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(54) Title: ANTI-FOLR1 IMMUNOCONJUGATE DOSING REGIMENS

(57) Abstract: Methods of administering immunoconjugates that bind to FOLR1 are provided. The methods comprise administering an anti-FOLR1 immunoconjugate to a person in need thereof, for example, a cancer patient, at a therapeutically effective dosing regimen that results in minimal adverse effects.



WO 2014/186403 A2

ANTI-FOLR1 IMMUNOCONJUGATE DOSING REGIMENS

Field of the Invention

[0001] The field of the invention generally relates to methods of administering anti-FOLR1 immunoconjugates for the treatment of diseases, such as cancer. The methods provide dosing regimens that minimize unwanted side-effects.

Background of the Invention

[0002] Cancer is one of the leading causes of death in the developed world, with over one million people diagnosed with cancer and 500,000 deaths per year in the United States alone. Overall it is estimated that more than 1 in 3 people will develop some form of cancer during their lifetime. There are more than 200 different types of cancer, four of which—breast, lung, colorectal, and prostate—account for over half of all new cases (Jemal et al., 2003, *Cancer J. Clin.* 53:5-26).

[0003] Folate Receptor 1 (FOLR1), also known as Folate Receptor-alpha, or Folate Binding Protein, is an N-glycosylated protein expressed on plasma membrane of cells. FOLR1 has a high affinity for folic acid and for several reduced folic acid derivatives. FOLR1 mediates delivery of the physiological folate, 5-methyltetrahydrofolate, to the interior of cells.

[0004] FOLR1 is overexpressed in vast majority of ovarian cancers, as well as in many uterine, endometrial, pancreatic, renal, lung, and breast cancers, while the 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 SD, *et al.*, *Cancer Res* 52: 3396-3401 (1992); Antony AC, *Annu Rev Nutr* 16: 501-521 (1996); Kalli KR, *et al. Gynecol Oncol* 108: 619-626 (2008)). This expression pattern of FOLR1 makes it a desirable target for FOLR1-directed cancer therapy.

[0005] Because ovarian cancer is typically asymptomatic until advanced stage, it is often diagnosed at a late stage and has poor prognosis when treated with currently available procedures, typically chemotherapeutic drugs after surgical de-bulking (von Gruenigen V *et al.*, *Cancer* 112: 2221-2227 (2008); Ayhan A *et al.*, *Am J Obstet Gynecol* 196: 81 e81-86 (2007); Harry VN *et al.*, *Obstet Gynecol Surv* 64: 548-560 (2009)). Thus there is a clear unmet medical need for more effective therapeutics for ovarian cancers.

[0006] Antibodies are emerging as a promising method to treat such cancers. In addition, immunoconjugates, which comprise an antibody conjugated to another compound, for example, a cytotoxin, are also being investigated as potential therapeutics. In particular, immunoconjugates comprising maytansinoids, which are plant derived anti-fungal and anti-tumor agents, have been shown to have some beneficial activities. The isolation of three ansa macrolides from ethanolic extracts of

Maytenus ovatus and *Maytenus buchananii* was first reported by S. M. Kupchan *et al.* and is the subject of U.S. Pat. No. 3,896,111 along with demonstration of their anti-leukemic effects in murine models at the microgram/kg dose range. Maytansinoids, however, have unacceptable toxicity, causing both central and peripheral neuropathies, and side effects: particularly nausea, vomiting, diarrhea, elevations of hepatic function tests and, less commonly, weakness and lethargy. This overall toxicity is reduced to some extent by the conjugation of maytansinoids to antibodies because an antibody conjugate has a toxicity which is several orders of magnitude lower on antigen-negative cells compared to antigen-positive cells. However, immunoconjugates comprising maytansinoids have still been associated with unacceptable levels of adverse side effects. For example, animals injected with high dosages of anti-FOLR1 immunoconjugates comprising a maytansinoid showed ocular toxicity. The cause of this toxicity, for example, whether it could be related to Cmax or AUC was not known. As a result, there is still a need to identify particular dosage regimens of anti-FOLR1 immunoconjugates that are therapeutically effective in humans but avoid adverse effects.

BRIEF SUMMARY OF THE INVENTION

[0007] Methods of administering an anti-FOLR1 immunoconjugate at a therapeutically effective dosing regimen that minimizes unwanted side-effects are provided herein. Thus, described herein are methods for treating a patient having cancer comprising administering to the patient an effective dose of an immunoconjugate which binds to FOLR1, wherein the immunoconjugate is administered at a dose of about 3.0 mg/kg to about 6 mg/kg. The anti-FOLR1 immunoconjugate can comprise a charged linker. In some embodiments, the anti-FOLR1 immunoconjugate comprises the antibody huMov19, the linker sulfo-SPDB, and the maytansinoid DM4.

[0008] In some embodiments, the immunoconjugate comprises an antibody or antigen-binding fragment thereof that competitively inhibits the binding of an antibody with the sequences of SEQ ID NO:3 and SEQ ID NO:5 to FOLR1. In some embodiments, the antibody or fragment thereof comprises the CDRs of huMov19 (i.e., SEQ ID NOs: 6-10 and 12 or SEQ ID NOs: 6-9, 11, and 12). In some embodiments, the antibodies or fragments do not comprise the six CDRs of murine Mov19 (i.e., SEQ ID NOs:6-9, 16, and 12). In some embodiments, the antibody is huMov19. In some embodiments, the immunoconjugate comprises a maytansinoid. In some embodiments, the maytansinoid is DM4. In some embodiments, the immunoconjugate comprises a linker that is sulfo-SPDB. In some embodiments, the immunoconjugate is IMGN853 (huMov19-sulfo-SPDB-DM4).

[0009] In some embodiments, the anti-FOLR1 binding agent (e.g., huMov19-sulfo-SPDB-DM4) is administered at a dose of about 3.0 mg/kg. In some embodiments, the anti-FOLR1 binding agent (e.g., huMov19-sulfo-SPDB-DM4) is administered at a dose of about 3.3 mg/kg. In some embodiments, the anti-FOLR1 binding agent (e.g., huMov19-sulfo-SPDB-DM4) is administered at a dose of about 4.0

mg/kg. In some embodiments, the anti-FOLR1 binding agent (e.g., huMov19-sulfo-SPDB-DM4) is administered at a dose of about 5 mg/kg. In some embodiments, the anti-FOLR1 binding agent (e.g., huMov19-sulfo-SPDB-DM4) is administered at a dose of about 5.5 mg/kg. In some embodiments, the anti-FOLR1 binding agent (e.g., huMov19-sulfo-SPDB-DM4) is administered at a dose of about 6 mg/kg.

