 to about 50 mg/kg/day, from about 0.1 to about 40 mg/kg/day, from about 0.5 to about 30 mg/kg/day, from about 0.5 to about 25 mg/kg/day, 1 to about 25 mg/kg/day, from about 1 to about 20 mg/kg/day, from about 1 to about 15 mg/kg/day, or from about 1 to about 10 mg/kg/day.

[00330] Depending on the disease to be treated and the subject's condition, the compound of formula (I), or a pharmaceutically acceptable salt thereof, may be administered by oral, parenteral (*e.g.*, intramuscular, intraperitoneal, intravenous, CIV, intracistemal injection or infusion, subcutaneous injection, or implant), inhalation, nasal, vaginal, rectal, sublingual, or topical (*e.g.*, transdermal or local) routes of administration. The compound of formula (I), or a pharmaceutically acceptable salt thereof, may be formulated, alone or together, in suitable dosage unit with pharmaceutically acceptable excipients, carriers, adjuvants and vehicles, appropriate for each route of administration.

[00331] The compound of formula (I), or a pharmaceutically acceptable salt thereof, can be delivered as a single dose such as, *e.g.*, a single bolus injection, or oral tablets or pills; or over time, such as, *e.g.*, continuous infusion over time or divided bolus doses over time. The compound can be administered repeatedly, if necessary, for example, until the patient experiences stable disease or regression, or until the patient experiences disease progression or unacceptable toxicity. For example, stable disease for solid tumors generally means that the perpendicular diameter of measurable lesions has not increased by 25% or more from the last measurement. Response Evaluation Criteria in Solid Tumors (RECIST) Guidelines, *Journal of the National Cancer Institute* 92(3): 205-216 (2000). Stable disease or lack thereof is determined by methods known in the art such as evaluation of patient symptoms, physical examination, visualization of the tumor that has been imaged using X-ray, CAT, PET, or MRI scan and other commonly accepted evaluation modalities.

[00332] The compound of formula (I), or a pharmaceutically acceptable salt thereof, can be administered once daily (QD), or divided into multiple daily doses such as twice daily (BID), three times daily (TID), and four times daily (QID). In addition, the administration can be continuous (*i.e.*, daily for consecutive days or every day), intermittent, *e.g.*, in cycles (*i.e.*, including days, weeks, or months of rest without drug). As used herein, the term "daily" is intended to mean that a therapeutic compound, such as the compound of formula (I), is administered once or more than once each day, for example, for a period of time. The term "continuous" is intended to mean that a therapeutic compound, such as the compound of formula (I), is administered daily for an uninterrupted period of at least 10 days to 52 weeks. The term "intermittent" or "intermittently" as used herein is intended to mean stopping and starting at either regular or irregular intervals. For example, intermittent administration of the compound of formula (I) is administration for one to six days per week, administration in cycles (*e.g.*, daily administration for two to eight consecutive weeks, then a rest period with no administration for up to one week), or administration on alternate days. The term "cycling" as used herein is intended to mean that a therapeutic compound, such as the compound of formula (I), is administered daily or continuously but with a rest period.

[00333] In embodiments, the frequency of administration is in the range of about a daily dose to about a monthly dose. In embodiments, administration is once a day, twice a day, three times

a day, four times a day, once every other day, twice a week, once every week, once every two weeks, once every three weeks, or once every four weeks. In embodiments, the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered once a week. In another embodiment, the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered twice a week. In embodiments, the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered three times a week. In embodiments, the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered once every two weeks. In embodiments, the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered once every three weeks. In embodiments, the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered once every four weeks. In embodiments, the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered once every eight weeks.

Combination therapy with a second active agent

[00334] The ADC of formula (I), or a pharmaceutically acceptable salt thereof, can also be combined or used in combination with other therapeutic agents useful in the treatment of cancer described herein.

[00335] In embodiments, provided herein is a method of treating cancer, comprising administering to a patient an ADC of formula (I), or a pharmaceutically acceptable salt thereof, in combination with one or more second active agents, and optionally in combination with radiation therapy, blood transfusions, or surgery. It is believed that certain combinations work synergistically in the treatment of particular types of cancer, and certain diseases and conditions associated with or characterized by undesired angiogenesis.

[00336] One or more second active ingredients or agents can be used in the methods and compositions provided herein with the compounds provided herein. In embodiments, the second active agent is an anti-cancer agent. Second active agents can be large molecules (*e.g.*, proteins) or small molecules (*e.g.*, synthetic inorganic, organometallic, or organic molecules).

[00337] Examples of large molecule active agents include, but are not limited to, hematopoietic growth factors, cytokines, and monoclonal and polyclonal antibodies. In certain embodiments, large molecule active agents are biological molecules, such as naturally occurring or artificially made proteins.

[00338] Antibodies that can be used in combination with the compounds provided herein include monoclonal and polyclonal antibodies. In embodiments, the antibodies include, but are not limited to anti-VEGF antibodies. Examples of anti-VEGF antibodies include, but are not limited to, bevacizumab and ramucirumab.

[00339] VEGF inhibitors may be used in methods and compositions provided herein. Inhibitors of VEGF receptors include anti-VEGF antibodies and small molecules (*e.g.*, synthetic inorganic, organometallic, or organic molecules). In embodiments, VEGF inhibitors are antibodies. In embodiments, VEGF inhibitors are small molecules.

[00340] In embodiments, VEGF inhibitors are anti-VEGF antibodies, including but not limited to, bevacizumab and ramucirumab. In embodiments, the anti-VEGF antibody is bevacizumab or ramucirumab. In embodiments, the anti-VEGF antibody is bevacizumab. In embodiments, the anti-VEGF antibody is ramucirumab.

[00341] In embodiments, VEGF inhibitors are small molecules, including but not limited to, sunitinib, sorafenib, axitinib, pazopanib and regorafenib. In embodiments, the small molecule is sunitinib, sorafenib, axitinib, pazopanib or regorafenib. In embodiments, the small molecule is sunitinib. In embodiments, the small molecule is sorafenib. In embodiments, the small molecule is axitinib. In embodiments, the small molecule is pazopanib. In embodiments, the small molecule is regorafenib.

[00342] As used herein, the term "in combination" includes the use of more than one therapy (*e.g.*, one or more therapeutic agents). However, the use of the term "in combination" does not restrict the order in which therapies (*e.g.*, therapeutic agents) are administered to a patient with a disease or disorder. A first therapy (*e.g.*, a therapeutic agent such as an ADC provided herein, *e.g.*, the compound of formula (I), or a pharmaceutically acceptable salt thereof) can be

administered prior to (*e.g.*, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (*e.g.*, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapy (*e.g.*, a therapeutic agent) to the subject. Triple therapy is also contemplated herein.

[00343] Administration of the compound of formula (I) and one or more second active agents to a patient can occur simultaneously or sequentially by the same or different routes of administration. Combinations of agents or compositions can be administered either concomitantly (e.g., as a mixture), separately but simultaneously (e.g., via separate intravenous lines) or sequentially (e.g., one agent is administered first followed by administration of the second agent). Thus, the term combination is used to refer to concomitant, simultaneous or sequential administration of two or more agents or compositions. The course of treatment is best determined on an individual basis depending on the particular characteristics of the subject and the type of treatment selected. The treatment, such as those disclosed herein, can be administered to the subject on a daily, twice daily, bi-weekly, monthly or any applicable basis that is therapeutically effective. The treatment can be administered alone or in combination with any other treatment disclosed herein or known in the art. The additional treatment can be administered simultaneously with the first treatment, at a different time, or on an entirely different therapeutic schedule (e.g., the first treatment can be daily, while the additional treatment is weekly).

[00344] The route of administration of the compound of formula (I) is independent of the route of administration of a second therapy. In one embodiment, the compound of formula (I) is administered orally. In another embodiment, the compound of formula (I) is administered intravenously. In another embodiment, the compound of formula (I) is administered intraperitoneally. Thus, in accordance with these embodiments, the compound of formula (I) is administered orally or intravenously, and the second therapy can be administered orally, parenterally, intraperitoneally, intravenously, intraarterially, transdermally, sublingually, intramuscularly, rectally, transbuccally, intranasally, liposomally, via inhalation, vaginally,

intraoccularly, via local delivery by catheter or stent, subcutaneously, intraadiposally, intraarticularly, intrathecally, or in a slow release dosage form. In embodiments, the compound of formula (I) and a second therapy are administered by the same mode of administration, orally or by IV. In another embodiment, the compound of formula (I) is administered by one mode of administration, *e.g.*, by IV, whereas the second agent (an anticancer agent) is administered by another mode of administration, *e.g.*, intraperitoneally.

[00345] In embodiments, the second active agent is administered intravenously or intraperitoneally and once every week or every two weeks in an amount of from about 1 to about 1000 mg, from about 5 to about 500 mg, from about 10 to about 500 mg, or from about 50 to about 500 mg. The specific amount of the second active agent will depend on the specific agent used, the type of disease being treated or managed, the severity and stage of disease, and the amount of the compound of formula (I) provided herein and any optional additional active agents concurrently administered to the patient. In certain embodiments, the second active agent is bevacizumab, ramucirumab, sunitinib, sorafenib, axitinib, pazopanib or regorafenib

[00346] In embodiments, a compound provided herein, *e.g.,* the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered with bevacizumab to patients with FOLR1-expressing cancer. In embodiments, a compound provided herein, *e.g.,* the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered with ramucirumab to patients with FOLR1-expressing cancer. In embodiments, a compound provided herein, *e.g.,* the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered with sunitinib to patients with FOLR1-expressing cancer. In embodiments, a compound provided herein, *e.g.,* the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered with sorafenib to patients with FOLR1-expressing cancer. In embodiments, a compound provided herein, *e.g.,* the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered with axitinib to patients with FOLR1-expressing cancer. In embodiments, a compound provided herein, *e.g.,* the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered with pazopanib to patients with FOLR1-expressing cancer. In embodiments, a compound provided herein, *e.g.,* the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered with regorafenib to patients with FOLR1-expressing cancer.

[00347] In embodiments, a compound provided herein, *e.g.,* the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered with bevacizumab to patients with multiple myeloma. In embodiments, a compound provided herein, *e.g.,* the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered with sunitinib to patients with multiple myeloma. In embodiments, a compound provided herein, *e.g.,* the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered with sorafenib to patients with multiple myeloma.

[00348] In embodiments, a compound provided herein, *e.g.,* the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered with bevacizumab to patients with lung cancer. In embodiments, a compound provided herein, *e.g.,* the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered with ramucirumab to patients with lung cancer. In embodiments, a compound provided herein, *e.g.,* the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered with sunitinib to patients with lung cancer. In embodiments, a compound provided herein, *e.g.,* the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered with sorafenib to patients with lung cancer.In embodiments, a compound provided herein, *e.g.,* the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered with axitinib to patients with lung cancer.In embodiments, a compound provided herein, *e.g.,* the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered with pazopanib to patients with lung cancer.In embodiments, a compound provided herein, *e.g.,* the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered with regorafenib to patients with lung cancer.

[00349] In embodiments, a compound provided herein, *e.g.,* the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered with bevacizumab to patients with ovarian cancer. In embodiments, a compound provided herein, *e.g.,* the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered with ramucirumab to patients with ovarian cancer. In embodiments, a compound provided herein, *e.g.,* the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered with sunitinib to patients with ovarian cancer. In embodiments, a compound provided herein, *e.g.,* the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered with sorafenib to

patients with ovarian cancer.In embodiments, a compound provided herein, *e.g.,* the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered with axitinib to patients with ovarian cancer. In embodiments, a compound provided herein, *e.g.,* the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered with pazopanib to patients with ovarian cancer. In embodiments, a compound provided herein, *e.g.,* the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered with regorafenib to patients with ovarian cancer.

Kits

[00350] In embodiments, active ingredients provided herein are not administered to a patient at the same time or by the same route of administration. Therefore, encompassed herein are kits which, when used by the medical practitioner, can simplify the administration of appropriate amounts of active ingredients to a patient.

[00351] In embodiments, a kit provided herein comprises a dosage form of a compound provided herein, *e.g.*, the compound of formula (I), or a pharmaceutically acceptable salt thereof. In embodiments, the kit provided herein further comprises additional active ingredients, such as a VEGF inhibitor. In embodiments, the kit provided herein further comprises additional active ingredients, such as an anti-VEGF antibody or a small molecule VEGF inhibitor. In embodiments, the kit provided herein further comprises additional active ingredients, such as bevacizumab, ramucirumab, sunitinib, sorafenib, axitinib, pazopanib or regorafenib

[00352] In embodiments, the kit provided herein may include a pharmaceutical composition comprising a dosage form of a compound provided herein, *e.g.*, the compound of formula (I), or a pharmaceutically acceptable salt thereof, together with a VEGF inhibitor. In embodiments, the kit provided herein may include a pharmaceutical composition comprising a dosage form of a compound provided herein, *e.g.*, the compound of formula (I), or a pharmaceutically acceptable salt thereof, together with an anti-VEGF antibody. In embodiments, the kit provided herein may include a pharmaceutical composition comprising a dosage form of a compound provided herein, *e.g.*, the compound of formula (I), or a pharmaceutically acceptable salt thereof, together with a small molecule VEGF inhibitor.

[00353] In embodiments, the kit provided herein may include a pharmaceutical composition

comprising a dosage form of a compound provided herein, *e.g.*, the compound of formula (I), or a pharmaceutically acceptable salt thereof, together with bevacizumab. In embodiments, the kit provided herein may include a pharmaceutical composition comprising a dosage form of a compound provided herein, *e.g.*, the compound of formula (I), or a pharmaceutically acceptable salt thereof, together with ramucirumab. In embodiments, the kit provided herein may include a pharmaceutical composition comprising a dosage form of a compound provided herein, *e.g.*, the compound of formula (I), or a pharmaceutically acceptable salt thereof, together with sunitinib. In embodiments, the kit provided herein may include a pharmaceutical composition comprising a dosage form of a compound provided herein, *e.g.*, the compound of formula (I), or a pharmaceutically acceptable salt thereof, together with sorafenib. In embodiments, the kit provided herein may include a pharmaceutical composition comprising a dosage form of a compound provided herein, *e.g.*, the compound of formula (I), or a pharmaceutically acceptable salt thereof, together with axitinib. In embodiments, the kit provided herein may include a pharmaceutical composition comprising a dosage form of a compound provided herein, *e.g.*, the compound of formula (I), or a pharmaceutically acceptable salt thereof, together with pazopanib. In embodiments, the kit provided herein may include a pharmaceutical composition comprising a dosage form of a compound provided herein, *e.g.*, the compound of formula (I), or a pharmaceutically acceptable salt thereof, together with regorafenib. In embodiments, the compound of formula (I) is ADC-1, ADC-2, ADC-3, ADC-4, ADC-5, or ADC-6. In embodiments, the compound of formula (I) is ADC-1. In embodiments, the compound of formula (I) is ADC-2. In embodiments, the compound of formula (I) is ADC-3. In embodiments, the compound of formula (I) is ADC-4. In embodiments, the compound of formula (I) is ADC-5. In embodiments, the compound of formula (I) is ADC-6.

[00354] In embodiments, the kit provided herein further comprises an additional active ingredient, such as bevacizumab. In embodiments, the kit provided herein further comprises an additional active ingredient, such as ramucirumab. In embodiments, the kit provided herein further comprises an additional active ingredient, such as sunitinib. In embodiments, the kit provided herein further comprises an additional active ingredient, such as sorafenib. In embodiments, the kit provided herein further comprises an additional active ingredient, such as axitinib. In embodiments, the kit provided herein further comprises an additional active

ingredient, such as pazopanib. In embodiments, the kit provided herein further comprises an additional active ingredient, such as regorafenib.

[00355] In embodiments, the kit provided herein further comprises a device that is used to administer the active ingredients. Examples of such devices include, but are not limited to, syringes, drip bags, patches, and inhalers.