[0010] According to the methods described herein, the anti-FOLR1 binding agent (e.g., huMov19-sulfo-SPDB-DM4) can be administered about once every 4 weeks. In some embodiments, the anti-FOLR1 binding agent (e.g., huMov19-sulfo-SPDB-DM4) is administered about once every 3 weeks. In some embodiments, the anti-FOLR1 binding agent (e.g., huMov19-sulfo-SPDB-DM4) is administered about once every 2 weeks. In some embodiments, the anti-FOLR1 binding agent (e.g., huMov19-sulfo-SPDB-DM4) is administered about once every 1 week. In some embodiments, the anti-FOLR1 binding agent (e.g., huMov19-sulfo-SPDB-DM4) is administered about twice a week.

[0011] In some embodiments, the anti-FOLR1 binding agent (e.g., huMov19-sulfo-SPDB-DM4) is administered once every 21 days by intravenous infusion.

[0012] According to the methods described herein, the administration can produce an $AUC_{(0-inf)}$ of about 10,000-18,000 hr• μ g/mL, about 10,000-17,500 hr• μ g/mL, about 10,000-17,000 hr• μ g/mL, or about 10,000-16,000 hr• μ g/mL. In some embodiments, the $AUC_{(0-inf)}$ is about 12,000 hr• μ g/mL to about 13,500 hr• μ g/mL. In some embodiments, the $AUC_{(0-inf)}$ is about 12,708 hr• μ g/mL. In some embodiments, the $AUC_{(0-inf)}$ is the $AUC_{(0-inf)}$ obtained in Example 1 and shown in Figure 1.

[0013] According to the methods described herein, the administration can produce an $AUC_{(0-168)}$ of about 7,500-12,500 hr• μ g/mL, about 7,500-12,000 hr• μ g/mL, about 7,500-10,000 hr• μ g/mL, or about 8,000-10,000 hr• μ g/mL. In some embodiments, the $AUC_{(0-168)}$ is about 8,000 hr• μ g/mL to about 8,500 hr• μ g/mL. In some embodiments, the $AUC_{(0-168)}$ is about 8,254 hr• μ g/mL. In some embodiments, the $AUC_{(0-168)}$ is the $AUC_{(0-168)}$ obtained in Example 1 and shown in Figure 1.

[0014] According to the methods described herein, the administration can produce a C_{max} of about 50-250 μ g/mL, about 50-200 μ g/mL, about 50-175 μ g/mL, about 50-150 μ g/mL, about 50-125 μ g/mL, about 75-250 μ g/mL, about 75-200 μ g/mL, about 75-175 μ g/mL, about 75-150 μ g/mL, or about 75-125 μ g/mL. In some embodiments, the C_{max} is about 100 μ g/mL to about 150 μ g/mL. In some embodiments, the C_{max} is about 100 μ g/mL to about 120 μ g/mL. In some embodiments, the C_{max} is about 108 μ g/mL. In some embodiments, the C_{max} is the C_{max} obtained in Example 1 and shown in Figure 1.

[0015] According to the methods described herein, the clearance of the anti-FOLR1 binding agent (e.g., huMov19-sulfo-SPDB-DM4) can be less than 1.0 mL/hr/kg. In some embodiments, the clearance of the anti-FOLR1 binding agent (e.g., huMov19-sulfo-SPDB-DM4) is less than 0.6 mL/hr/kg. In some embodiments, the clearance of the anti-FOLR1 binding agent (e.g., huMov19-sulfo-SPDB-DM4) is about 0.2 mL/hr/kg to about 0.6 mL/hr/kg. In some embodiments, the clearance of the anti-FOLR1 binding

agent (e.g., huMov19-sulfo-SPDB-DM4) is about 0.3 mL/hr/kg to about 0.4 mL/hr/kg. In some embodiments, the clearance of the anti-FOLR1 binding agent (e.g., huMov19-sulfo-SPDB-DM4) is about 0.3 mL/hr/kg. In some embodiments, the clearance of the anti-FOLR1 binding agent (e.g., huMov19-sulfo-SPDB-DM4) is about 0.4 mL/hr/kg. In some embodiments, the clearance is the clearance obtained in Example 1 and shown in Figure 1.

[0016] According to the methods described herein, the half-life of the anti-FOLR1 binding agent (e.g., huMov19-sulfo-SPDB-DM4) can be at least about 4 days. In some embodiments, the half-life of the anti-FOLR1 binding agent (e.g., huMov19-sulfo-SPDB-DM4) is about 3 to about 5 days, or about 4 to about 4.5 days. In some embodiments, the half-life is about 4.4 days. In some embodiments, the half-life is the half-life obtained in Example 1 and shown in Figure 1.

[0017] According to the methods described herein, the apparent volume of distribution at steady state (V_{ss}) of the anti-FOLR1 binding agent (e.g., huMov19-sulfo-SPDB-DM4) can be about 25 to about 100 mL/kg, about 25 to about 75 mL/kg, about 30 to about 75 mL/kg, or about 35 to about 70 mL/kg. In some embodiments, the V_{ss} is about 55 mL/kg to about 65 mL/kg. In some embodiments, the V_{ss} is about 61 mL/kg. In some embodiments, the V_{ss} is the V_{ss} obtained in Example 1 and shown in Figure 1.

[0018] In some embodiments, the anti-FOLR1 binding agent (e.g., huMov19-sulfo-SPDB-DM4) is administered intravenously.

[0019] The methods described herein can be used to treat cancer. In some embodiments, the cancer is selected from the group consisting of ovarian, brain, breast, uterine, endometrial, pancreatic, renal (e.g., renal cell carcinoma), and lung cancer (e.g., non small cell lung cancer, or bronchioloalveolar carcinoma (BAC)). In some embodiments, the cancer is ovarian cancer or lung cancer. In some embodiments, the cancer is epithelial ovarian cancer.

[0020] In some embodiments, the cancer expresses FOLR1 polypeptide or nucleic acid. In some embodiments, the cancer has an increased expression level of FOLR1 polypeptide as measured by immunohistochemistry (IHC). For example, in some embodiments, the cancer is a cancer that expresses FOLR1 polypeptide at a level of 2 hetero or higher by IHC. In some embodiments, the cancer is a cancer that expresses FOLR1 polypeptide at a level of 2 homo or higher by IHC. In some embodiments, the cancer is a cancer that expresses FOLR1 polypeptide at a level of 3 hetero or higher by IHC. In some embodiments, the cancer is a cancer that expresses FOLR1 polypeptide at a level of 3 homo or higher by IHC. In some embodiments, the cancer is a lung cancer that expresses FOLR1 polypeptide at a level of 2 hetero or higher by IHC. In some embodiments, the cancer is a lung cancer that expresses FOLR1 polypeptide at a level of 3 hetero or higher by IHC. In some embodiments, the cancer is an epithelial ovarian cancer (e.g., platinum resistant or relapsed or refractory) that expresses FOLR1 polypeptide at a level of 3 hetero or higher.

[0021] In some embodiments, the methods further comprise administering a steroid to the patient. The steroid can be administered as a pre-treatment, i.e., prior to the administration of the anti-FOLR1 binding agent. The steroid can be dexamethasone.

[0022] The methods described herein can result in a decrease in tumor size. The methods described herein can result in a decrease in CA125 levels in ovarian cancer patients. In one example, CA125 levels are measured in a sample from an ovarian cancer patient prior to treatment and then one or more times after treatment, and a decrease in the CA125 level over time is indicative of therapeutic efficacy. The methods described herein can result in an increased time between cancer treatments. The methods described herein can result in increased progression free survival (PFS). The methods described herein can result in increased disease-free survival (DFS). The methods described herein can result in increased overall survival (OS). The methods described herein can result in increased complete response (CR). The methods described herein can result in increased partial response (PR). The methods described herein can result in increased stable disease (SD). The methods described herein can result in increased decrease in progressive disease (PD). The methods described herein can result in a reduced time to progression (TTP).

[0023] The methods described herein can also result in a decrease in adverse effects.

[0024] In particular, the dosing regimens provided herein achieve an optimal balance between efficacy (e.g., PR) and reduced toxicity as demonstrated, for instance, in Examples 1 and 2 and Figure 1.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0025] Figure 1 provides pharmacokinetic data resulting from the administration of IMG853 (0.15 mg/kg to 7.0 mg/kg) as described in Example 1.

DETAILED DESCRIPTION OF THE INVENTION

[0026] The present invention provides new dosing regimens for FOLR1 binding immunoconjugates.

I. Definitions

[0027] To facilitate an understanding of the present invention, a number of terms and phrases are defined below.

[0028] The terms "human folate receptor 1," "FOLR1," or "folate receptor alpha (FR- α)", as used herein, refers to any native human FOLR1, unless otherwise indicated. Thus, all of these terms can refer to either a protein or nucleic acid sequence as indicated herein. The term "FOLR1" encompasses "full-length," unprocessed FOLR1 as well as any form of FOLR1 that results from processing within the cell. The term also encompasses naturally occurring variants of FOLR1, e.g., splice variants, allelic variants

and isoforms. The FOLR1 polypeptides described herein can be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant or synthetic methods. Examples of FOLR1 sequences include, but are not limited to NCBI reference numbers P15328, NP_001092242.1, AAX29268.1, AAX37119.1, NP_057937.1, and NP_057936.1.

[0029] The term "antibody" means an immunoglobulin molecule that recognizes and specifically binds to a target, such as a protein, polypeptide, peptide, carbohydrate, polynucleotide, lipid, or combinations of the foregoing through at least one antigen recognition site within the variable region of the immunoglobulin molecule. As used herein, the term "antibody" encompasses intact polyclonal antibodies, intact monoclonal antibodies, antibody fragments (such as Fab, Fab', F(ab')₂, and Fv fragments), single chain Fv (scFv) mutants, multispecific antibodies such as bispecific antibodies generated from at least two intact antibodies, chimeric antibodies, humanized antibodies, human antibodies, fusion proteins comprising an antigen determination portion of an antibody, and any other modified immunoglobulin molecule comprising an antigen recognition site so long as the antibodies exhibit the desired biological activity. An antibody can be of any the five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, or subclasses (isotypes) thereof (e.g. IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2), based on the identity of their heavy-chain constant domains referred to as alpha, delta, epsilon, gamma, and mu, respectively. The different classes of immunoglobulins have different and well known subunit structures and three-dimensional configurations. Antibodies can be naked or conjugated to other molecules such as toxins, radioisotopes, etc.

[0030] A "blocking" antibody or an "antagonist" antibody is one which inhibits or reduces biological activity of the antigen it binds, such as FOLR1. In some embodiments, blocking antibodies or antagonist antibodies substantially or completely inhibit the biological activity of the antigen. The biological activity can be reduced by 10%, 20%, 30%, 50%, 70%, 80%, 90%, 95%, or even 100%.

[0031] The term "anti-FOLR1 antibody" or "an antibody that binds to FOLR1" refers to an antibody that is capable of binding FOLR1 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting FOLR1. The extent of binding of an anti-FOLR1 antibody to an unrelated, non-FOLR1 protein can be 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 (K_d) of $\leq 1 \mu\text{M}$, $\leq 100 \text{ nM}$, $\leq 10 \text{ nM}$, $\leq 1 \text{ nM}$, or $\leq 0.1 \text{ nM}$.

[0032] The term "antibody fragment" refers to a portion of an intact antibody and refers to the antigenic determining variable regions of an intact antibody. Examples of antibody fragments include, but are not limited to Fab, Fab', F(ab')₂, and Fv fragments, linear antibodies, single chain antibodies, and multispecific antibodies formed from antibody fragments.

[0033] A "monoclonal antibody" refers to a homogeneous antibody population involved in the highly specific recognition and binding of a single antigenic determinant, or epitope. This is in contrast to

polyclonal antibodies that typically include different antibodies directed against different antigenic determinants. The term "monoclonal antibody" encompasses both intact and full-length monoclonal antibodies as well as antibody fragments (such as Fab, Fab', F(ab')₂, Fv), single chain (scFv) mutants, fusion proteins comprising an antibody portion, and any other modified immunoglobulin molecule comprising an antigen recognition site. Furthermore, "monoclonal antibody" refers to such antibodies made in any number of manners including but not limited to by hybridoma, phage selection, recombinant expression, and transgenic animals.

[0034] The term "humanized antibody" refers to forms of non-human (e.g. murine) antibodies that are specific immunoglobulin chains, chimeric immunoglobulins, or fragments thereof that contain minimal non-human (e.g., murine) sequences. Typically, humanized antibodies are human immunoglobulins in which residues from the complementary determining region (CDR) are replaced by residues from the CDR of a non-human species (e.g. mouse, rat, rabbit, hamster) that have the desired specificity, affinity, and capability (Jones et al., 1986, *Nature*, 321:522-525; Riechmann et al., 1988, *Nature*, 332:323-327; Verhoeyen et al., 1988, *Science*, 239:1534-1536). In some instances, the Fv framework region (FR) residues of a human immunoglobulin are replaced with the corresponding residues in an antibody from a non-human species that has the desired specificity, affinity, and capability. The humanized antibody can be further modified by the substitution of additional residues either in the Fv framework region and/or within the replaced non-human residues to refine and optimize antibody specificity, affinity, and/or capability. In general, the humanized antibody will comprise substantially all of at least one, and typically two or three, variable domains containing all or substantially all of the CDR regions that correspond to the non-human immunoglobulin whereas all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody can also comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin. Examples of methods used to generate humanized antibodies are described in U.S. Pat. 5,225,539. In some embodiments, a "humanized antibody" is a resurfaced antibody.