[00356] In embodiments, the kit provided herein further comprises cells or blood for transplantation as well as pharmaceutically acceptable vehicles that can be used to administer one or more active ingredients. For example, if an active ingredient is provided in a solid form that must be reconstituted for parenteral administration, the kit can comprise a sealed container of a suitable vehicle in which the active ingredient can be dissolved to form a particulate-free sterile solution that is suitable for parenteral administration. Examples of pharmaceutically acceptable vehicles include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

LIST OF SEQUENCES:

[00357] Human FOLR1 sequence **SEQ ID NO:9**

[00358] MAQRMTTQLLLLLVWVAVVGEAQTRIAWARTELLNVCMNAKHHKEKPGPE
DKLHEQCRPWRKNACCSTNTSQEAHKDVSYLYRFNWNHCGEMAPACKRHFIQDTCLY
ECSPNLGPWIQQVDQSWRKERVLNVPLCKEDCEQWWEDCRTSYTCKSNWHKGWNWT
SGFNKCAVGAACQPFHFYFPTPTVLCNEIWTHSYKVSNYSRGSGRCIQMWFDPAQGNN
EEVARFYAAAMSGAGPWAAWPFLLSLALMLLWLLS

[00359] **Tables of Sequences:**

Table 2:

Light chain variable region:	**Heavy chain variable region:**
(a) AMT-151 SEQ ID NO:7 DIVMTQSPDSLAVSLGERATINCKSS**QSL** **LYSSSQKNY**LAWYQQKPGQSPKLLIY**W** **AS**TRESGVPDRFSGSGSGTDFTLTISSLQA EDVAVYYC**QQYYSYPWT**FGGGTKVEIK	(a) AMT-151 SEQ ID NO:8 QVQLVQSGSELKKPGASVKVSCKAS**GY** **TFTSYW**MNWVRQAPGQGLEWMGR**IN** **PYDSET**HSNQKFKDRFVFSLDTSVSTAY LQISSLKAEDTAVYYC**ARSGGYAWFAY** WGQGTLVTVSS
(b) FOLR1-Ab1 SEQ ID NO:23 DIVMSQSPSSLAVSVGEKVTMSCKSS**QSL** **LYSSSQKNY**LAWYQQKPGQSPKLLIY**W** **AS**TRESGVPDRFTGSGSGTDFTLTISSVKA EDLAVYYC**QQYYSYPWT**FGGGTKLEIK	(b) FOLR1-Ab1 SEQ ID NO:24 QVQLQQPGAELVRPGASVKLSCKAS**GY** **TFTSYW**MNWVKQRPEQGLEWIGR**INP** **YDSET**HSNQKFKDKAILTVDKSSTTAY MQLSSLTSEDSAVYYC**ARSGGYAWFA** **Y**WGQGTLVTVSA
(c) FOLR1-Ab4 SEQ ID NO:25 DIVLTQSPASLAVSLGQRATISCRAS**ESVD** **NYGISF**MNWFQQKPGQPPKLLIY**AAS**NQ GSGVPARFSDSGSGTDFSLNIHPMEEDDT AMYFC**QQSKEVPYT**FGGGTKLEIK	(c) FOLR1-Ab4 SEQ ID NO:26 QIQLVQSGPELKKPGETVKISCKAS**IYT** **FTDYS**IQWVKQAPGKGLKWMGW**INTE** **TGEP**TYADDFKGRFAFSLESSASTAFLQ INNLKNEDTATYFC**ARMGYYGPKIMD** **Y**WGQGTSVTVSS
(d) FOLR1-Ab14 SEQ ID NO:27 DIVLTQSPASLAVSLGQRATISCRAS**ESVD** **NYGISF**MNWFQQKPGQPPKLLIY**VAS**NQ GSGVPARFSGSGSGTDFSLNIHPMEEDDT AMYFC**QQSKEVPT**FGGGTKLEIK	(d) FOLR1-Ab14 SEQ ID NO:28 EVLLQQSGPELVKPGASVKIPCKAS**GYT** **FTDYN**MDWVKQSHGKSLEWIGD**INPN** **NGGT**IYNQKFKGKATLTVDKSSSTAYM ELRSLTSEDTAVYYC**ARKPYYGPRYFD** **V**WGAGTTVTVSS

Light chain variable region:	**Heavy chain variable region:**
(e) FOLR1-Ab20 SEQ ID NO:29 DIVLTQFPASLAVSLGQRATISCRAS**ESVD** **NYGISF**MNWFQQKPGQPPKLLIY**VAS**NQ GSGVPARFSGSGFGTDFSLNIHPMEEDDT AMYFC**QQSKEVPT**FGGGTKLEIK	(e) FOLR1-Ab20 SEQ ID NO:28 EVLLQQSGPELVKPGASVKIPCKAS**GY** **TFTDYN**MDWVKQSHGKSLEWIGD**INP** **NNGGT**IYNQKFKGKATLTVDKSSSTAY MELRSLTSEDTAVYYC**ARKPYYGPRY** **FDV**WGAGTTVTVSS
(f) FOLR1-Ab23 SEQ ID NO:30 DIVLTQFPAFLAVFLGQRATISCRAS**ESVD** **NYGISF**MNWFQQKPGQPPKLLIY**VAS**NQ GSGVPARFSGSGFGTEFSLNIHPMEEDDS AMYFC**QQSKEVPT**FGGGTKLEIK	(f) FOLR1-Ab23 SEQ ID NO:28 EVLLQQSGPELVKPGASVKIPCKAS**GYT** **FTDYN**MDWVKQSHGKSLEWIGD**INPN** **NGGT**IYNQKFKGKATLTVDKSSSTAYM ELRSLTSEDTAVYYC**ARKPYYGPRYFD** **V**WGAGTTVTVSS

Table 3:

CDRs 1, 2 and 3:
(a) AMT-151/FOLR1-Ab1:
AMT-151 (VL – CDR1) SEQ ID NO:1 QSLLYSSSQKNY
AMT-151 (VL – CDR2) SEQ ID NO:2 WAS
AMT-151 (VL – CDR3) SEQ ID NO:3 QQYYSYPWT
AMT-151 (VH – CDR1) SEQ ID NO:4 GYTFTSYW
AMT-151 (VH – CDR2) SEQ ID NO:5 INPYDSET
AMT-151 (VH – CDR3) SEQ ID NO:6 ARSGGYAWFAY
(b) FOLR1-Ab4:
FOLR1-Ab4 (VL – CDR1) SEQ ID NO:12 ESVDNYGISF
FOLR1-Ab4 (VL – CDR2) SEQ ID NO:13 AAS
FOLR1-Ab4 (VL – CDR3) SEQ ID NO:14 QQSKEVPYT
FOLR1-Ab4 (VH – CDR1) SEQ ID NO:15 IYTFTDYS

CDRs 1, 2 and 3:
FOLR1-Ab4 (VH – CDR2) SEQ ID NO:16 INTETGEP FOLR1-Ab4 (VH – CDR3) SEQ ID NO:17 ARMGYYGPKIMDY
(c) FOLR1-Ab14/FOLR1-Ab20/FOLR1-Ab23: FOLR1-Ab14 /FOLR1-Ab20/FOLR1-Ab23(VL – CDR1) SEQ ID NO:12 ESVDNYGISF FOLR1-Ab14/FOLR1-Ab20/FOLR1-Ab23 (VL – CDR2) SEQ ID NO:18 VAS FOLR1-Ab14 /FOLR1-Ab20/FOLR1-Ab23 (VL – CDR3) SEQ ID NO:19 QQSKEVPT FOLR1-Ab14/FOLR1-Ab20/FOLR1-Ab23 (VH – CDR1) SEQ ID NO:20 GYTFTDYN FOLR1-Ab14/FOLR1-Ab20/FOLR1-Ab23 (VH – CDR2) SEQ ID NO:21 INPNNGGT FOLR1-Ab14/FOLR1-Ab20/FOLR1-Ab23 (VH – CDR3) SEQ ID NO:22 ARKPYYGPRYFDV

Note: The anti-FOLR1 antibodies FOLR1-Ab1, FOLR1-Ab4, FOLR1-Ab14, FOLR1-Ab20 and FOLR1-Ab23 were disclosed in WO2020/016661, among which AMT-151 and FOLR1-Ab1 share the same 6 CDRs, and FOLR1-Ab14, FOLR1-Ab20 and FOLR1-Ab23 share the same 6 CDRs.

Table 4:

Light chain constant region:	**Heavy chain constant region:**
AMT-151 SEQ ID NO:10 RTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESV TEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC	AMT-151 SEQ ID NO:11 ASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVVTVPSSSLGTQTYIC NVNHKPSNTKVDKKVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQV SLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLS PGK

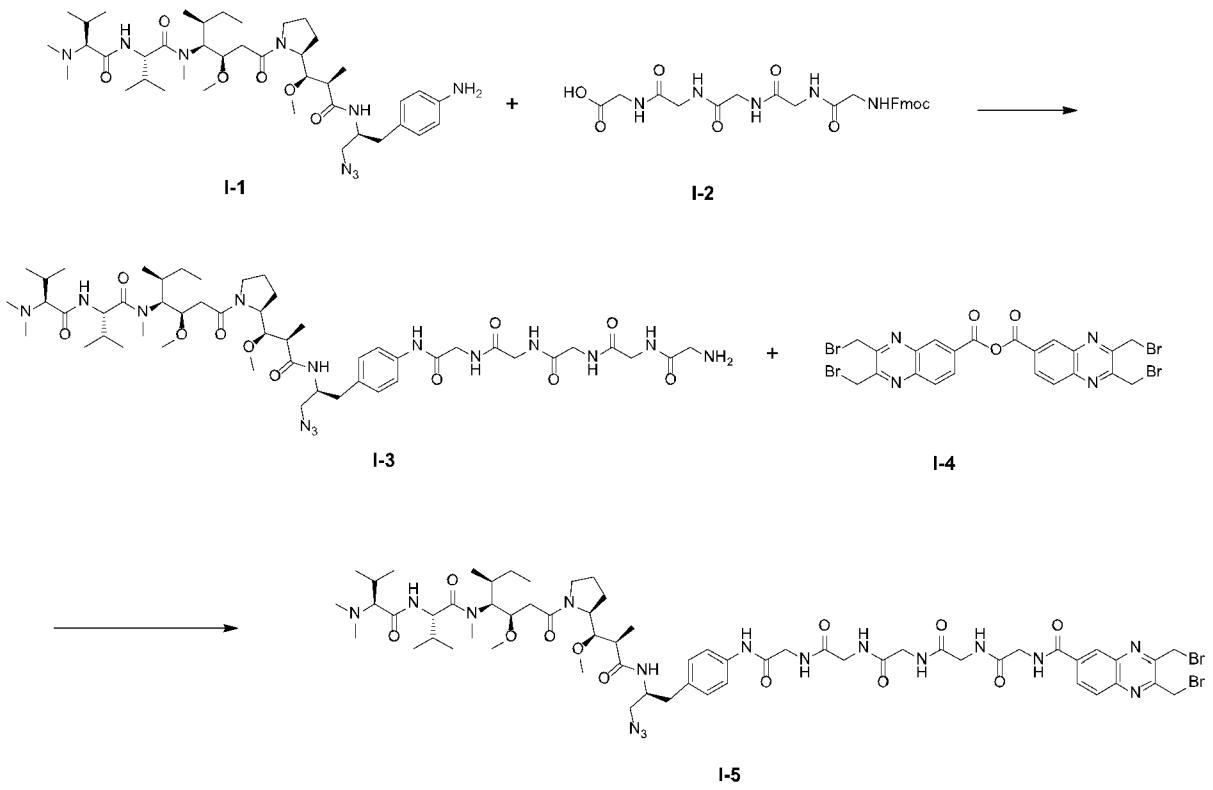
EXAMPLES

[00360] The following examples are meant to be illustrative and can be used to further understand embodiments of the present disclosure and should not be construed as limiting the scope of the present teachings in any way.

[00361] The chemical reactions described in the Examples can be readily adapted to prepare a number of other compounds of the present disclosure, and alternative methods for preparing the compounds of this disclosure are deemed to be within the scope of this disclosure. For example, the synthesis of non-exemplified compounds according to the present disclosure can be successfully performed by modifications apparent to those skilled in the art, e.g., by utilizing other suitable reagents known in the art other than those described, or by making routine modifications of reaction conditions, reagents, and starting materials. Alternatively, other reactions disclosed herein or known in the art will be recognized as having applicability for preparing other compounds of the present disclosure. Synthesis of compound **I-1** and related compounds was disclosed in US Patent Nos. 10,590165 and 9,981,046, which are incorporated herein in their entireties.

Synthetic Examples

Example S1: Synthesis of Compound I-5.

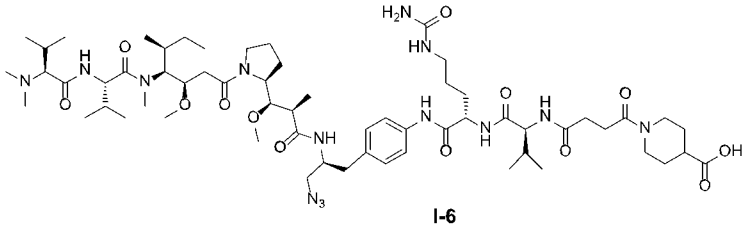


[00362] To compound **I-1** (TFA salt, 250 mg, 0.25 mmol) in 2 mL of DMF was added a solution of HATU (103 mg, 0.27 mmol), DIEA (188 μL, 1.08 mmol), and acid **I-2** (142 mg, 0.27 mmol) in 2 mL of DMF. The mixture was stirred for 30 min, then 160 μL of DBU was added and stirred for 10 min. The mixture was purified by HPLC to give compound **I-3** (214 mg). MS m/z 1057.6 (M+H).

[00363] To compound **I-3** (TFA salt, 10 mg, 7.8 μmol) in 0.5 mL of DMF was added a solution of anhydride **I-4** (16.5 mg, 23.5 μmol) and DIEA (5.4 μL, 31 μmol) in 0.5 mL of DMF. The mixture was stirred for 10 min, then purified by HPLC to give compound **I-5** (8.5 mg). MS m/z 1397.5 (M+H).

Example S2: Synthesis of Compound I-6.

[00364] The synthesis of compound I-6 is described in Example 3 of US patent No. 10,590,165, which is incorporated herein in its entirety.



I-6

[00365] Antibody-Drug Conjugates (ADCs) were prepared by conjugating compound I-5 or compound I-6 with **AMT-151** clone of anti-FOLR1 antibody. **AMT-151** is a human IgG1 antibody.

Example S3: Preparation of Antibody-Drug Conjugate (ADC) anti-FOLR1-Compound I-5 (151-C-LOCK-D5; ADC-1).

[00366] The 6 CDRs of anti-FOLR1 antibody used in this Example have identical antibody CDR sequences of the FOLR1 antibody anti-FLOR1-Ab1 described in WIPO publication No. WO 2020/016661, which is incorporated herein in its entirety. The heavy chain sequences and the light chain sequences of the anti-FOLR1 antibody used in this Example are respectively shown in SEQ ID NO:7 and SEQ ID NO:8 in Table 2, and SEQ ID NO:10 and SEQ ID NO:11 in Table 4. Affinity purified anti-FOLR1 antibody was buffer exchanged into Conjugation Buffer (50 mM sodium phosphate buffer, pH 7.0-7.2, 4 mM EDTA) at a concentration of 5 mg/mL. To a portion of this antibody stock was added a freshly prepared 10 mM water solution of tris(2-carboxyethyl)phosphine) (TCEP) at 20-fold molar excess. The resulting mixture was incubated at 4-8 °C overnight. The excess TCEP was then removed by several rounds of centrifugal filtration with fresh Conjugation Buffer. UV-Vis quantification of recovered, reduced antibody material was followed by confirmation of sufficient free thiol-to-antibody ratio (SH/Ab). Briefly, a 1 mM aliquot of freshly prepared Ellman's Reagent (5,5'-dithiobis-(2-nitrobenzoic acid) in Conjugation Buffer was mixed with an equal volume of purified antibody solution. The resulting absorbance at 412 nm was measured and the reduced cysteine content was determined

using the extinction coefficient of 14,150 $M^{-1}cm^{-1}$. Under these conditions, SH/Ab ratio measured ~6.

[00367] To initiate conjugation of toxin-linker material to anti-FOLR1 antibody, compound **I-5** was freshly dissolved in a 3:2 acetonitrile/water mixture to a concentration of 5 mM. Propylene glycol (PG) was then added to a portion of the reduced, purified (TCEP removed) anti-FOLR1 antibody to give a final concentration of 30% (v/v) PG immediately prior to addition of **I-5** in 4.5-fold molar excess. After thorough mixing and incubation at ambient temperature for 2 h, the crude conjugation reaction was analyzed by HIC-HPLC chromatography to confirm reaction completion (disappearance of starting antibody peak) at 280 nm wavelength detection. Purification of the resulting **ADC-1** conjugate was then carried out by gel-filtration chromatography using an AKTA system equipped with a Superdex 200 pg column (GE Healthcare) equilibrated with PBS. The average drug-to-antibody ratio (DAR) was calculated to be 4 based on comparative peak area integration of the HIC-HPLC chromatogram. Confirmation of low percent (<5%) high molecular weight (HMW) aggregates for the resulting **ADC-1** was determined using analytical SEC-HPLC.

Example S4: Preparation of Antibody-Drug Conjugate (ADC) anti-FOLR1-Compound I-6 (151-K-LOCK-D5; ADC-5).

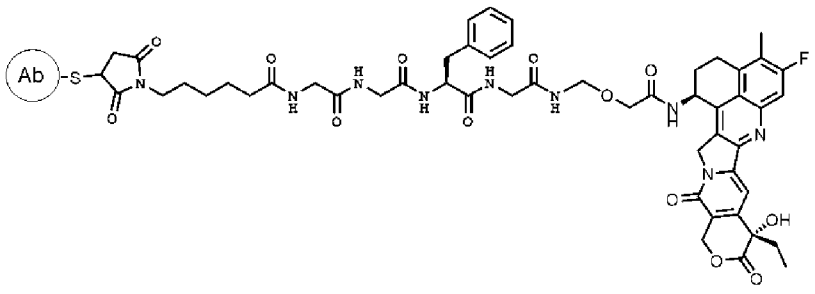
[00368] The ADC anti-FOLR1-Compound **I-6** was prepared as described in US patent No. 10,590,165, which is incorporated herein in its entirety. To a solution of 0.5-50 mgs/mL of antibody in buffer at pH 6.0-9.0 with 0-30% organic solvent, was added 0.1-10 eq of activated drug linker conjugate (I-6) in a manner of portion wise or continuous flow. The reaction was performed at 0-40° C. for 0.5-50 hours with gentle stirring or shaking, monitored by HIC-HPLC. The resultant crude ADC product underwent necessary down-stream steps of desalt, buffer changes/formulation, and optionally, purification, using the state-of-art procedures. The ADC product was characterized by HIC-HPLC, SEC, RP-HPLC, and optionally LC-MS. According to HIC-HPLC analysis, the resulting average DAR for **ADC-5** was 2.

[00369] Example S5: Preparation of Antibody-Drug Conjugate (ADC) anti-FOLR1-GGFG-Dxd (151-GGFG-Dxd).

[00370] Reduction of the antibody: 151 antibody was exchanged with PBS 7.0/EDTA. 7 equivalents TCEP was added to 151 antibody solution, and the reaction mixture was allowed to react for 2 hours at 37°C.

[00371] Conjugation of antibody and linker-payload: the reduced antibody mixture was incubated at 4°C for 10 minutes. The linker-payload GGFG-DXd (developed by Daiichi Sankyo was purchased from DC Chemicals, DC50025) was dissolved in dimethyl acetamide, and 12 equivalents of GGFG-DXd solution was added to the reduced antibody mixture. The reaction was allowed to proceed for 60 minutes at 22 °C.

[00372] The antibody-drug conjugate was desalted, buffet changes/formulation, and purified using state-of-art procedures.



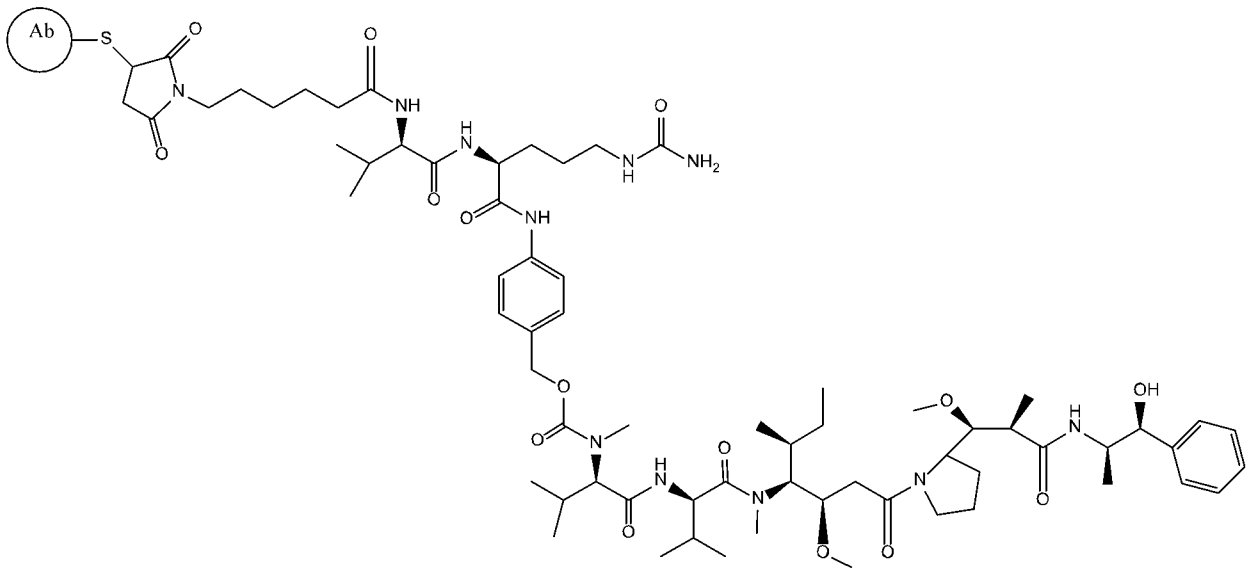
151-GGFG-Dxd.

[00373] Example S6: Preparation of Antibody-Drug Conjugate (ADC) anti-FOLR1-VC-MMAE (151-VC-MMAE).

[00374] Reduction of the antibody: 151 antibody was exchanged with PBS 7.0/EDTA. 2.5 equivalents TCEP was added to 151 antibody solution, and the reaction mixture was allowed to react for 2 hours at 37°C.

[00375] Conjugation of antibody and linker-payload: the reduced antibody mixture was incubated at 4°C for 10 minutes. The linker-payload VC-MMAE (purchased from DC Chemicals, DC7556) was dissolved in dimethyl acetamide, and 7 equivalents of VC-MMAE solution was added to the reduced antibody mixture. The reaction was allowed to proceed for 30 minutes at 22 °C.

[00376] The antibody-drug conjugate was desalted, buffet changes/formulation, and purified using state-of-art procedures.



151-VC-MMAE.

Biological Examples

In vitro and *in vivo* Efficacy of Antibody-Drug Conjugates (ADCs) was assessed using AMT-151 clone of anti-FOLR1 antibody.

Example B1: In vitro Efficacy of Antibody-Drug Conjugates (ADCs) 151-C-Lock-D5 (ADC-1) and 151-K-Lock-D5 (ADC-5).

[00377] The *in vitro* efficacies of ADCs 151-C-Lock-D5 and 151-K-Lock-D5, were evaluated using the following human cancer cell lines: IGROV1, SKOV-3 and A549 (which are ovarian and lung carcinoma cell lines). The cells were cultured in RPMI-1640 medium (Gibco ThermoFisher; Waltham, MA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Corning; Corning, NY, USA) and maintained at 37°C in a 5% CO_2 humidified environment.

[00378] The *in vitro* assays were performed as follows: Tumor cells were harvested by centrifugation at 300*g* for 5 minutes and plated into 96-well clear bottom white-walled plates (2,000 to 3,000 cells/well in 50 µL complete medium) and maintained at 37°C. Cells were then

treated in duplicate with 50 µL of test articles prepared at 2X final concentration that were serially diluted in complete medium and incubated at 37°C for up to 120 hrs. After treatment, inhibition of cancer cell growth was determined using CCK-8 (Cell Counting Kit-8) viability assays as described by the manufacturers' protocol.

[00379] Data were normalized to non-treated controls using Microsoft Excel (Redmond, WA, USA) and analyzed using GraphPad Prism software (version 8; La Jolla, CA, USA). Half-maximal inhibitory concentrations (IC_{50}) were derived from dose response curves.

[00380] The cell viability for 151-C-Lock-D5 and 151-K-Lock-D5 is shown in **FIG. 1**.

[00381] The *in vitro* cytotoxic activities and targeting specificity of the ADCs described herein were evaluated against high FOLR1-expressing IGROV1, low FOLR1-expressing SKOV-3 and FOLR1 negative A549 (negative control) cancer cell lines using standard cell viability assays. As shown in **FIG. 1**, anti-FOLR1 151-C-LOCK-D5 and 151-K-LOCK-D5 (where 151 is AMT-151 clone of FOLR1 antibody) dose-dependently reduced IGROV1 and SKOV-3 cell viability, and did not show activity against A549 cells in 5-day assays. A range in potency as determined by IC50 of ~0.35 to 99 nM against FOLR1-expressing cell lines were observed among the ADCs with different conjugation chemistries to the anti-FOLR1 antibody and in cell lines with different amounts of FOLR1 expression (**Table 5**).

[00382] Summary of IC_{50} Values (nM) of anti-FOLR1 151-C-LOCK-D5 and 151-K-LOCK-D5 in Human Tumor Cells is presented in **Table 5**.

Table 5: IC_{50} Values (nM) of anti-**FOLR1 151-C-LOCK-D5**, **151-K-LOCK-D5** and their corresponding controls in Human Tumor Cells

Test Article	**Cell Line**	
	IGROV1	**SKOV-3**
FOLR1-151-C-LOCK-D5	0.35	45
IgG-C-LOCK-D5	>100	>100
FOLR1-151-K-LOCK-D5	0.55	15
IgG-K-LOCK-D5	>100	NA

[00383] In IGROV1 (high FOLR1 expressing) cell line, 151-C-LOCK-D5 and 151-K-LOCK-D5 exhibited comparable cell killing activity. In SKOV-3 (low FOLR1expressing) cell line, 151-C-LOCK-D5 exhibited a stronger cell killing activity than 151-K-LOCK-D5.

[00384] The control ADCs (IgG-C-LOCK-D5 and IgG-K-LOCK-D5) led to no significant losses in cellular viability as shown in FIG. 1.

[00385] Example B2: In vitro Efficacy of Antibody-Drug Conjugates (ADCs) 151-C-Lock-D5 (ADC-1) and 151-GGFG-Dxd.

[00386] The *in vitro* efficacies of ADCs 151-C-Lock-D5 and 151-K-Lock-D5, were evaluated using SKOV-3 cell line.

[00387] The *in vitro* assays were performed as described above in Example B1. Cell viability was determined after a 5-day incubation period. 151-mAb was used as a negative control in the assay. 151-mAb is the antibody comprising SEQ ID NO: 7 as light chain variable region and SEQ ID NO:8 as heavy chain variable region.

[00388] Results of cell viability assay for 151-C-Lock-D5 and 151-GGFG-Dxd are shown in **FIG. 5**. **FIG. 5** shows that 151-C-Lock-D5 exhibited better cell killing activity than 151-GGFG-Dxd in SKOV-3 cell line. 151-mAb alone did not exhibit losses in cellular viability.

Example B3: In vivo Efficacy of Antibody-Drug Conjugates (ADCs) 151-C-Lock-D5 (ADC-1) and 151-K-Lock-D5 (ADC-5).

[00389] 6-8 weeks female Balb/c mice were purchased from JSJ Laboratory (Shanghai, China).

[00390] Human ovarian cancer tumor cell lines IGROV1 and SKOV-3 were cultured and expanded in RPMI 1640 medium supplemented with 10% FBS at 37°C in a 5% CO_2 humidified environment for a period of 2-3 weeks before harvesting for implantation. Cell viability determined by Trypan blue dye exclusion assay was >90% before implantation. 5-10 million of

IGROV1 or SKOV-3 cells in 100 μl of PBS were inoculated to the right upper flank of each mouse by s.c. injection.

[00391] Tumor volume measurement was started at day 4 after tumor cell inoculation and performed twice weekly. The longest longitudinal diameter as length and the widest transverse diameter as width were measured by using a digital caliper. Tumor volume (TV) was then calculated by the formula: TV = [length x $(width)^2$] / 2 and was analyzed in Excel.

[00392] The treatment was started when average tumor size reaches around 150 mm^3 for IGROV1 and SKOV-3.

[00393] Mice were euthanized when tumor size reached 2000 mm^3.

[00394] After tumor-bearing mice were randomized, 151-C-LOCK-D5, 151-K-LOCK-D5 or isotype ADC IgG-C-LOCK-D5 and IgG-K-LOCK-D5 diluted in PBS were administered to mice through i.p. injections. In experiment I, the treatment regimen included 1.5, 3 or 6 mg/kg of 151-C-LOCK-D5 administered once on day 8 after inoculation with tumor (IGROV1), or 3 or 6 mg/kg of 151-K-LOCK-D5 administered once on day 8 after inoculation with tumor (IGROV1). FIG. 2A shows IGROV1 tumor volume over time; FIG. 2B shows IGROV1 tumor volume on day 46 after inoculation with tumor.

[00395] The body weight of all mice was measured twice weekly after the single ADC dose as shown in FIG. 2C.

[00396] In experiment II, the treatment regimen included 1.5, 3 or 6 mg/kg of 151-C-LOCK-D5 administered biweekly with two doses (Q2W*2), or 3 or 6 mg/kg of 151-K-LOCK-D5 administered biweekly with two doses (Q2W*2), the ADCs were administered on day 15 and day 29 after inoculation with tumor (SKOV-3). FIG. 3A shows SKOV-3 tumor volume over time; FIG. 3B shows SKOV-3 tumor volume on day 49 after inoculation with tumor.

[00397] The body weight of all mice was measured twice weekly after the administration of the first dose of ADC as shown in FIG. 3C.

[00398] Raw data of tumor measurements were analyzed in Excel. Tumor growth curves were plotted using GraphPad Prism 8.0 software and values were presented as mean ± SEM.

[00399] In IGROV1 tumor xenograft model of **Experiment I** (as shown in **FIG. 2A** and **FIG. 2B**), all treatment regimens with anti-FOLR1-151-K-LOCK-D5 (151-K-LOCK-D5; ADC-5) and with anti-FOLR1-151-C-LOCK-D5 (151-C-LOCK-D5; ADC-1) significantly inhibited tumor growth as compared to IgG-C-LOCK-D5 isotype control. All regimens induced dramatic tumor regressions even at low dose and eliminated all tumors about five weeks after initial treatment. Tumor growth inhibition is shown in **Table 6**.

Table 6: Tumor growth inhibition

Tumor Volume	TGI	P value
151-K-LOCK-D5 3 mg/kg	97.13%	0.0008
151-K-LOCK-D5 6 mg/kg	99.60%	0.0007
151-C-LOCK-D5 1.5 mg/kg	95.31%	0.0010
151-C-LOCK-D5 3 mg/kg	100.00%	0.0007
151-C-LOCK-D5 6 mg/kg	100.00%	0.0007
IgG-C-LOCK-D5 6 mg/kg	0.00%	0.0000

[00400] No toxicity was observed with any of the treatment regimens as evidenced by the absence of weight loss (**FIG. 2C**).

[00401] In SKOV-3 tumor xenograft model of **Experiment II** (as shown in **FIG. 3A** and **FIG. 3B**), anti-FOLR1-151-C-LOCK-D5 (151-C-LOCK-D5; ADC-1) showed antitumor activity compared to the negative control (IgG-K-LOCK-D5).

[00402] No toxicity was observed with any of the treatment regimens as evidenced by the absence of significant weight loss (**FIG. 3C**).

Example B4. *In vivo* antitumor effect of 151-K-Lock-D5 (ADC-5) on NSCLC cancer PDX model LU-01-1618

[00403] The anti-tumor efficacy of a single dose of 1.5 mg/kg, 3 mg/kg, 6 mg/kg and 10mg/kg 151-K-Lock-D5 (ADC-5) was evaluated on a FRα positive NSCLC LU-01-1618 PDX tumor (WuXi AppTec (Shanghai) Co., Ltd.) implanted on female BALB/c nude mice. Balb/c nude mice were purchased from Shanghai Lingchang Laboratory Animal Technology Co., LTD.. Tumors of LU-01-1618 were sliced into about ~30 mm^3 fragments and implanted subcutaneously into the flank of mice. On day 18 (mean tumor size ~170 mm^3) following implanting, mice were injected intravenously with one of the following substances: 151-K-Lock-D5 (1.5 mg/kg), 151-K-Lock-D5 (3 mg/kg), 151-K-Lock-D5 (6 mg/kg), 151-K-Lock-D5 (10 mg/kg), isotype ADC (10 mg/kg) and vehicle. Tumor volumes were measured two times per week from day of treatment initiation by Caliper and calculated as follows: $TV=(Width*Length)^2/2$.

[00404] The results of *in vivo* antitumor efficacy of 151-K-Lock-D5 (1.5 mg/kg), 151-K-Lock-D5 (3 mg/kg), 151-K-Lock-D5 (6 mg/kg), 151-K-Lock-D5 (10 mg/kg), isotype ADC (10 mg/kg) and vehicle were shown in **FIG. 4A** and **Table 7.** On day 14 post treatment, when compared with the vehicle group, 151-K-Lock-D5 showed significant dose-dependent anti-tumor activity at doses of 1.5 mg/kg, 3 mg/kg, 6 mg/kg and 10 mg/kg with the TGI of 77.29% (p=0.012), 105.97% (p=0.004), 111.94% (p=0.003), 116.16% (p=0.002), respectively. The significant anti-tumor activity of 3, 6, and 10 mg/kg dosing groups persisted to end of this study (day 28), the TGI were 79.44% (p=0.021), 85.57% (p=0.019) and 93.33% (p=0.010). The AMT-151 at doses of 1.5 mg/kg showed moderate anti-tumor activity (TGI=48.55%, p=0.139) relative to the Vehicle group. No significant weight loss, morbidity or mortality was observed in any group in this study. The weight loss changes of mice were shown in **FIG. 4B**. The arrowhead in the figures indicates the time for dosing.

Table 7: Tumor growth inhibition

Treatment	Tumor Size (mm^3)[a]	T/C (%)	TGI (%)	*p* value
151-K-LOCK-D5, 1.5 mg/kg	311±57	40.49	77.29	0.012
151-K-LOCK-D5, 3 mg/kg	142±11	18.42	105.97	0.004
151-K-LOCK-D5, 6 mg/kg	106±25	13.8	111.94	0.003
151-K-LOCK-D5, 10 mg/kg	81±28	10.55	116.16	0.002
Isotype-ADC, 10 mg/kg	767±198	--	--	--
Vehicle	768±123	--	--	--

Example B5: In vivo Efficacy of Antibody-Drug Conjugates (ADCs) 151-C-Lock-D5 (ADC-1) and 151-VC-MMAE.

[00405] BALB/c Nude mice with established SKOV-3 ovarian tumors were treated once with 2 doses (3mg/kg or 6mg/kg) of 151-C-lock-D5 or 151-VC-MMAE on Day 15 and Day 29 after tumor inoculation. In vivo assays were performed as described above in Example B3.

[00406] **FIG. 6A** showed the effects of treatment on SKOV-3 tumor growth. In SKOV-3 tumor xenograft model, both anti-FOLR1-C-LOCK-D5 (151-C-LOCK-D5; ADC-1) and anti-FOLR1-VC-MMAE (151-VC-MMAE) showed significantly more potent antitumor activity, on day 39, compared to the negative control (IgG-VC-MMAE) at the same dose. 151-C-LOCK-D5 exhibited stronger antitumor activity than 151-VC-MMAE both at the lower dose (3 mg/kg) and higher dose (6 mg/kg).