[0035] A "variable region" of an antibody refers to the variable region of the antibody light chain or the variable region of the antibody heavy chain, either alone or in combination. The variable regions of the heavy and light chain each consist of four framework regions (FR) connected by three complementarity determining regions (CDRs) also known as hypervariable regions. The CDRs in each chain are held together in close proximity by the FRs and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies. There are at least two techniques for determining CDRs: (1) an approach based on cross-species sequence variability (i.e., Kabat et al. Sequences of Proteins of Immunological Interest, (5th ed., 1991, National Institutes of Health, Bethesda Md.)); and (2) an approach based on crystallographic studies of antigen-antibody complexes (Al-lazikani

et al (1997) *J. Molec. Biol.* 273:927-948)). In addition, combinations of these two approaches are sometimes used in the art to determine CDRs.

[0036] The Kabat numbering system is generally used when referring to a residue in the variable domain (approximately residues 1-107 of the light chain and residues 1-113 of the heavy chain) (e.g., Kabat et al., *Sequences of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)).

[0037] The amino acid position numbering as in Kabat, refers to the numbering system used for heavy chain variable domains or light chain variable domains of the compilation of antibodies in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991). Using this numbering system, the actual linear amino acid sequence can contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or CDR of the variable domain. For example, a heavy chain variable domain can include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (e.g. residues 82a, 82b, and 82c, etc according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues can be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence. Chothia refers instead to the location of the structural loops (Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)). The end of the Chothia CDR-H1 loop when numbered using the Kabat numbering convention varies between H32 and H34 depending on the length of the loop (this is because the Kabat numbering scheme places the insertions at H35A and H35B; if neither 35A nor 35B is present, the loop ends at 32; if only 35A is present, the loop ends at 33; if both 35A and 35B are present, the loop ends at 34). The AbM hypervariable regions represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software.

Loop	Kabat	AbM	Chothia
L1	L24-L34	L24-L34	L24-L34
L2	L50-L56	L50-L56	L50-L56
L3	L89-L97	L89-L97	L89-L97
H1	H31-H35B	H26-H35B (Kabat Numbering)	H26-H32..34
H1	H31-H35	H26-H35 (Chothia Numbering)	H26-H32
H2	H50-H65	H50-H58	H52-H56
H3	H95-H102	H95-H102	H95-H102

[0038] The term "human antibody" means an antibody produced by a human or an antibody having an amino acid sequence corresponding to an antibody produced by a human made using any technique

known in the art. This definition of a human antibody includes intact or full-length antibodies, fragments thereof, and/or antibodies comprising at least one human heavy and/or light chain polypeptide such as, for example, an antibody comprising murine light chain and human heavy chain polypeptides.

[0039] The term "chimeric antibodies" refers to antibodies wherein the amino acid sequence of the immunoglobulin molecule is derived from two or more species. Typically, the variable region of both light and heavy chains corresponds to the variable region of antibodies derived from one species of mammals (e.g. mouse, rat, rabbit, etc) with the desired specificity, affinity, and capability while the constant regions are homologous to the sequences in antibodies derived from another (usually human) to avoid eliciting an immune response in that species.

[0040] The term "epitope" or "antigenic determinant" are used interchangeably herein and refer to that portion of an antigen capable of being recognized and specifically bound by a particular antibody. When the antigen is a polypeptide, epitopes can be formed both from contiguous amino acids and noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained upon protein denaturing, whereas epitopes formed by tertiary folding are typically lost upon protein denaturing. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

[0041] "Binding affinity" generally 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 (K_d). Affinity can be measured by common methods known in the art, including those described herein. Low-affinity antibodies generally bind antigen slowly and tend to dissociate readily, whereas high-affinity antibodies generally bind antigen faster and tend to remain bound longer. A variety of methods of measuring binding affinity are known in the art, any of which can be used for purposes of the present invention. Specific illustrative embodiments are described in the following.

[0042] "Or better" when used herein to refer to binding affinity refers to a stronger binding between a molecule and its binding partner. "Or better" when used herein refers to a stronger binding, represented by a smaller numerical K_d value. For example, an antibody which has an affinity for an antigen of "0.6 nM or better", the antibody's affinity for the antigen is <0.6 nM, i.e. 0.59 nM, 0.58 nM, 0.57 nM etc. or any value less than 0.6 nM.

[0043] By "specifically binds," it is generally meant that an antibody binds to an epitope via its antigen binding domain, and that the binding entails some complementarity between the antigen binding domain and the epitope. According to this definition, an antibody is said to "specifically bind" to an epitope when it binds to that epitope, via its antigen binding domain more readily than it would bind to a

random, unrelated epitope. The term "specificity" is used herein to qualify the relative affinity by which a certain antibody binds to a certain epitope. For example, antibody "A" may be deemed to have a higher specificity for a given epitope than antibody "B," or antibody "A" may be said to bind to epitope "C" with a higher specificity than it has for related epitope "D."

[0044] By "preferentially binds," it is meant that the antibody specifically binds to an epitope more readily than it would bind to a related, similar, homologous, or analogous epitope. Thus, an antibody which "preferentially binds" to a given epitope would more likely bind to that epitope than to a related epitope, even though such an antibody may cross-react with the related epitope.

[0045] An antibody is said to "competitively inhibit" binding of a reference antibody to a given epitope if it preferentially binds to that epitope to the extent that it blocks, to some degree, binding of the reference antibody to the epitope. Competitive inhibition may be determined by any method known in the art, for example, competition ELISA assays. An antibody may be said to competitively inhibit binding of the reference antibody to a given epitope by at least 90%, at least 80%, at least 70%, at least 60%, or at least 50%.

[0046] The phrase "substantially similar," or "substantially the same", as used herein, denotes a sufficiently high degree of similarity between two numeric values (generally one associated with an antibody of the invention and the other associated with a reference/comparator antibody) such that one of skill in the art would consider the difference between the two values to be of little or no biological and/or statistical significance within the context of the biological characteristic measured by said values (e.g., K_d values). The difference between said two values can be less than about 50%, less than about 40%, less than about 30%, less than about 20%, or less than about 10% as a function of the value for the reference/comparator antibody.

[0047] A polypeptide, antibody, polynucleotide, vector, cell, or composition which is "isolated" is a polypeptide, antibody, polynucleotide, vector, cell, or composition which is in a form not found in nature. Isolated polypeptides, antibodies, polynucleotides, vectors, cell or compositions include those which have been purified to a degree that they are no longer in a form in which they are found in nature. In some embodiments, an antibody, polynucleotide, vector, cell, or composition which is isolated is substantially pure.