[00407] No toxicity was observed with any of the treatment regimens as evidenced by the absence of significant weight loss (**FIG. 6B**).

Example B6: In vivo Efficacy of Antibody-Drug Conjugates (ADCs) 151-K-Lock-D5 (ADC-5), bevacizumab, and 151-K-Lock-D5 (ADC-5)+bevacizumab.

[00408] BALB/c Nude mice with established OVCAR-3 ovarian tumors were treated once with a single dose of 151-K-Lock-D5 (5mg/kg administered intravenously), bevacizumab

(5mg/kg administered intraperitoneally), combination of 151-K-Lock-D5 (5mg/kg administered intravenously) and bevacizumab (5mg/kg intraperitoneally), or isotype ADC (IgG-K-LOCK-D5 administered intravenously). In vivo assays were performed as described above in Example B3.

[00409] **FIGS. 7A** and **7B** showed the effects of treatment on OVCAR-3 tumor growth. In OVCAR-3 tumor xenograft model, anti-FOLR1-K-LOCK-D5 (151-K-LOCK-D5; ADC-5) showed significantly more potent antitumor activity, on day 48, compared to the negative control (Isotype ADC; IgG-K-LOCK-D5), whereas bevacizumab treatment alone showed antitumor activity, which was comparable to the negative control, on day 48. Unexpectedly, however, combination treatment with 151-K-LOCK-D5 and bevacizumab, resulted in significantly higher antitumor activity than 151-K-LOCK-D5 or bevacizumab alone, or what would be expected from additive combination of the two treatments, without further increasing the side effect profile of the single agents. The 151-K-LOCK-D5/bevacizumab combination treatment demonstrated synergistic killing and inhibitory effect on ovarian tumor cells.

[00410] No toxicity was observed with any of the treatment regimens as evidenced by the absence of significant weight loss (**FIG. 7C**).

Example B7: Toxicity Study of 151-K-LOCK-D5 in Cynomolgus Monkeys.

[00411] Six Cynomolgus monkeys (purchased from Guangdong Chunsheng Biotechnology Development Co., Ltd) were randomly divided into three groups, with one male and one female per group. 10 mg/kg was administered to group 1 (for each of the 3 administrations). 20 mg/kg was administered to group 2 (for each of the 3 administrations). 30 mg/kg was administered to group 3 (for each of the 3 administrations). On day 1, day 22 and day 43, intravenous infusions of 10 mg/kg, 20 mg/kg or 30 mg/kg of 151-K-LOCK-D5 were administered, respectively. During the experiment, the animals were observed for any abnormalities; Blood samples were collected for hematology and blood biochemical analysis.

[00412] On day 50, the animals were euthanized and sampled for pathological analysis. As shown in **Table 8**, no animal death was observed at any doses used in this experiment. The

relevant target organs of the 151-K-LOCK-D5 were bone marrow, spleen and eyes. HNSTD (maximum dose without severe toxicity) was 30 mg/kg.

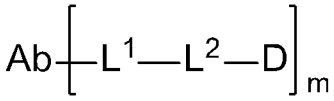
Table 8: Summary of toxicological results following administration of 151-K-LOCK-D5 to Cynomolgus monkeys

	Cynomolgus monkeys
Regimens	10, 20 and 30 mg/kg Intravenous, every 3 weeks for 2 months (thrice in total)
Lethal dose	>30 mg/kg
Body weight	≤30 mg/kg: normal
Hematology	≥10 mg/kg: Anemia
Blood chemistry	≤30 mg/kg: normal
Target organs and tissues	≥10 mg/kg: thymus ≥20 mg/kg: eyes ≥30 mg/kg: bone narrow
HNSTD	**30 mg/kg**

[00413] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated herein in their entirety by reference. To the extent any material incorporated by reference is inconsistent with the express content of this disclosure, the express content controls.

WHAT IS CLAIMED IS:

1. An antibody drug conjugate (ADC) of formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

Ab is an anti-FOLR1 antibody;

m is an integer from 1 to 20;

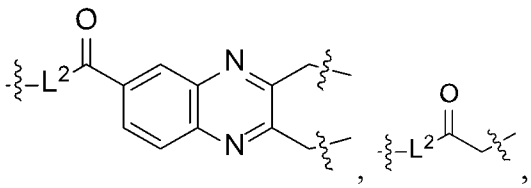
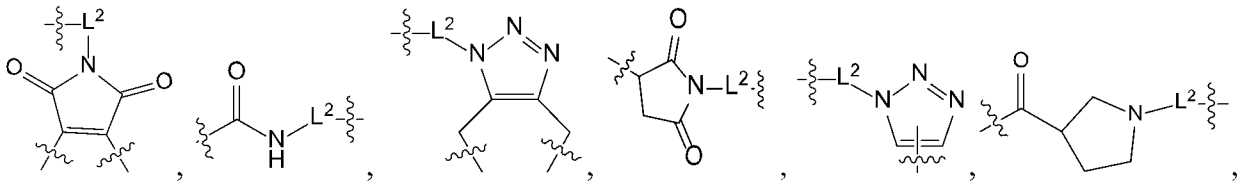
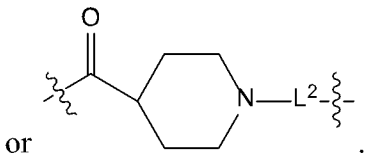
L^1 is a linker bound to the anti-FOLR1 antibody;

L^2 comprises or is a bond, -C(O)-, -NH-, Amino Acid Unit, $-(CH_2CH_2O)_n-$, $-(CH_2)_n-$, –(4-aminobenzyloxycarbonyl)–, $-(C(O)CH_2CH_2NH)-$, or any combination thereof; wherein n is an integer from 1 to 24; and

D is a drug moiety.

2. The ADC of claim 1, wherein L^1 is a linker bound to one or two sulfur or nitrogen atoms of the anti-FOLR1 antibody.

3. The ADC of claim 1 or 2, wherein $-L^1-L^2-$ is:

or .

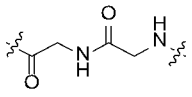
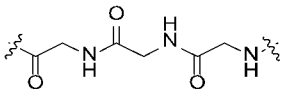
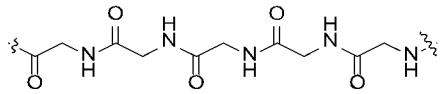
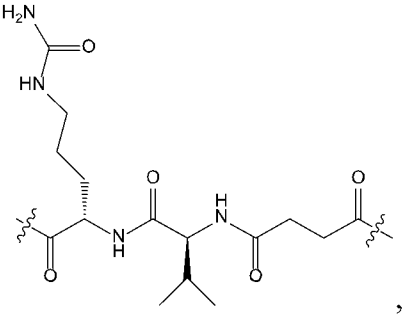
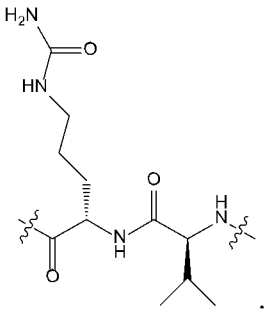
4. The ADC of any one of claims 1-3, wherein m is 1, 2, 3, 4, 5, 6, 7, or 8.

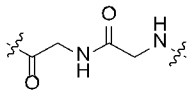
5. The ADC of claim 4, wherein m is from 2 to 4.

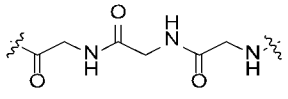
6. The ADC of any one of claims 1-5, wherein L^2 comprises or is a bond, -C(O)-, -NH-, Val, Phe, Lys, –(4-aminobenzyloxycarbonyl)–, Gly, Ser, Thr, Ala, β-Ala, citrulline (Cit), $–(CH_2)_n–$, $–(CH_2CH_2O)_n–$, or any combination thereof.

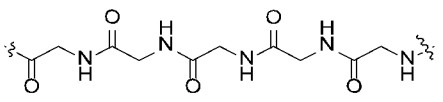
7. The ADC of claim 6, wherein L^2 comprises or is a bond, -C(O)-, -NH-, Val, Gly, Lys, Cit, $–(CH_2)_n–$, $–(CH_2CH_2O)_n–$, or any combination thereof.

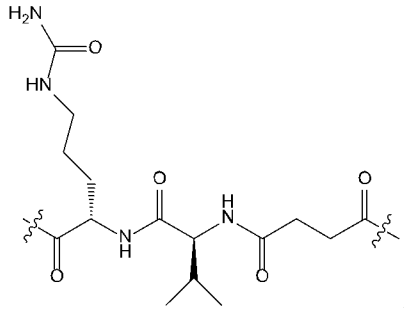
8. The ADC of claim 7, wherein L^2 comprises or is a bond, -C(O)-, Val, Gly, Cit, $–(CH_2)_n–$, or any combination thereof.

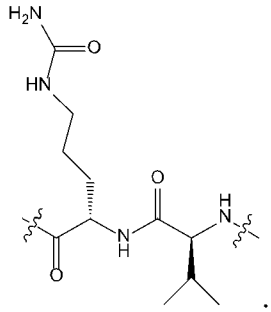
9. The ADC of claim 8, wherein L^2 comprises or is a bond, , , , , or .

10. The ADC of claim 9, wherein L^2 is .

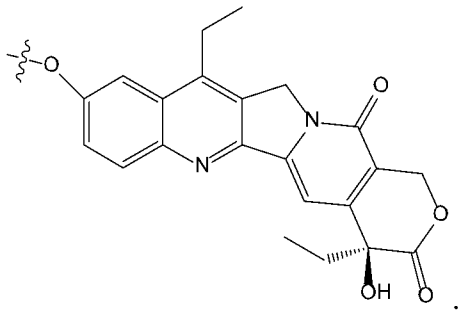
11. The ADC of claim 9, wherein L^2 is .

12. The ADC of claim 9, wherein L^2 is .

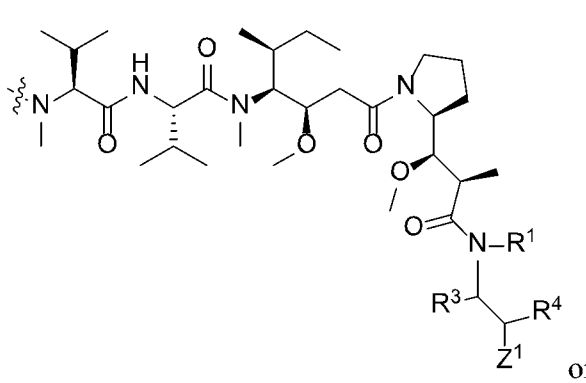
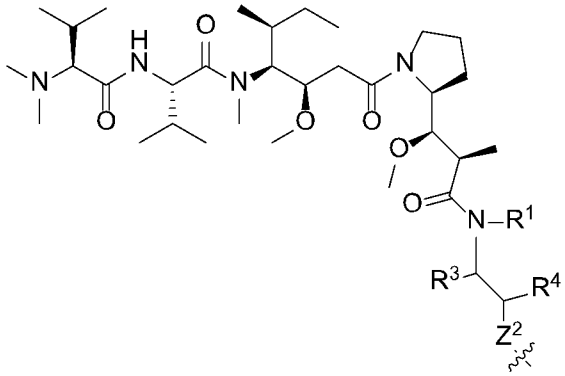
13. The ADC of claim 9, wherein L^2 is .

14. The ADC of claim 9, wherein L^2 is .

15. The ADC of any one of claims 1-14, wherein D is

.

16. The ADC of any one of claims 1-14, wherein D is

or

wherein:

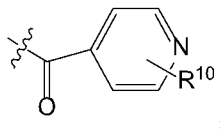
R^1 is H or $-C_1$-C_8 alkyl;

R^3 is H, halogen, -CCl_3, -CBr_3, -CF_3, -CI_3, -$CHCl_2$, -$CHBr_2$, -CHF_2, -CHI_2, -CH_2Cl, -CH_2Br, -CH_2F, -CH_2I, -CN, -OR^{3A}, -$NR^{3A}R^{3B}$, -$(CH_2)_vOR^6$, -$C(O)NHSO_2R^7$, substituted or unsubstituted alkyl, or substituted or unsubstituted heteroalkyl;

R^4 is H, halogen, -OR^{4A}, -$NR^{4A}R^{4B}$, substituted or unsubstituted alkyl, or substituted or unsubstituted heteroalkyl;

Z^1 is a substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, or substituted or unsubstituted heterocycloalkyl;

Z^2 is a substituted aryl, substituted heteroaryl, substituted cycloalkyl, or substituted heterocycloalkyl;

R^6 is H, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, -$CO(CH_2CH_2O)_wCH_2CH_2Y$, -$CONH(CH_2CH_2O)_wCH_2CH_2Y$, , a Charged Group, or a saccharide derivative, wherein

v is an integer from 1 to 24; w is an integer from 1 to 24; Y is -NH_2, -OH, -COOH, or -OCH_3;

R^7 is independently H, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl;

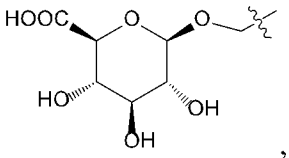
R^{10} is -OH, -OCH_3 or -COOH; and

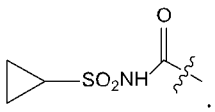
each R^{3A}, R^{3B}, R^{4A}, and R^{4B} is independently H or substituted or unsubstituted alkyl.

17. The ADC of claim 16, wherein R^1 is H.

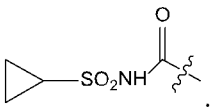
18. The ADC of claims 16 or 17, wherein R^3 is H, $-OR^{3A}$, $-(CH_2)_vOR^6$, $-C(O)NHSO_2R^7$, substituted or unsubstituted alkyl, or substituted or unsubstituted heteroalkyl.

19. The ADC of claim 18, wherein R^3 is H, $-OR^{3A}$, $-(CH_2)_vOR^6$, $-C(O)NHSO_2R^7$, unsubstituted C_1-C_6 alkyl, or substituted C_1-C_6 alkyl.

20. The ADC of claim 18, wherein R^3 is H, methyl, ethyl, propyl, butyl, $-CH_2OH$, $-CH_2CH_2OH$, $-CH_2N_3$, $-CH_2CH_2N_3$, $-CH_2OCH_3$, $-CH_2OCH_2CH_3$, $-CH_2CH_2OCH_3$, $-CH_2CH_2OCH_2CH_3$, 

, or 

.

21. The ADC of claim 20, wherein R^3 is $-CH_2N_3$ or 

.

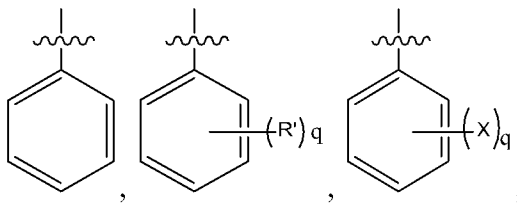
22. The ADC of any one of claims 16-21, wherein R^4 is H, $-OR^{4A}$, substituted or unsubstituted alkyl, or substituted or unsubstituted heteroalkyl.

23. The ADC of claim 22, wherein R^4 is H, -OH, methyl, ethyl, propyl or butyl.

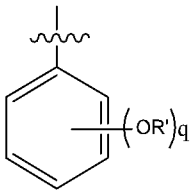
24. The ADC of claim 23, wherein R^4 is H or -OH.

25. The ADC of any one of claims 16-24, wherein Z^1 is a substituted or an unsubstituted aryl.

26. The ADC of any one of claims 16-24, wherein Z^2 is a substituted aryl.

27. The ADC of claim 25, wherein Z^1 is 

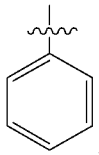
, or

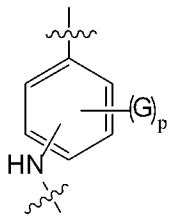
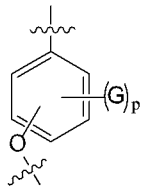

; wherein

each X is independently Cl, Br, I, or F;

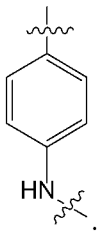
each R' is independently $-CH_3$, $-CH_2CH_3$ or $-CH_2CH_2CH_3$; and

q is an integer from 1 to 5.

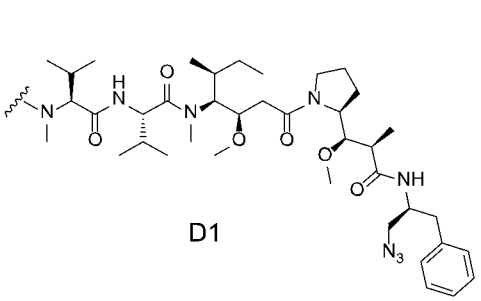
28. The ADC of claim 27, wherein Z^1 is .

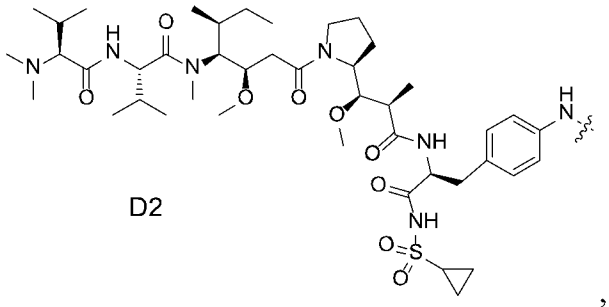
29. The ADC of claim 26, wherein Z^2 is  or  ; wherein

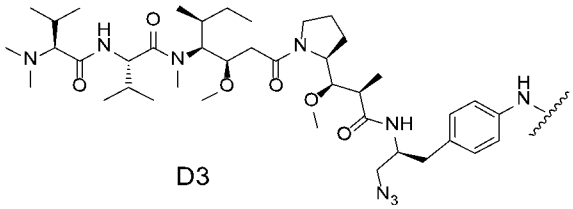
each G is independently Cl, Br, I, F, $-CH_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, $-OCH_3$, $-OCH_2CH_3$, -OH, or $-NH_2$; and p is an integer from 0-4.