[0048] As used herein, "substantially pure" refers to material which is at least 50% pure (i.e., free from contaminants), at least 90% pure, at least 95% pure, at least 98% pure, or at least 99% pure.

[0049] The term "immunoconjugate" or "conjugate" as used herein refers to a compound or a derivative thereof that is linked to a cell binding agent (i.e., an anti-FOLR1 antibody or fragment thereof) and is defined by a generic formula: C-L-A, wherein C = cytotoxin, L = linker, and A = anti-FOLR1 antibody or antibody fragment. Immunoconjugates can also be defined by the generic formula in reverse order: A-L-C.

[0050] The term "IMGN853" refers to the immunoconjugate described herein containing the huMov19 antibody, the sulfoSPDB linker, and the DM4 maytansinoid.

[0051] A "linker" is any chemical moiety that is capable of linking a compound, usually a drug, such as a maytansinoid, to a cell-binding agent such as an anti FOLR1 antibody or a fragment thereof in a stable, covalent manner. Linkers can be susceptible to or be substantially resistant to acid-induced cleavage, light-induced cleavage, peptidase-induced cleavage, esterase-induced cleavage, and disulfide bond cleavage, at conditions under which the compound or the antibody remains active. Suitable linkers are well known in the art and include, for example, disulfide groups, thioether groups, acid labile groups, photolabile groups, peptidase labile groups and esterase labile groups. Linkers also include charged linkers, and hydrophilic forms thereof as described herein and known in the art.

[0052] The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals in which a population of cells are characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancers. The cancer can be a cancer that expresses FOLR1.

[0053] "Tumor" and "neoplasm" refer to any mass of tissue that results from excessive cell growth or proliferation, either benign (noncancerous) or malignant (cancerous) including pre-cancerous lesions.

[0054] The terms "cancer cell," "tumor cell," and grammatical equivalents refer to the total population of cells derived from a tumor or a pre-cancerous lesion, including both non-tumorigenic cells, which comprise the bulk of the tumor cell population, and tumorigenic stem cells (cancer stem cells). As used herein, the term "tumor cell" will be modified by the term "non-tumorigenic" when referring solely to those tumor cells lacking the capacity to renew and differentiate to distinguish those tumor cells from cancer stem cells.

[0055] The term "subject" refers to any animal (e.g., a mammal), including, but not limited to humans, non-human primates, rodents, and the like, which is to be the recipient of a particular treatment. Typically, the terms "subject" and "patient" are used interchangeably herein in reference to a human subject.

[0056] Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

[0057] The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of the active ingredient to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. The formulation can be sterile.

[0058] An "effective amount" of an antibody or immunoconjugate as disclosed herein is an amount sufficient to carry out a specifically stated purpose. An "effective amount" can be determined empirically and in a routine manner, in relation to the stated purpose.

[0059] The term "therapeutically effective amount" refers to an amount of an antibody or other drug effective to "treat" a disease or disorder in a subject or mammal. In the case of cancer, the therapeutically effective amount of the drug can reduce the number of cancer cells; reduce the tumor size; inhibit (i.e., slow to some extent and in a certain embodiment, stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and in a certain embodiment, stop) tumor metastasis; inhibit, to some extent, tumor growth; relieve to some extent one or more of the symptoms associated with the cancer; and/or result in a favorable response such as increased progression-free survival (PFS), disease-free survival (DFS), or overall survival (OS), complete response (CR), partial response (PR), or, in some cases, stable disease (SD), a decrease in progressive disease (PD), a reduced time to progression (TTP), a decrease in CA125 in the case of ovarian cancer, or any combination thereof.

[0060] See the definition herein of "treating." To the extent the drug can prevent growth and/or kill existing cancer cells, it can be cytostatic and/or cytotoxic. In certain embodiments, identification of increased FOLR1 levels allows for administration of decreased amounts of the FOLR1-targeting therapeutic to achieve the same therapeutic effect as seen with higher dosages. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically but not necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[0061] The term "respond favorably" generally refers to causing a beneficial state in a subject. With respect to cancer treatment, the term refers to providing a therapeutic effect on the subject. Positive therapeutic effects in cancer can be measured in a number of ways (*See*, W.A. Weber, J. Nucl. Med. 50:1S-10S (2009)). For example, tumor growth inhibition, molecular marker expression, serum marker expression, and molecular imaging techniques can all be used to assess therapeutic efficacy of an anti-cancer therapeutic. With respect to tumor growth inhibition, according to NCI standards, a $T/C \leq 42\%$ is the minimum level of anti-tumor activity. A $T/C < 10\%$ is considered a high anti-tumor activity level, with $T/C (\%) = \text{Median tumor volume of the treated} / \text{Median tumor volume of the control} \times 100$. A favorable response can be assessed, for example, by increased progression-free survival (PFS), disease-free survival (DFS), or overall survival (OS), complete response (CR), partial response (PR), or, in some

cases, stable disease (SD), a decrease in progressive disease (PD), a reduced time to progression (TTP), a decrease in CA125 in the case of ovarian cancer or any combination thereof.

[0062] PFS, DFS, and OS can be measured by standards set by the National Cancer Institute and the U.S. Food and Drug Administration for the approval of new drugs. See Johnson et al, (2003) J. Clin. Oncol. 21(7):1404-1411.

[0063] "Progression free survival" (PFS) refers to the time from enrollment to disease progression or death. PFS is generally measured using the Kaplan-Meier method and Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 standards. Generally, progression free survival refers to the situation wherein a patient remains alive, without the cancer getting worse.

[0064] "Time to Tumor Progression" (TTP) is defined as the time from enrollment to disease progression. TTP is generally measured using the RECIST 1.1 criteria.

[0065] A "complete response" or "complete remission" or "CR" indicates the disappearance of all signs of tumor or cancer in response to treatment. This does not always mean the cancer has been cured.

[0066] A "partial response" or "PR" refers to a decrease in the size or volume of one or more tumors or lesions, or in the extent of cancer in the body, in response to treatment.

[0067] "Stable disease" refers to disease without progression or relapse. In stable disease there is neither sufficient tumor shrinkage to qualify for partial response nor sufficient tumor increase to qualify as progressive disease.

[0068] "Progressive disease" refers to the appearance of one more new lesions or tumors and/or the unequivocal progression of existing non-target lesions. Progressive disease can also refer to a tumor growth of more than 20 percent since treatment began, either due to an increases in mass or in spread of the tumor.

[0069] "Disease free survival" (DFS) refers to the length of time during and after treatment that the patient remains free of disease.

[0070] "Overall Survival" (OS) refers to the time from patient enrollment to death or censored at the date last known alive. OS includes a prolongation in life expectancy as compared to naive or untreated individuals or patients. Overall survival refers to the situation wherein a patient remains alive for a defined period of time, such as one year, five years, etc., e.g., from the time of diagnosis or treatment.

[0071] A "decrease in CA125 levels" can be assessed according to the Gynecologic Cancer Intergroup (GCIg) guidelines. For example, CA125 levels can be measured prior to treatment to establish a baseline CA125 level. CA125 levels can be measured one or more times during or after treatment, and a reduction in the CA125 levels over time as compared to the baseline level is considered a decrease in CA125 levels.

[0072] Terms such as "treating" or "treatment" or "to treat" or "alleviating" or "to alleviate" refer to therapeutic measures that cure, slow down, lessen symptoms of, and/or halt progression of a diagnosed

pathologic condition or disorder. Thus, those in need of treatment include those already diagnosed with or suspected of having the disorder. In certain embodiments, a subject is successfully "treated" for cancer according to the methods of the present invention if the patient shows one or more of the following: a reduction in the number of or complete absence of cancer cells; a reduction in the tumor size; inhibition of or an absence of cancer cell infiltration into peripheral organs including, for example, the spread of cancer into soft tissue and bone; inhibition of or an absence of tumor metastasis; inhibition or an absence of tumor growth; relief of one or more symptoms associated with the specific cancer; reduced morbidity and mortality; improvement in quality of life; reduction in tumorigenicity, tumorigenic frequency, or tumorigenic capacity, of a tumor; reduction in the number or frequency of cancer stem cells in a tumor; differentiation of tumorigenic cells to a non-tumorigenic state; increased progression-free survival (PFS), disease-free survival (DFS), or overall survival (OS), complete response (CR), partial response (PR), stable disease (SD), a decrease in progressive disease (PD), a reduced time to progression (TTP), a decrease in CA125 in the case of ovarian cancer, or any combination thereof.

[0073] Prophylactic or preventative measures refer to therapeutic measures that prevent and/or slow the development of a targeted pathologic condition or disorder. Thus, those in need of prophylactic or preventative measures include those prone to have the disorder and those in whom the disorder is to be prevented.

[0074] The terms "pre-treat" and "pre-treatment" refer to therapeutic measures that occur prior to the administration of an anti-FOLR1 therapeutic. For example, as described in more detail herein, a prophylactic such as a steroid can administered within about a week, about five days, about three days, about two days, or about one day or 24 hours prior to the administration of the anti-FOLR1 therapeutic. The prophylactic can also be administered prior to the anti-FOLR1 therapeutic on the same day as the anti-FOLR1 therapeutic.

[0075] A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer, regardless of mechanism of action. Chemotherapeutic agents include, for example, antagonists of CD20 such as Rituximab and cyclophosphamide, doxorubicin, vincristine, prednisone, fludarabine, etoposide, methotrexate, lenalidomide, chlorambucil, bentamustine and/or modified versions of such chemotherapeutics.

[0076] The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The polymer can be linear or branched, it can comprise modified amino acids, and it can be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as

well as other modifications known in the art. It is understood that, because the polypeptides of this invention are based upon antibodies, in certain embodiments, the polypeptides can occur as single chains or associated chains.

[0077] The terms "identical" or percent "identity" in the context of two or more nucleic acids or polypeptides, refer to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned (introducing gaps, if necessary) for maximum correspondence, not considering any conservative amino acid substitutions as part of the sequence identity. The percent identity can be measured using sequence comparison software or algorithms or by visual inspection. Various algorithms and software are known in the art that can be used to obtain alignments of amino acid or nucleotide sequences. One such non-limiting example of a sequence alignment algorithm is the algorithm described in Karlin et al., 1990, *Proc. Natl. Acad. Sci.*, 87:2264-2268, as modified in Karlin et al., 1993, *Proc. Natl. Acad. Sci.*, 90:5873-5877, and incorporated into the NBLAST and XBLAST programs (Altschul et al., 1991, *Nucleic Acids Res.*, 25:3389-3402). In certain embodiments, Gapped BLAST can be used as described in Altschul et al., 1997, *Nucleic Acids Res.* 25:3389-3402. BLAST-2, WU-BLAST-2 (Altschul et al., 1996, *Methods in Enzymology*, 266:460-480), ALIGN, ALIGN-2 (Genentech, South San Francisco, California) or Megalign (DNASTAR) are additional publicly available software programs that can be used to align sequences. In certain embodiments, the percent identity between two nucleotide sequences is determined using the GAP program in GCG software (e.g., using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 90 and a length weight of 1, 2, 3, 4, 5, or 6). In certain alternative embodiments, the GAP program in the GCG software package, which incorporates the algorithm of Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) can be used to determine the percent identity between two amino acid sequences (e.g., using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5). Alternatively, in certain embodiments, the percent identity between nucleotide or amino acid sequences is determined using the algorithm of Myers and Miller (CABIOS, 4:11-17 (1989)). For example, the percent identity can be determined using the ALIGN program (version 2.0) and using a PAM120 with residue table, a gap length penalty of 12 and a gap penalty of 4. Appropriate parameters for maximal alignment by particular alignment software can be determined by one skilled in the art. In certain embodiments, the default parameters of the alignment software are used. In certain embodiments, the percentage identity "X" of a first amino acid sequence to a second sequence amino acid is calculated as $100 \times (Y/Z)$, where Y is the number of amino acid residues scored as identical matches in the alignment of the first and second sequences (as aligned by visual inspection or a particular sequence alignment program) and Z is the total number of residues in the second sequence. If the length of a first sequence is longer than the second sequence, the percent identity of the first sequence to the second sequence will be longer than the percent identity of the second sequence to the first sequence.

[0078] As a non-limiting example, whether any particular polynucleotide has a certain percentage sequence identity (e.g., is at least 80% identical, at least 85% identical, at least 90% identical, and in some embodiments, at least 95%, 96%, 97%, 98%, or 99% identical) to a reference sequence can, in certain embodiments, be determined using the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). Bestfit uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics* 2: 482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

[0079] In some embodiments, two nucleic acids or polypeptides of the invention are substantially identical, meaning they have at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, and in some embodiments at least 95%, 96%, 97%, 98%, 99% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using a sequence comparison algorithm or by visual inspection. Identity can exist over a region of the sequences that is at least about 10, about 20, about 40-60 residues in length or any integral value there between, and can be over a longer region than 60-80 residues, for example, at least about 90-100 residues, and in some embodiments, the sequences are substantially identical over the full length of the sequences being compared, such as the coding region of a nucleotide sequence for example.