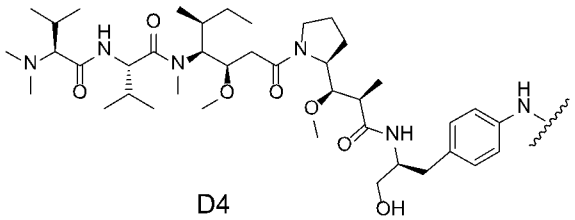
30. The ADC of claim 29, wherein Z^2 is .

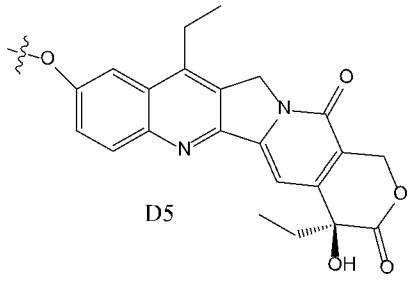
31. The ADC of any one of claims 1-30, wherein D is:

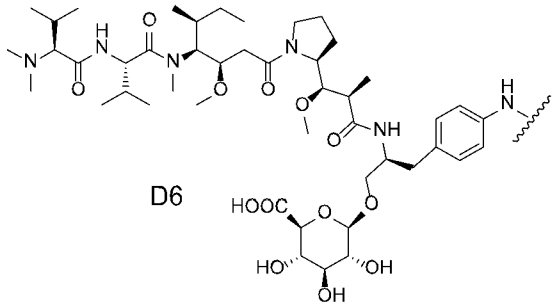

D1 ,


D2 ,

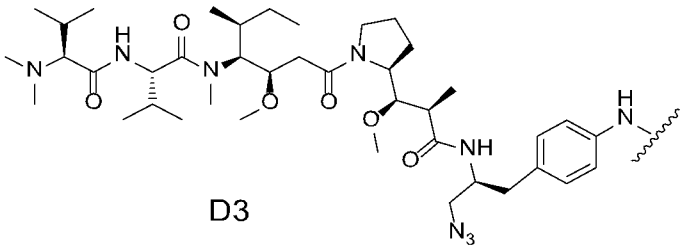

D3 ,


D4 ,

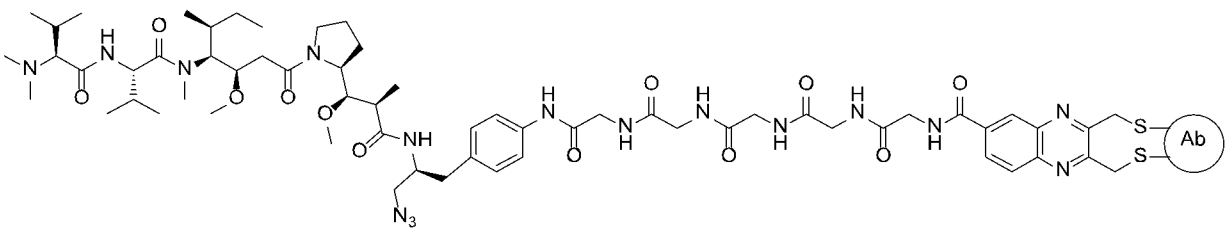

D5 , or


D6 .

32. The ADC of claim 31, wherein D is

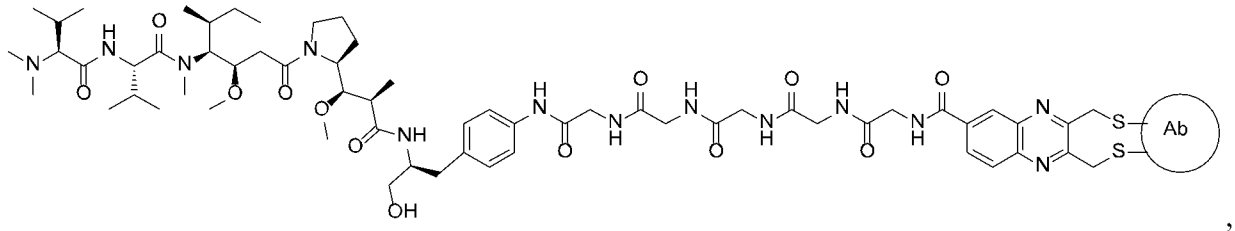

D3 .

33. The ADC of any one of claims 1-32, wherein the ADC is:



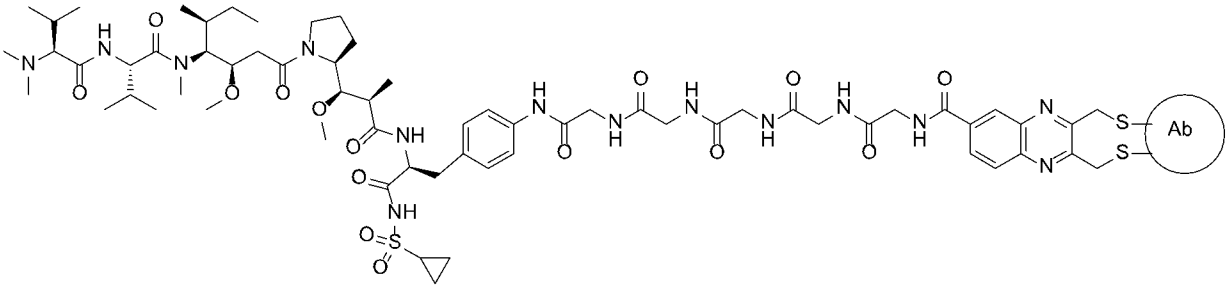
,

ADC-1



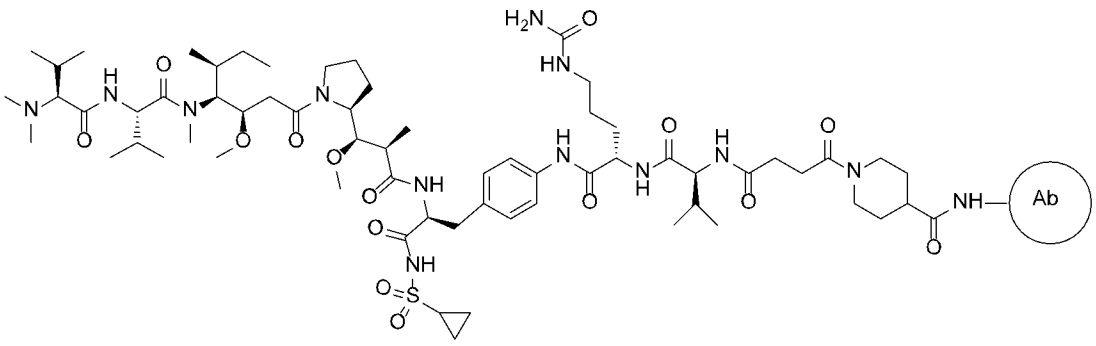
,

ADC-2



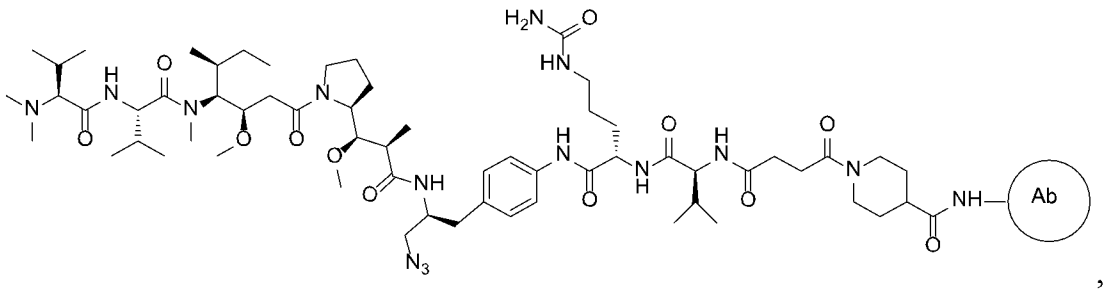
,

ADC-3



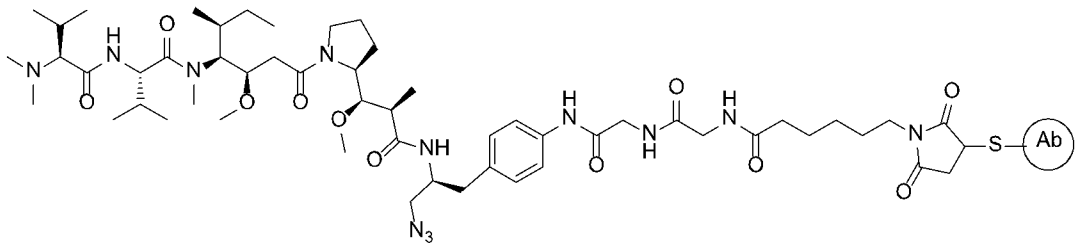
,

ADC-4



,

ADC-5



,

ADC-6

or a pharmaceutically acceptable salt thereof, wherein m is an integer from 1 to 8.

34. The ADC of any one of claims 1-33, wherein the anti-FOLR1 antibody comprises (a) a VL CDR1 comprising the sequence of SEQ ID NO: 1, a VL CDR2 comprising the sequence of SEQ ID NO: 2, a VL CDR3 comprising the sequence of SEQ ID NO: 3, a VH CDR1 comprising the sequence of SEQ ID NO: 4, a VH CDR2 comprising the sequence of SEQ ID NO: 5, and a VH CDR3 comprising the sequence of SEQ ID NO: 6; (b) a VL CDR1 comprising the sequence of SEQ ID NO: 12, a VL CDR2 comprising the sequence of SEQ ID NO: 13, a VL CDR3 comprising the sequence of SEQ ID NO: 14, a VH CDR1 comprising the sequence of SEQ ID NO: 15, a VH CDR2 comprising the sequence of SEQ ID NO: 16, and a VH CDR3 comprising the sequence of SEQ ID NO: 17; or (c) a VL CDR1 comprising the sequence of SEQ ID NO: 12, a VL CDR2 comprising the sequence of SEQ ID NO: 18, a VL CDR3 comprising the sequence of SEQ ID NO: 19, a VH CDR1 comprising the sequence of SEQ ID NO: 20, a VH CDR2 comprising the sequence of SEQ ID NO: 21, and a VH CDR3 comprising the sequence of SEQ ID NO: 22.

35. The ADC of any one of claims 1-34, wherein the anti-FOLR1 antibody comprises a VL having a sequence with at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 7, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29 or SEQ ID NO: 30.

36. The ADC of any one of claims 1-35, wherein the anti-FOLR1 antibody comprises a VH having a sequence with at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 8, , SEQ ID NO: 24, SEQ ID NO: 26 or SEQ ID NO: 28.

37. The ADC of any one of claims 1-36, wherein the anti-FOLR1 antibody comprises a VL having the sequence of SEQ ID NO: 7, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29 or SEQ ID NO: 30..

38. The ADC of any one of claims 1-37, wherein the anti-FOLR1 antibody comprises a VH having the sequence of SEQ ID NO: 8, SEQ ID NO: 24, SEQ ID NO: 26 or SEQ ID NO: 28.

39. The ADC of any one of claims 1-38, wherein the anti-FOLR1 antibody is an IgG antibody, optionally wherein the anti-FOLR1 antibody is an IgG1 antibody.

40. The ADC of any one of claims 1-39, wherein the anti-FOLR1 antibody binds a human FOLR1, optionally wherein the human FOLR1 has the amino acid sequence of SEQ ID NO: 9.

41. The ADC of any one of claims 1-40, for use in therapy.

42. The ADC of claim 41, for use in treating a FOLR1-expressing cancer , optionally wherein the FOLR1-expressing cancer is multiple myeloma, lung cancer, or ovarian cancer.

43. The ADC of claim 42, for use in treating epithelial-derived tumors.

44. A method of treating a FOLR1-expressing cancer in a subject, comprising administering the ADC of any one of claims 1-40 to a subject in need thereof.

45. Use of the ADC of any one of claims 1-40 for the manufacture of a medicament.

46. Use of the ADC of any one of claims 1-40 for the manufacture of a medicament for treating a FOLR1-expressing cancer, optionally wherein the FOLR1-expressing cancer is multiple myeloma, lung cancer, or ovarian cancer.

47. The ADC for use, use, or method of any one of claims 42, 44, or 46, wherein the FOLR1-expressing cancer is multiple myeloma.

48. The ADC for use of claim 43, wherein the epithelial-derived tumors are uterine, breast, endometrial, pancreatic, renal, colorectal, or brain tumors.

49. The ADC for use, use, or method of any one of claims 42, 44, 46, or 47, wherein the FOLR1-expressing cancer is in a mammal, optionally wherein the mammal is a human.

50. The ADC for use, use, or method of any one of claims 41-49, further comprising the administration of a therapeutically effective amount of one or more additional active agents.

51. The ADC for use, use, or method of claim 50, wherein the additional active agent is a VEGF inhibitor.

52. The ADC for use, use, or method of claim 51, wherein the VEGF inhibitor is an anti-VEGF antibody or a small molecule VEGF inhibitor.

53. The ADC for use, use, or method of claim 51 or 52, wherein the VEGF inhibitor is bevacizumab, ramucirumab, sunitinib, sorafenib, axitinib, pazopanib or regorafenib.

54. A method of inhibiting proliferation of a FOLR1-expressing cell, comprising contacting the FOLR1-expressing cell with the ADC of any one of claims 1-40.

55. The use of claim 45, wherein the medicament is for inhibiting proliferation of a FOLR1-expressing cell.

56. The ADC of any one of claims 1-40, for use in inhibiting proliferation of a FOLR1-expressing cell.

57. The method, use, or ADC for use of any one of claims 54-56, wherein the FOLR1-expressing cell is a FOLR1-expressing cancer cell, optionally wherein the FOLR1-expressing cancer is multiple myeloma, lung cancer, or ovarian cancer.

58. The method, use, or ADC for use of any one of claims 54-56, wherein the FOLR1-expressing cell is a FOLR1-expressing multiple myeloma cell.

59. The method of any one of claims 54, 57, or 58, which is in vitro.

60. The method of any one of claims 54, 57, or 58, which is in vivo.

61. A kit for treating a FOLR1-expressing cancer, comprising:

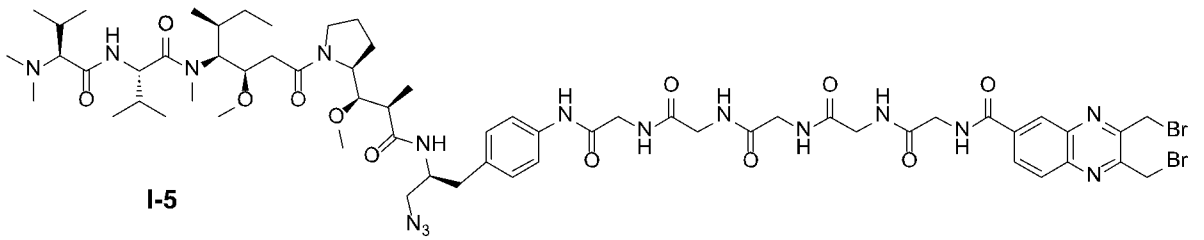
(i) the ADC of any one of claims 1-40; and

(ii) a VEGF inhibitor.

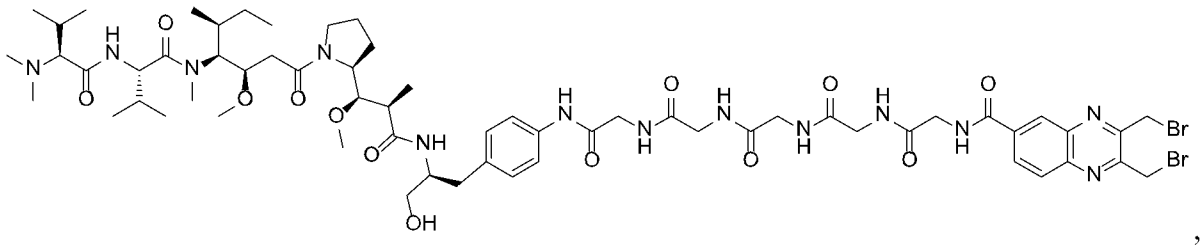
62. The kit of claim 61, wherein the VEGF inhibitor is an anti-VEGF antibody or a small molecule VEGF inhibitor.

63. The kit of claim 61 or 62, wherein the VEGF inhibitor is bevacizumab, ramucirumab, sunitinib, sorafenib, axitinib, pazopanib or regorafenib..