[0080] A "conservative amino acid substitution" is one in which one amino acid residue is replaced with another amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). For example, substitution of a phenylalanine for a tyrosine is a conservative substitution. In some embodiments, conservative substitutions in the sequences of the polypeptides and antibodies of the invention do not abrogate the binding of the polypeptide or antibody containing the amino acid sequence, to the antigen(s), i.e., the FOLR1 to which the polypeptide or antibody binds. Methods of identifying nucleotide and amino acid conservative substitutions which do not eliminate antigen binding are well-known in the art (see, e.g., Brummell et al., *Biochem.* 32: 1180-1187 (1993); Kobayashi et al. *Protein Eng.* 12(10):879-884 (1999); and Burks et al. *Proc. Natl. Acad. Sci. USA* 94:412-417 (1997)).

[0081] As used in the present disclosure and claims, the singular forms "a," "an," and "the" include plural forms unless the context clearly dictates otherwise.

[0082] It is understood that wherever embodiments are described herein with the language "comprising," otherwise analogous embodiments described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[0083] The term "and/or" as used in a phrase such as "A and/or B" herein is intended to include both "A and B," "A or B," "A," and "B." 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 embodiments: 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).

II. FOLR1 binding agents

[0084] The methods described herein provide methods of administering sequences that specifically bind FOLR1 ("FOLR1 binding agents"). In certain embodiments, the FOLR1 binding agents are antibodies, immunoconjugates or polypeptides. The amino acid and nucleotide sequences for human FOLR1 are known in the art and are also provided herein as represented by SEQ ID NO:1 and SEQ ID NO:2. Thus, in some embodiments, the FOLR1 binding agents can bind to an epitope of SEQ ID NO:1.

[0085] Examples of therapeutically effective anti-FOLR1 antibodies can be found in US Appl. Pub. No. US 2012/0009181 which is herein incorporated by reference. An example of a therapeutically effective anti-FOLR1 antibody is huMov19 (M9346A). The polypeptides of SEQ ID NOs: 3-5 comprise the variable domain of the heavy chain of huMov19 (M9346A), and the variable domain light chain version 1.00, the variable domain light chain version 1.60 of huMov19, respectively. In certain embodiments, the huMov19 (M9346A) antibody is encoded by the plasmids deposited with the American Type Culture Collection (ATCC), located at 10801 University Boulevard, Manassas, VA 20110 on April 7, 2010 under the terms of the Budapest Treaty and having ATCC deposit nos. PTA-10772 and PTA-10773 or 10774. Examples of FOLR1 immunoconjugates useful in the therapeutic methods of the invention are provided below.

[0086] In some embodiments, the FOLR1 binding agents are humanized antibodies or antigen-binding fragments thereof. In some embodiments, the humanized antibody or fragment is a resurfaced antibody or antigen-binding fragment thereof. In other embodiments, the FOLR1 binding agent is a fully human antibody or antigen-binding fragment thereof.

[0087] In certain embodiments, the FOLR1-binding agents have one or more of the following effects: induce stable disease, inhibit proliferation of tumor cells, reduce the tumorigenicity of a tumor by reducing the frequency of cancer stem cells in the tumor, inhibit tumor growth, increase survival, trigger cell death of tumor cells, differentiate tumorigenic cells to a non-tumorigenic state, or prevent metastasis of tumor cells.

[0088] In certain embodiments, a FOLR1-binding agent that is an antibody that has antibody-dependent cellular cytotoxicity (ADCC) activity.

[0089] In some embodiments, the FOLR1-binding agents are capable of reducing tumor volume. The ability of a FOLR1-binding agent to reduce tumor volume can be assessed, for example, by measuring a %T/C value, which is the median tumor volume of treated subjects divided by the median tumor volume of the control subjects. In certain embodiments, immunoconjugates or other agents that specifically bind human FOLR1 trigger cell death via a cytotoxic agent. For example, in certain embodiments, an antibody to a human FOLR1 antibody is conjugated to a maytansinoid that is activated in tumor cells expressing the FOLR1 by protein internalization. In certain embodiments, the FOLR1-binding agents are capable of inhibiting tumor growth. In certain embodiments, the FOLR1-binding agents are capable of inhibiting tumor growth *in vivo* (e.g., in a xenograft mouse model and/or in a human having cancer).

[0090] The FOLR1 binding molecules can be antibodies or antigen binding fragments that specifically bind to FOLR1 that comprise the CDRs of huMov19 (M9346A) with up to four (i.e. 0, 1, 2, 3, or 4) conservative amino acid substitutions per CDR, e.g., wherein the antibodies or fragments do not comprise the six CDRs of murine Mov19 (i.e., SEQ ID NOs:6-9, 16, and 12). Polypeptides can comprise one of the individual variable light chains or variable heavy chains described herein. Antibodies and polypeptides can also comprise both a variable light chain and a variable heavy chain.

[0091] In some embodiments, the FOLR1 binding molecule is an antibody or antigen-binding fragment comprising the sequences of SEQ ID NOs:6-10 and the sequence of SEQ ID NO:12. In some embodiments, the FOLR1 binding molecule is an antibody or antigen-binding fragment comprising the sequences of SEQ ID NOs:6-9 and the sequences of SEQ ID NOs:11 and 12.

[0092] Also provided are polypeptides that comprise a polypeptide having at least about 90% sequence identity to SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5. In certain embodiments, the polypeptide comprises a polypeptide having at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5. Thus, in certain embodiments, the polypeptide comprises (a) a polypeptide having at least about 95% sequence identity to SEQ ID NO:3 and/or (b) a polypeptide having at least about 95% sequence identity to SEQ ID NO:4 or SEQ ID NO:5. In certain embodiments, the polypeptide comprises (a) a polypeptide having the amino acid sequence of SEQ ID NO:3; and/or (b) a polypeptide having the amino acid sequence of SEQ ID NO:4 or SEQ ID NO:5. In certain embodiments, the polypeptide is an antibody and/or the polypeptide specifically binds FOLR1. In certain embodiments, the polypeptide is a murine, chimeric, or humanized antibody that specifically binds FOLR1. In certain embodiments, the polypeptide having a certain percentage of sequence identity to SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5 differs from SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5 by conservative amino acid substitutions only.

[0093] Polypeptides can comprise one of the individual light chains or heavy chains described herein. Antibodies and polypeptides can also comprise both a light chain and a heavy chain.