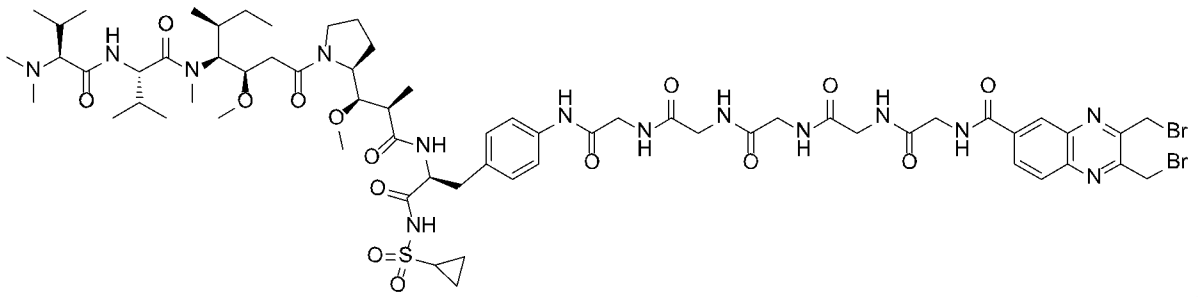
64. A compound selected from:



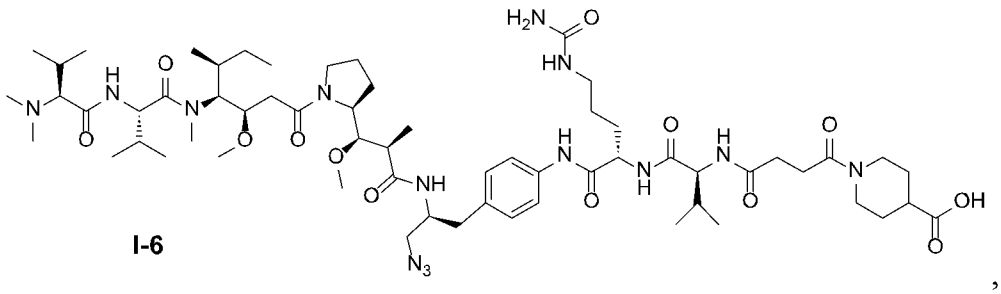
I-5 ,



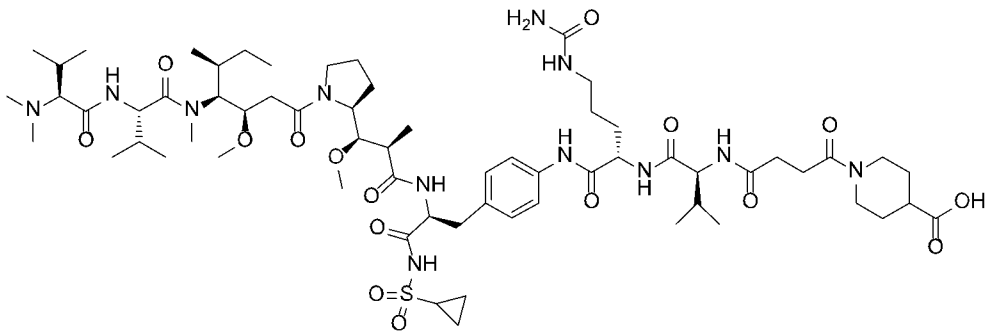
,



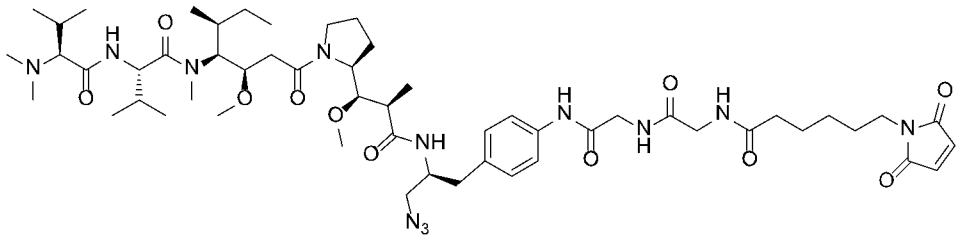
,



I-6 ,



, and



,

or a pharmaceutically acceptable salt thereof.

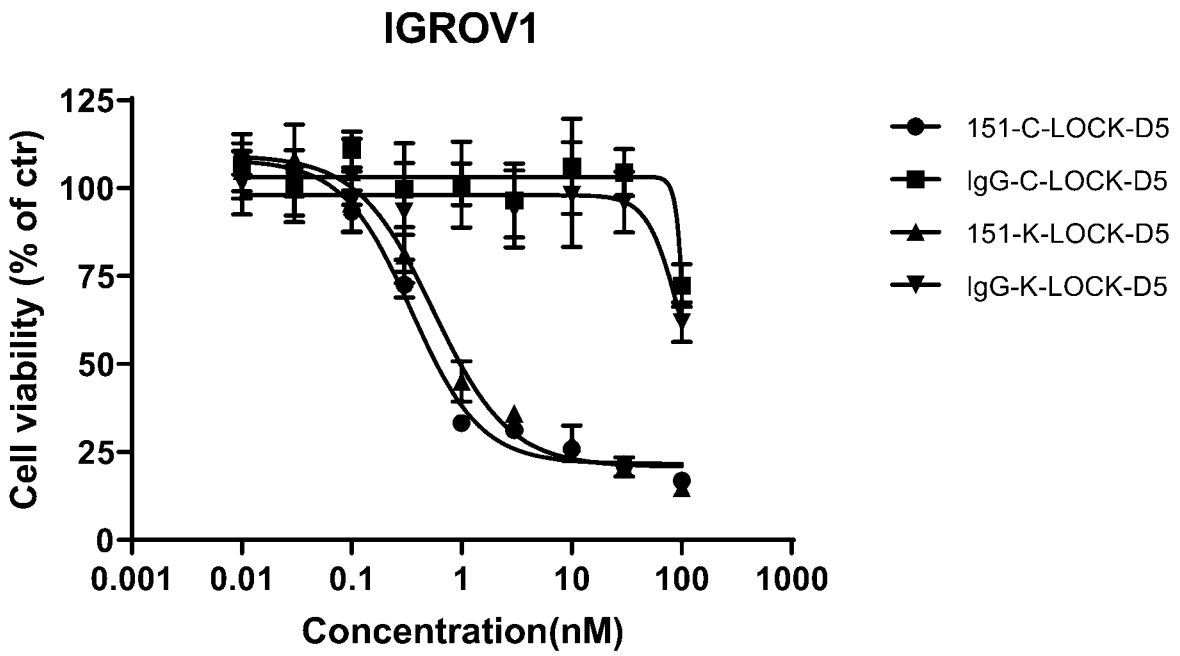

IGROV1
Cell viability (% of ctr)
125
100
75
50
25
0
0.001
0.01
0.1
1
10
100
1000
Concentration(nM)
151-C-LOCK-D5
IgG-C-LOCK-D5
151-K-LOCK-D5
IgG-K-LOCK-D5

FIG. 1A

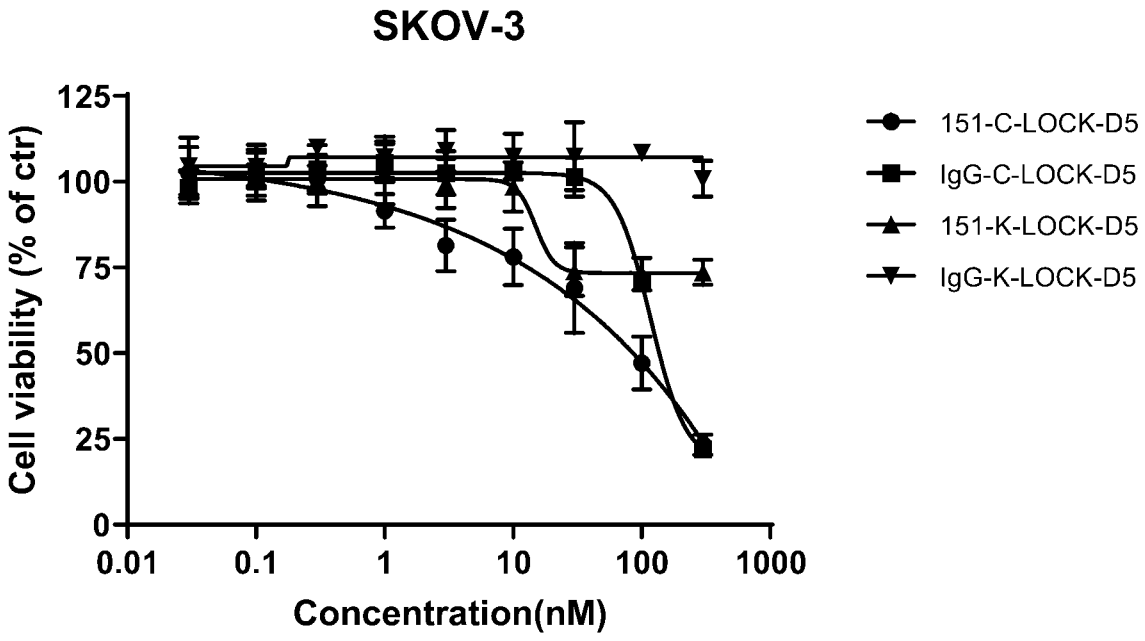

SKOV-3
Cell viability (% of ctr)
125
100
75
50
25
0
0.01
0.1
1
10
100
1000
Concentration(nM)
151-C-LOCK-D5
IgG-C-LOCK-D5
151-K-LOCK-D5
IgG-K-LOCK-D5

FIG. 1B

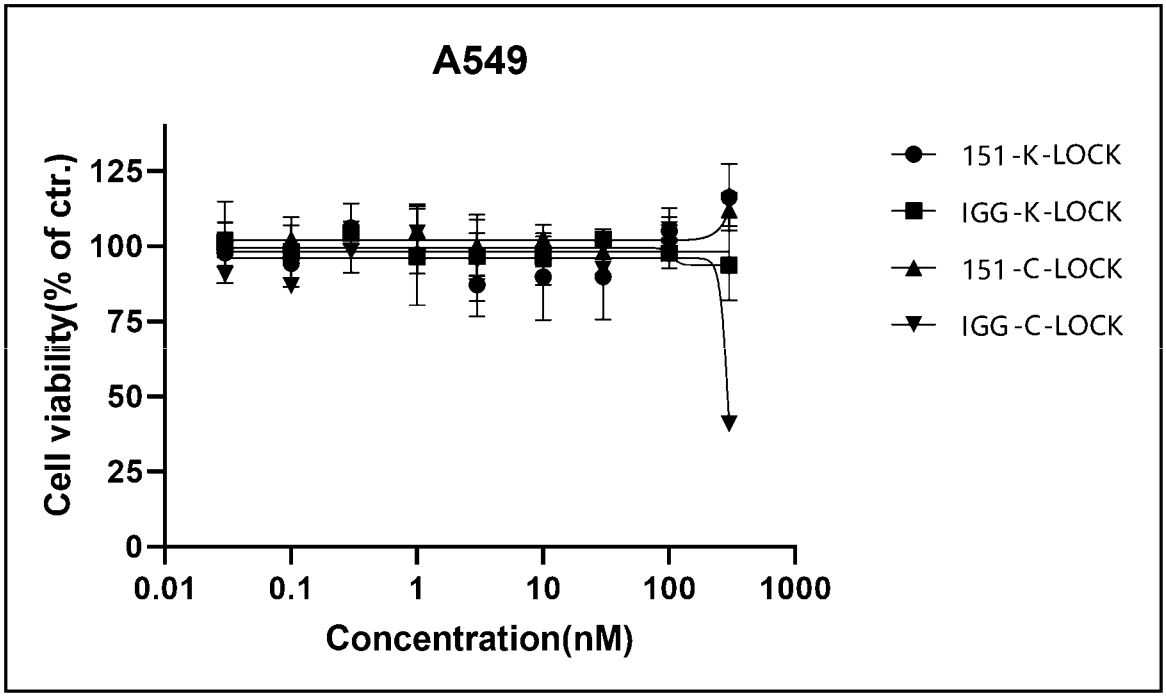

A549
Cell viability(% of ctr.)
125
100
75
50
25
0
0.01
0.1
1
10
100
1000
Concentration(nM)
151-K-LOCK
IGG-K-LOCK
151-C-LOCK
IGG-C-LOCK

FIG. 1C

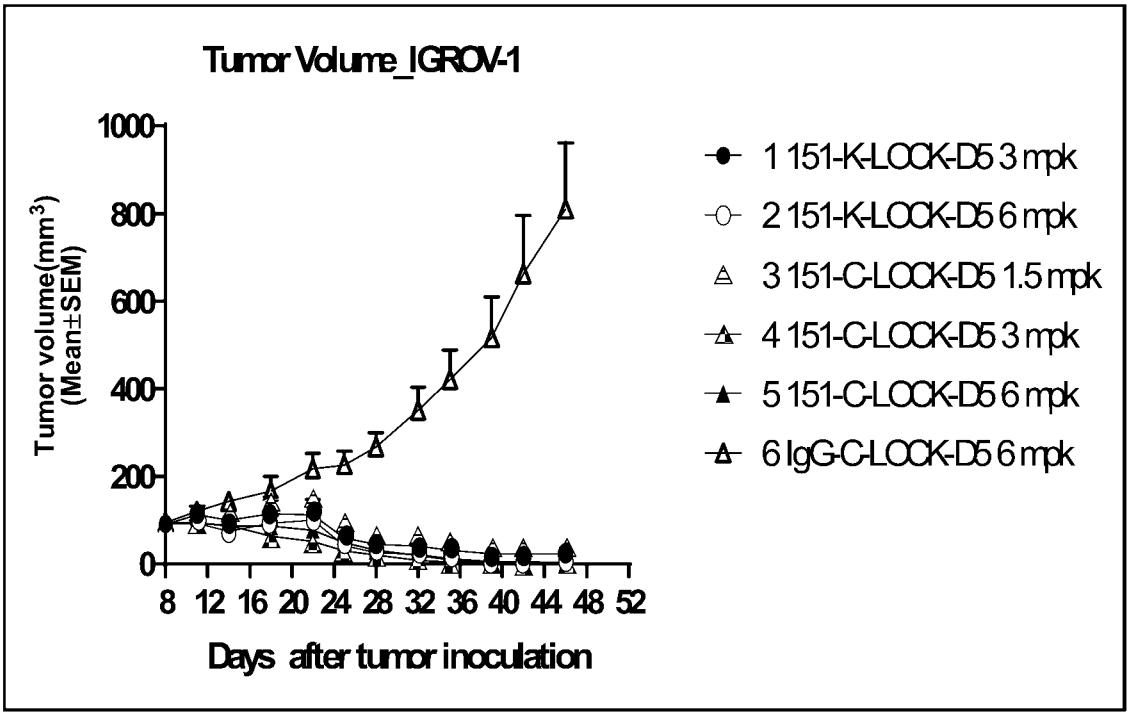

Tumor Volume_IGROV-1
Tumor volume(mm3)
(Mean±SEM)
1000
800
600
400
200
0
8 12 16 20 24 28 32 36 40 44 48 52
Days after tumor inoculation
1 151-K-LOCK-D5 3 mpk
2 151-K-LOCK-D5 6 mpk
3 151-C-LOCK-D5 1.5 mpk
4 151-C-LOCK-D5 3 mpk
5 151-C-LOCK-D5 6 mpk
6 IgG-C-LOCK-D5 6 mpk

FIG. 2A

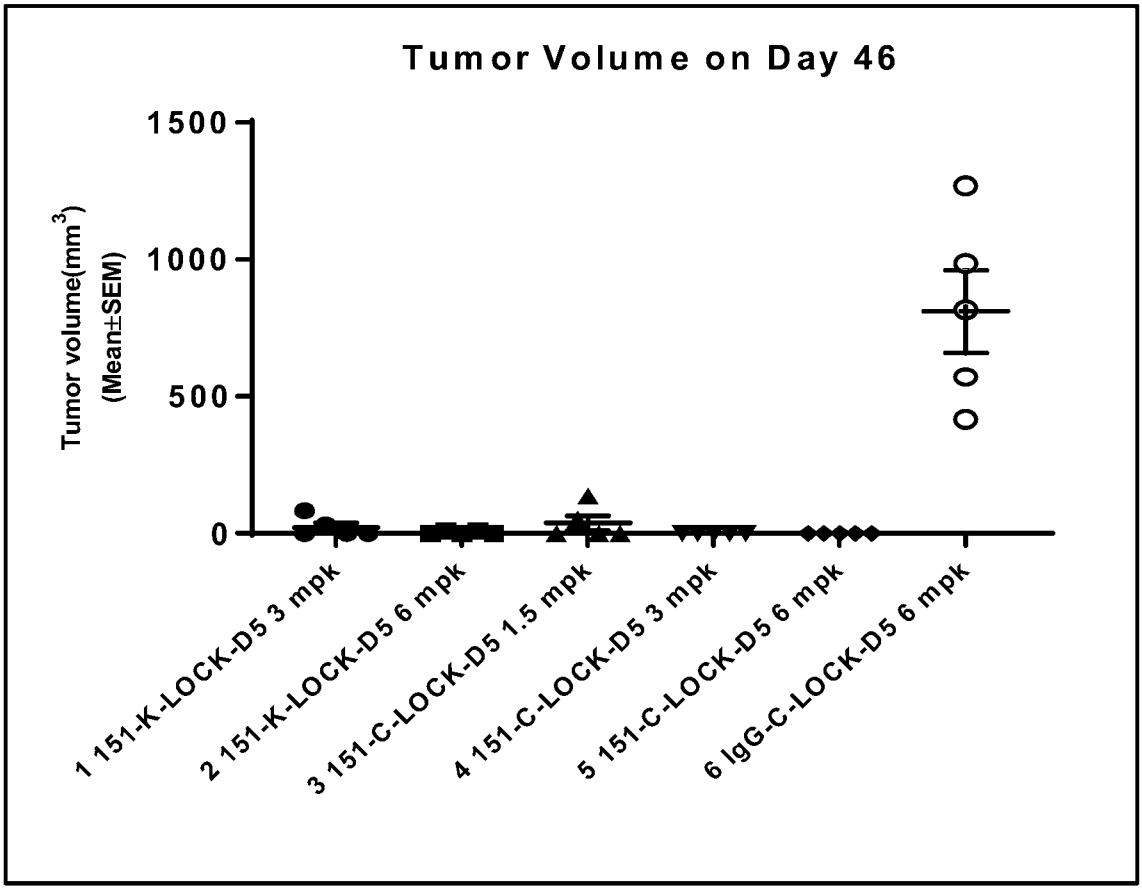

Tumor Volume on Day 46
Tumor volume(mm3)
(Mean±SEM)
1500
1000
500
0
1 151-K-LOCK-D5 3 mpk
2 151-K-LOCK-D5 6 mpk
3 151-C-LOCK-D5 1.5 mpk
4 151-C-LOCK-D5 3 mpk
5 151-C-LOCK-D5 6 mpk
6 IgG-C-LOCK-D5 6 mpk

FIG. 2B

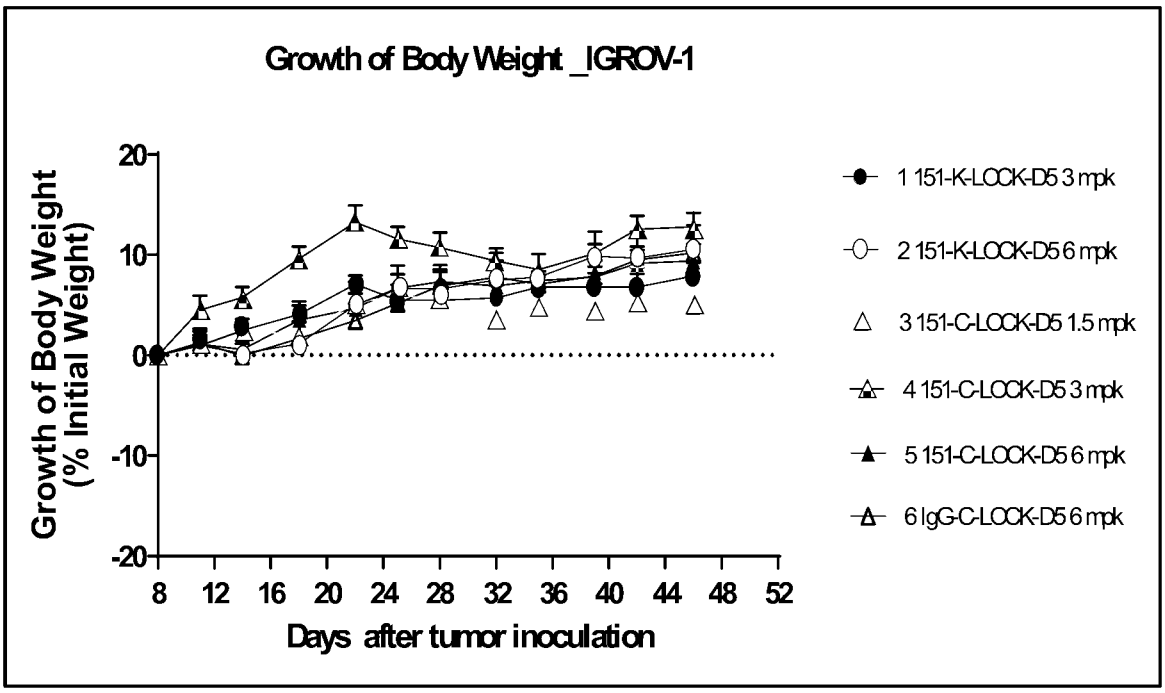

Growth of Body Weight _IGROV-1
Growth of Body Weight
(% Initial Weight)
20
10
0
-10
-20
8
12
16
20
24
28
32
36
40
44
48
52
Days after tumor inoculation
1 151-K-LOCK-D5 3 mpk
2 151-K-LOCK-D5 6 mpk
3 151-C-LOCK-D5 1.5 mpk
4 151-C-LOCK-D5 3 mpk
5 151-C-LOCK-D5 6 mpk
6 IgG-C-LOCK-D5 6 mpk

FIG. 2C

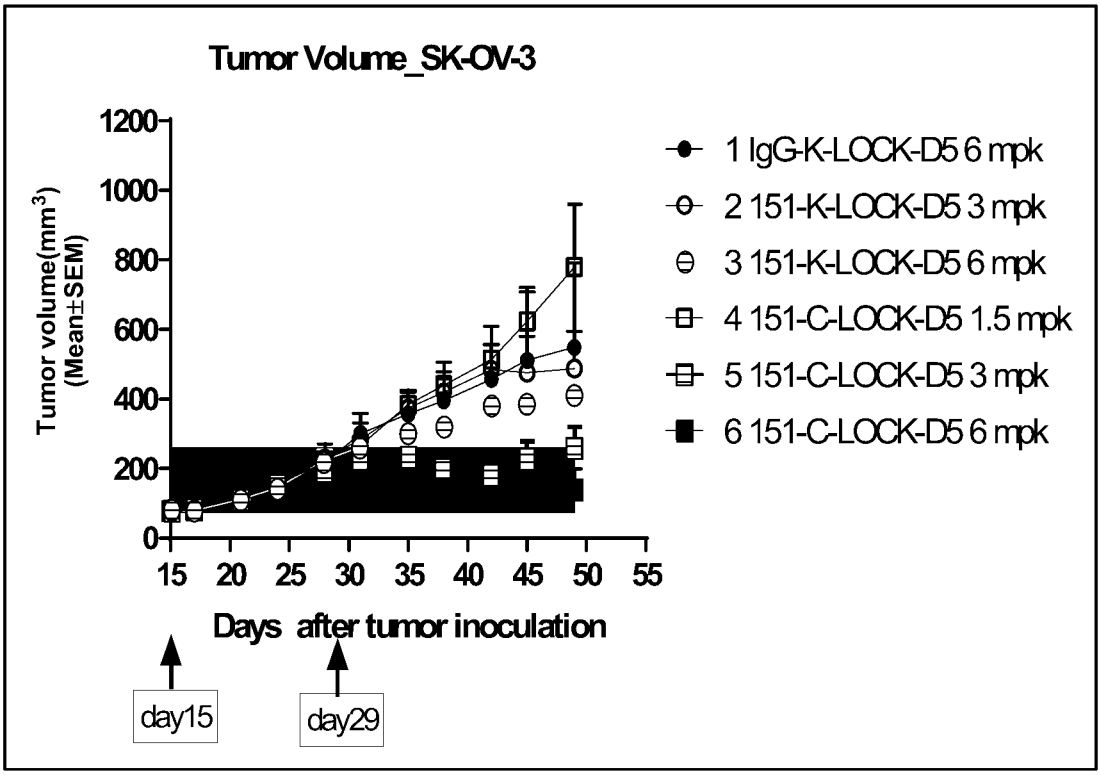

Tumor Volume_SK-OV-3
Tumor volume(mm3)
(Mean±SEM)
1200
1000
800
600
400
200
0
15 20 25 30 35 40 45 50 55
Days after tumor inoculation
day15
day29
1 IgG-K-LOCK-D5 6 mpk
2 151-K-LOCK-D5 3 mpk
3 151-K-LOCK-D5 6 mpk
4 151-C-LOCK-D5 1.5 mpk
5 151-C-LOCK-D5 3 mpk
6 151-C-LOCK-D5 6 mpk

FIG. 3A

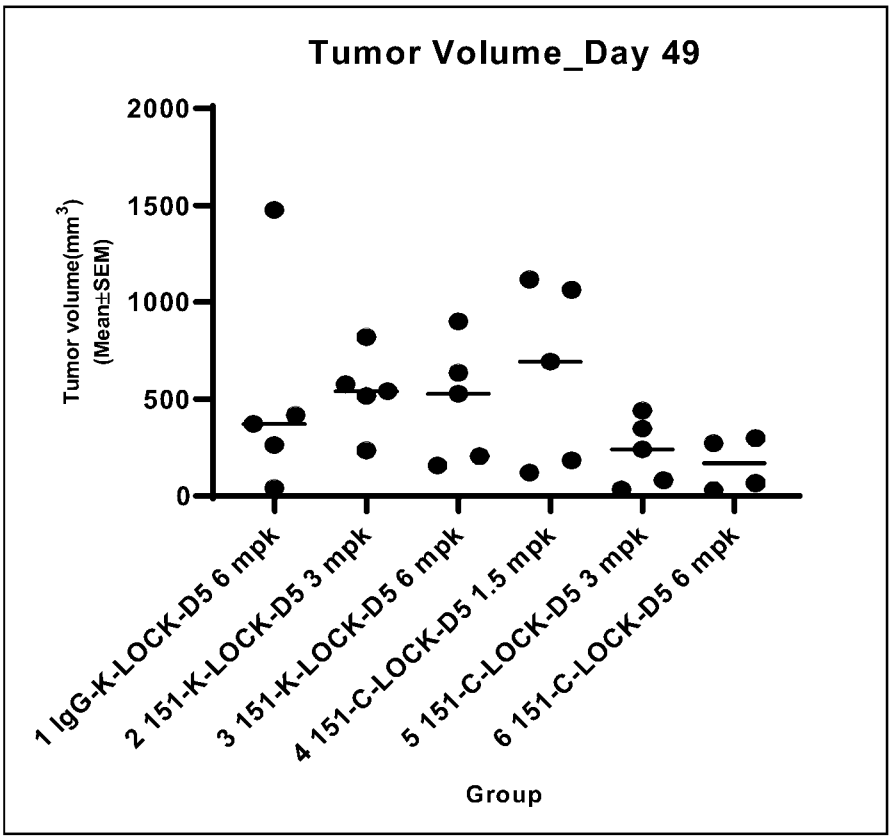

Tumor Volume_Day 49
Tumor volume(mm3)
(Mean±SEM)
2000
1500
1000
500
0
1 IgG-K-LOCK-D5 6 mpk
2 151-K-LOCK-D5 3 mpk
3 151-K-LOCK-D5 6 mpk
4 151-C-LOCK-D5 1.5 mpk
5 151-C-LOCK-D5 3 mpk
6 151-C-LOCK-D5 6 mpk
Group

FIG. 3B

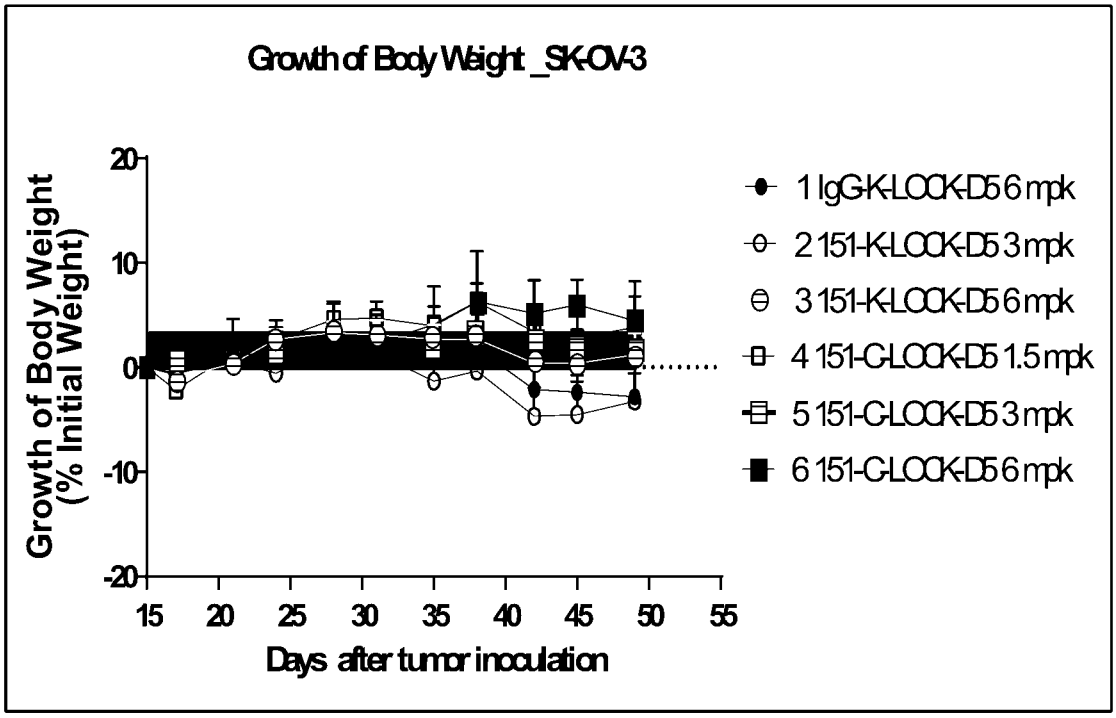

Growth of Body Weight _SK-OV-3
Growth of Body Weight
(% Initial Weight)
20
10
0
-10
-20
15
20
25
30
35
40
45
50
55
Days after tumor inoculation
1 IgG-K-LOCK-D5 6 mpk
2 151-K-LOCK-D5 3 mpk
3 151-K-LOCK-D5 6 mpk
4 151-C-LOCK-D5 1.5 mpk
5 151-C-LOCK-D5 3 mpk
6 151-C-LOCK-D5 6 mpk

FIG. 3C

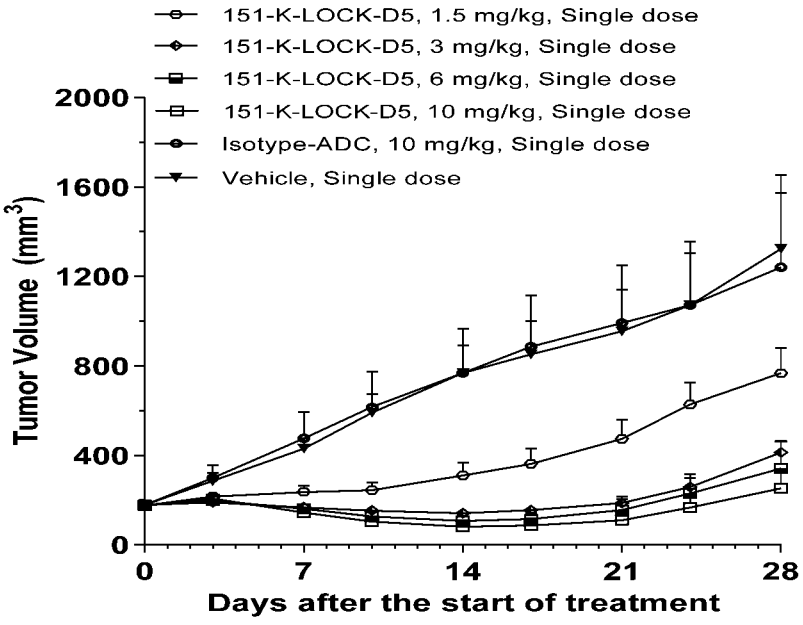

151-K-LOCK-D5, 1.5 mg/kg, Single dose
151-K-LOCK-D5, 3 mg/kg, Single dose
151-K-LOCK-D5, 6 mg/kg, Single dose
151-K-LOCK-D5, 10 mg/kg, Single dose
Isotype-ADC, 10 mg/kg, Single dose
Vehicle, Single dose
Tumor Volume (mm3)
2000
1600
1200
800
400
0
0
7
14
21
28
Days after the start of treatment

FIG. 4A

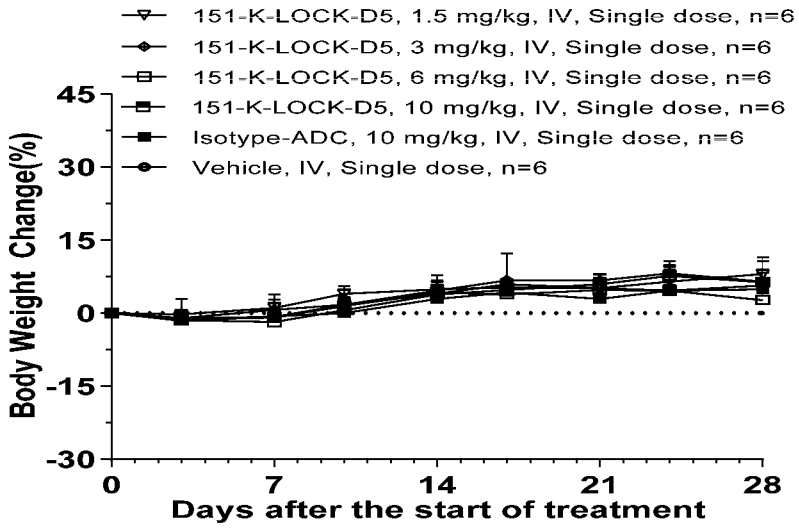

151-K-LOCK-D5, 1.5 mg/kg, IV, Single dose, n=6
151-K-LOCK-D5, 3 mg/kg, IV, Single dose, n=6
151-K-LOCK-D5, 6 mg/kg, IV, Single dose, n=6
151-K-LOCK-D5, 10 mg/kg, IV, Single dose, n=6
Isotype-ADC, 10 mg/kg, IV, Single dose, n=6
Vehicle, IV, Single dose, n=6
Body Weight Change(%)
45
30
15
0
-15
-30
0
7
14
21
28
Days after the start of treatment

FIG. 4B

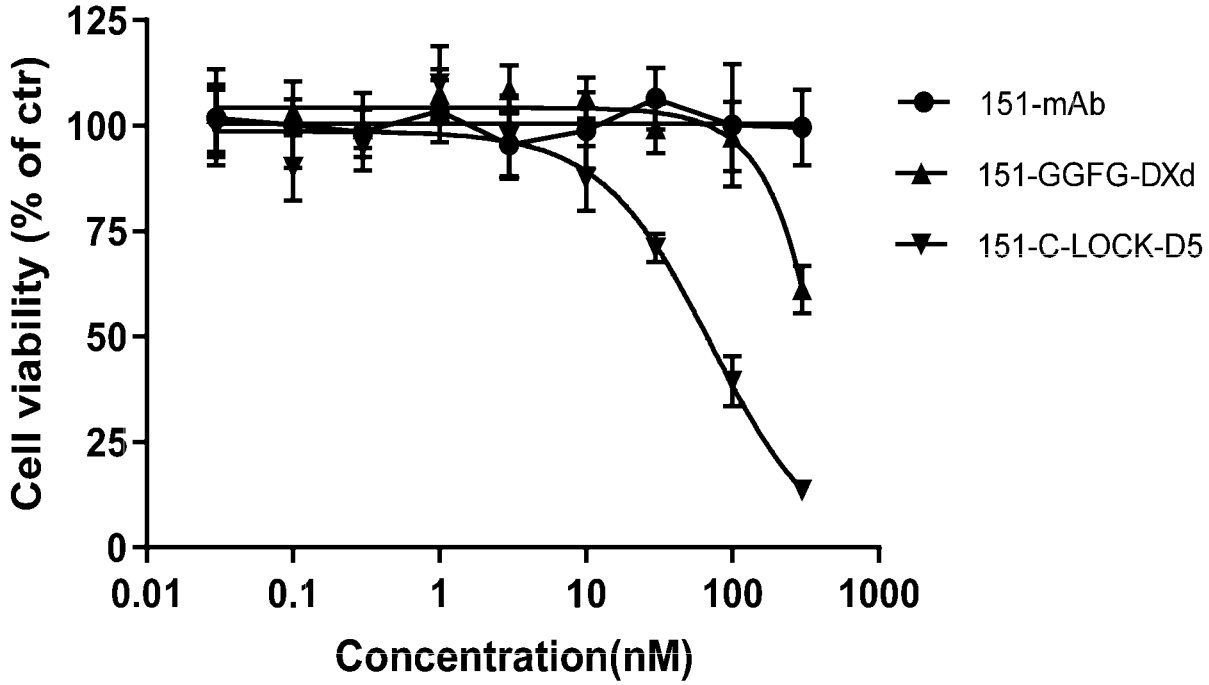

Cell viability (% of ctr)
125
100
75
50
25
0
0.01
0.1
1
10
100
1000
Concentration(nM)
151-mAb
151-GGFG-DXd
151-C-LOCK-D5

FIG. 5

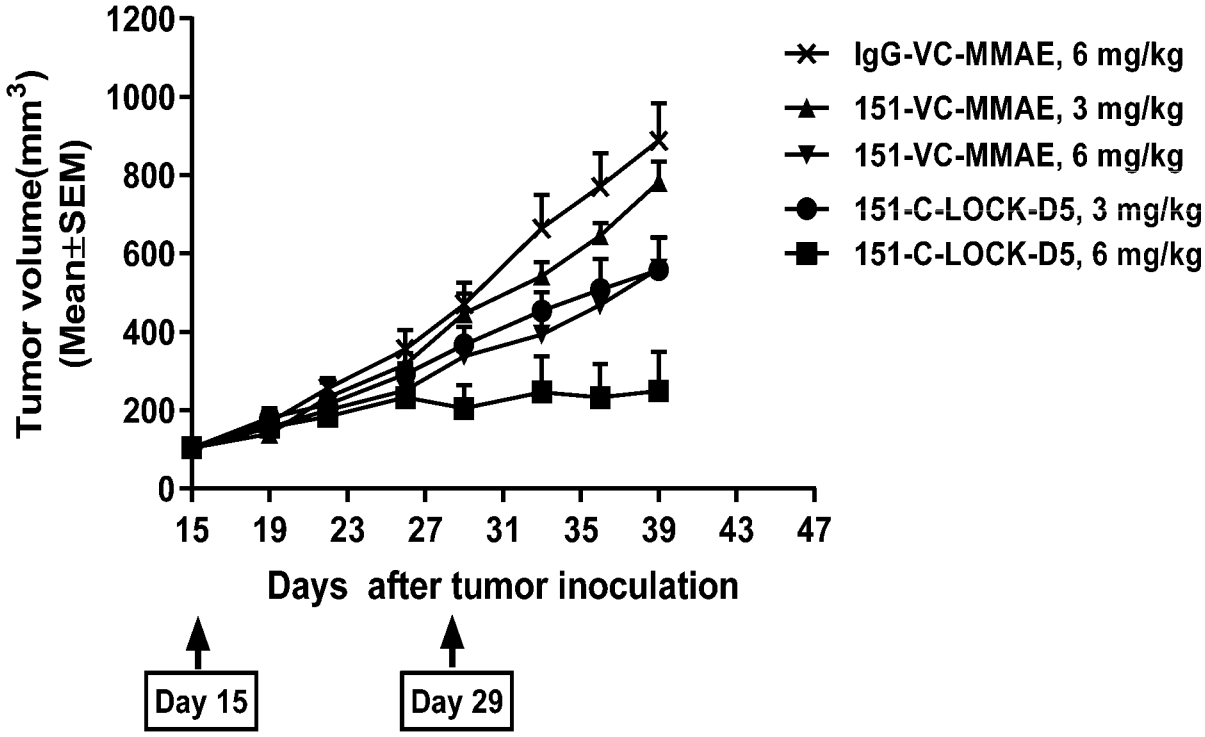

Tumor volume(mm3)
(Mean±SEM)
1200
1000
800
600
400
200
0
15 19 23 27 31 35 39 43 47
Days after tumor inoculation
Day 15
Day 29
IgG-VC-MMAE, 6 mg/kg
151-VC-MMAE, 3 mg/kg
151-VC-MMAE, 6 mg/kg
151-C-LOCK-D5, 3 mg/kg
151-C-LOCK-D5, 6 mg/kg

FIG. 6A

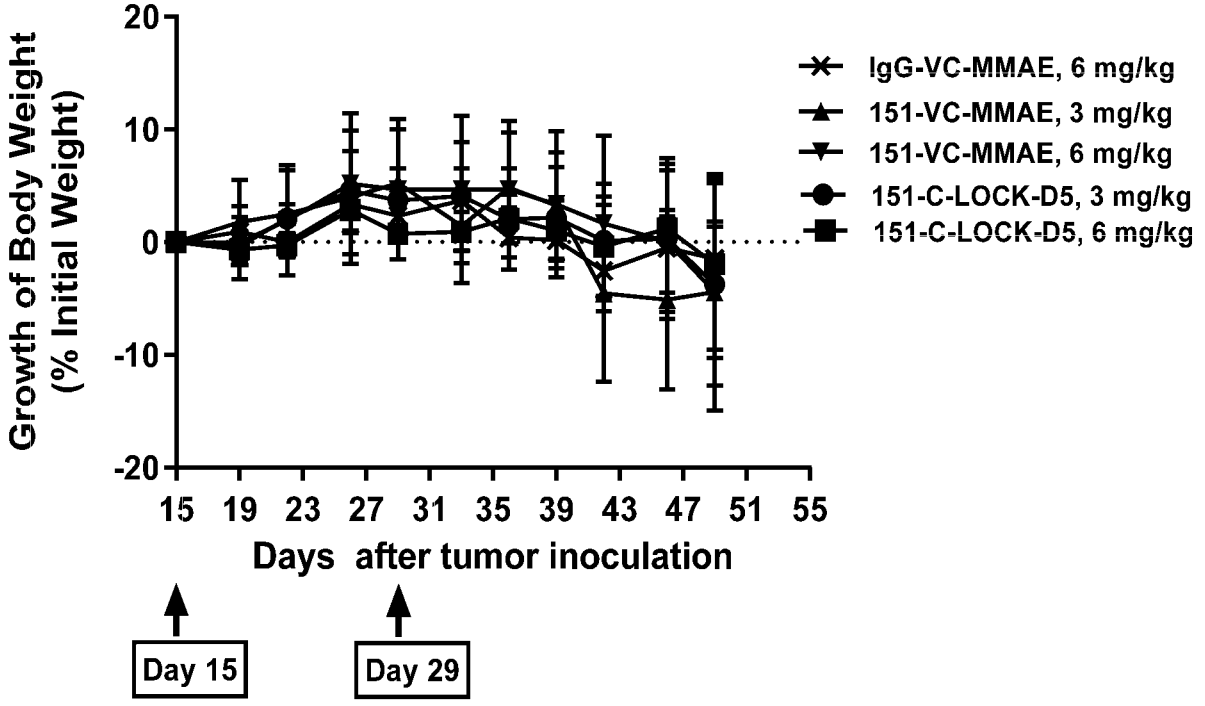

Growth of Body Weight
(% Initial Weight)
20
10
0
-10
-20
15 19 23 27 31 35 39 43 47 51 55
Days after tumor inoculation
Day 15
Day 29
IgG-VC-MMAE, 6 mg/kg
151-VC-MMAE, 3 mg/kg
151-VC-MMAE, 6 mg/kg
151-C-LOCK-D5, 3 mg/kg
151-C-LOCK-D5, 6 mg/kg

FIG. 6B

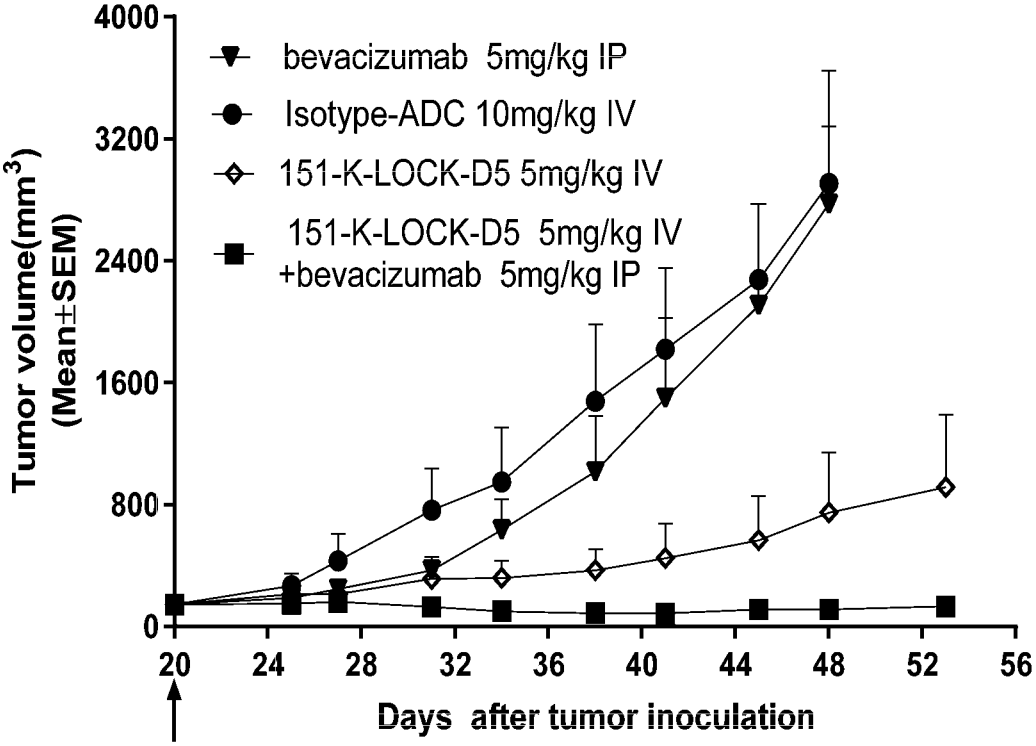

4000
3200
2400
1600
800
0
Tumor volume(mm3)
(Mean±SEM)
bevacizumab 5mg/kg IP
Isotype-ADC 10mg/kg IV
151-K-LOCK-D5 5mg/kg IV
151-K-LOCK-D5 5mg/kg IV
+bevacizumab 5mg/kg IP
20
24
28
32
36
40
44
48
52
56
Days after tumor inoculation

FIG. 7A

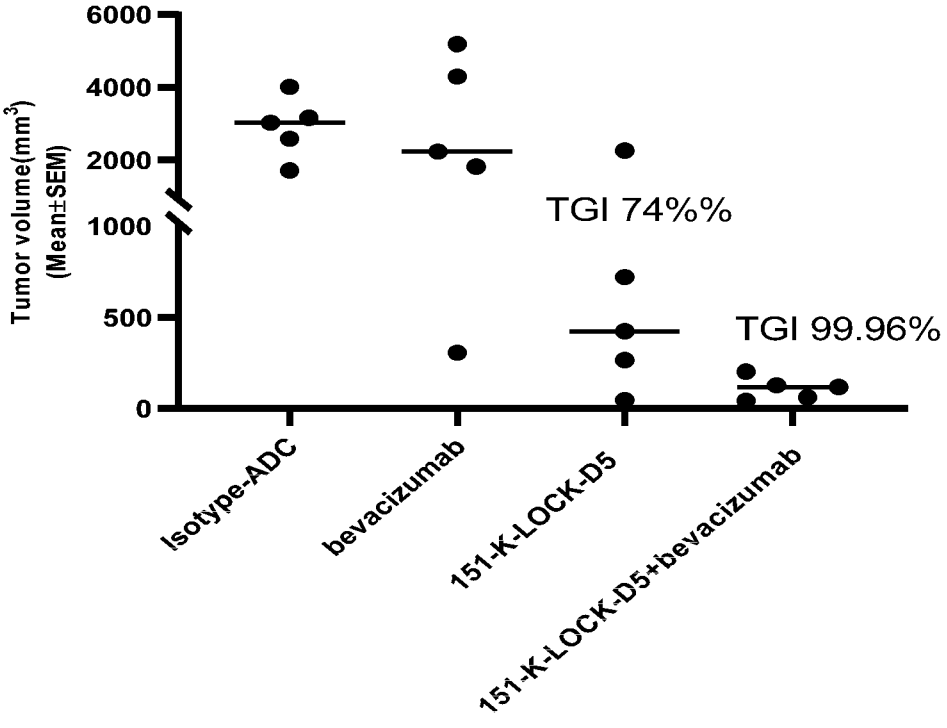

6000
4000
2000
1000
500
0
Tumor volume(mm3)
(Mean±SEM)
TGI 74%%
TGI 99.96%
Isotype-ADC
bevacizumab
151-K-LOCK-D5
151-K-LOCK-D5+bevacizumab

FIG. 7B

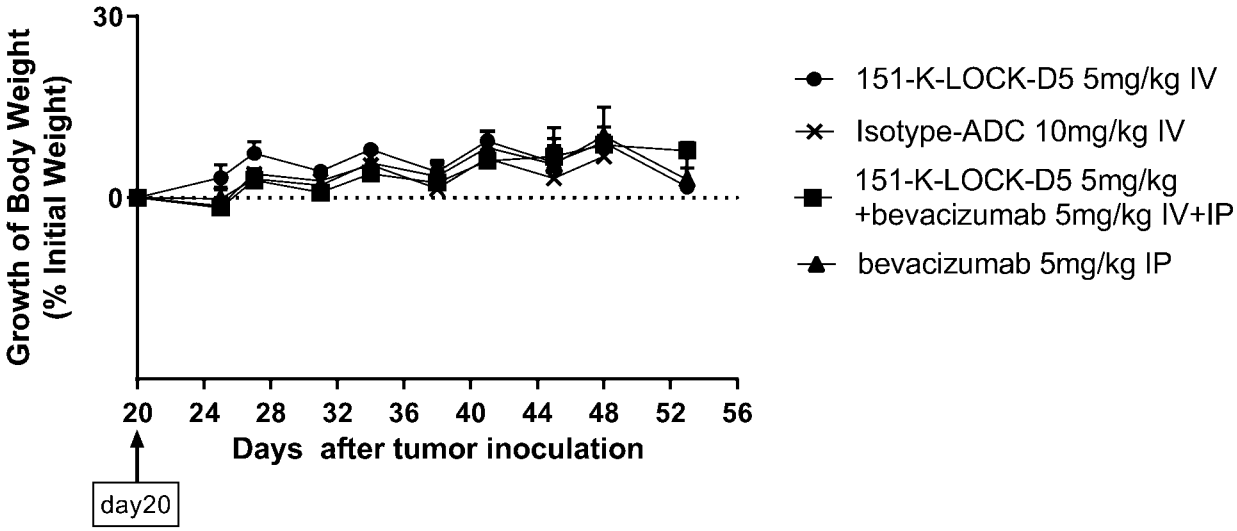

30
0
Growth of Body Weight
(% Initial Weight)
20
24
28
32
36
40
44
48
52
56
Days after tumor inoculation
day20
151-K-LOCK-D5 5mg/kg IV
Isotype-ADC 10mg/kg IV
151-K-LOCK-D5 5mg/kg
+bevacizumab 5mg/kg IV+IP
bevacizumab 5mg/kg IP

FIG. 7C