[0094] Monoclonal antibodies can be prepared using hybridoma methods, such as those described by Kohler and Milstein (1975) *Nature* 256:495. Using the hybridoma method, a mouse, hamster, or other appropriate host animal, is immunized as described above to elicit the production by lymphocytes of antibodies that will specifically bind to an immunizing antigen. Lymphocytes can also be immunized *in vitro*. Following immunization, the lymphocytes are isolated and fused with a suitable myeloma cell line using, for example, polyethylene glycol, to form hybridoma cells that can then be selected away from unfused lymphocytes and myeloma cells. Hybridomas that produce monoclonal antibodies directed specifically against a chosen antigen as determined by immunoprecipitation, immunoblotting, or by an *in vitro* binding assay (e.g. radioimmunoassay (RIA); enzyme-linked immunosorbent assay (ELISA)) can then be propagated either *in vitro* culture using standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press, 1986) or *in vivo* as ascites tumors in an animal. The monoclonal antibodies can then be purified from the culture medium or ascites fluid as described for polyclonal antibodies above.

[0095] Alternatively monoclonal antibodies can also be made using recombinant DNA methods as described in U.S. Patent 4,816,567. The polynucleotides encoding a monoclonal antibody are isolated from mature B-cells or hybridoma cell, such as by RT-PCR using oligonucleotide primers that specifically amplify the genes encoding the heavy and light chains of the antibody, and their sequence is determined using conventional procedures. The isolated polynucleotides encoding the heavy and light chains are then cloned into suitable expression vectors, which when transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, monoclonal antibodies are generated by the host cells. Also, recombinant monoclonal antibodies or fragments thereof of the desired species can be isolated from phage display libraries expressing CDRs of the desired species as described (McCafferty et al., 1990, *Nature*, 348:552-554; Clackson et al., 1991, *Nature*, 352:624-628; and Marks et al., 1991, *J. Mol. Biol.*, 222:581-597).

[0096] The polynucleotide(s) encoding a monoclonal antibody can further be modified in a number of different manners using recombinant DNA technology to generate alternative antibodies. In some embodiments, the constant domains of the light and heavy chains of, for example, a mouse monoclonal antibody can be substituted 1) for those regions of, for example, a human antibody to generate a chimeric antibody or 2) for a non-immunoglobulin polypeptide to generate a fusion antibody. In some embodiments, the constant regions are truncated or removed to generate the desired antibody fragment of a monoclonal antibody. Site-directed or high-density mutagenesis of the variable region can be used to optimize specificity, affinity, etc. of a monoclonal antibody.

[0097] In some embodiments, the monoclonal antibody against the human FOLR1 is a humanized antibody. In some embodiments, the humanized antibody is a resurfaced antibody. In certain embodiments, such antibodies are used therapeutically to reduce antigenicity and HAMA (human anti-mouse antibody) responses when administered to a human subject. Humanized antibodies can be produced using various techniques known in the art. In certain alternative embodiments, the antibody to FOLR1 is a human antibody.

[0098] Human antibodies can be directly prepared using various techniques known in the art. Immortalized human B lymphocytes immunized *in vitro* or isolated from an immunized individual that produce an antibody directed against a target antigen can be generated (See, e.g., Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boemer et al., 1991, *J. Immunol.*, 147 (1):86-95; and U.S. Patent 5,750,373). Also, the human antibody can be selected from a phage library, where that phage library expresses human antibodies, as described, for example, in Vaughan et al., 1996, *Nat. Biotech.*, 14:309-314, Sheets et al., 1998, *Proc. Nat'l. Acad. Sci.*, 95:6157-6162, Hoogenboom and Winter, 1991, *J. Mol. Biol.*, 227:381, and Marks et al., 1991, *J. Mol. Biol.*, 222:581). Techniques for the generation and use of antibody phage libraries are also described in U.S. Patent Nos. 5,969,108, 6,172,197, 5,885,793, 6,521,404; 6,544,731; 6,555,313; 6,582,915; 6,593,081; 6,300,064; 6,653,068; 6,706,484; and 7,264,963; and Rothe et al., 2007, *J. Mol. Bio.*, doi:10.1016/j.jmb.2007.12.018 (each of which is incorporated by reference in its entirety). Affinity maturation strategies and chain shuffling strategies (Marks et al., 1992, *Bio/Technology* 10:779-783, incorporated by reference in its entirety) are known in the art and can be employed to generate high affinity human antibodies.

[0099] Humanized antibodies can also be made in transgenic mice containing human immunoglobulin loci that are capable upon immunization of producing the full repertoire of human antibodies in the absence of endogenous immunoglobulin production. This approach is described in U.S. Patents 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016.

[00100] This invention also encompasses bispecific antibodies that specifically recognize a FOLR1. Bispecific antibodies are antibodies that are capable of specifically recognizing and binding at least two different epitopes. The different epitopes can either be within the same molecule (e.g. the same FOLR1) or on different molecules such that both, for example, the antibodies can specifically recognize and bind a FOLR1 as well as, for example, 1) an effector molecule on a leukocyte such as a T-cell receptor (e.g. CD3) or Fc receptor (e.g. CD64, CD32, or CD16) or 2) a cytotoxic agent as described in detail below.

[00101] The polypeptides of the present invention can be recombinant polypeptides, natural polypeptides, or synthetic polypeptides comprising an antibody, or fragment thereof, against a human FOLR1.

[00102] The polypeptides and analogs can be further modified to contain additional chemical moieties not normally part of the protein. Those derivatized moieties can improve the solubility, the biological half

life or absorption of the protein. The moieties can also reduce or eliminate any desirable side effects of the proteins and the like. An overview for those moieties can be found in REMINGTON'S PHARMACEUTICAL SCIENCES, 20th ed., Mack Publishing Co., Easton, PA (2000).

[00103] The isolated polypeptides described herein can be produced by any suitable method known in the art. Such methods range from direct protein synthetic methods to constructing a DNA sequence encoding isolated polypeptide sequences and expressing those sequences in a suitable transformed host. In some embodiments, a DNA sequence is constructed using recombinant technology by isolating or synthesizing a DNA sequence encoding a wild-type protein of interest. Optionally, the sequence can be mutagenized by site-specific mutagenesis to provide functional analogs thereof. See, e.g. Zoeller et al., Proc. Nat'l. Acad. Sci. USA 81:5662-5066 (1984) and U.S. Pat. No. 4,588,585.

[00104] In some embodiments a DNA sequence encoding a polypeptide of interest would be constructed by chemical synthesis using an oligonucleotide synthesizer. Such oligonucleotides can be designed based on the amino acid sequence of the desired polypeptide and selecting those codons that are favored in the host cell in which the recombinant polypeptide of interest will be produced. Standard methods can be applied to synthesize an isolated polynucleotide sequence encoding an isolated polypeptide of interest. For example, a complete amino acid sequence can be used to construct a back-translated gene. Further, a DNA oligomer containing a nucleotide sequence coding for the particular isolated polypeptide can be synthesized. For example, several small oligonucleotides coding for portions of the desired polypeptide can be synthesized and then ligated. The individual oligonucleotides typically contain 5' or 3' overhangs for complementary assembly.

[00105] Once assembled (by synthesis, site-directed mutagenesis or another method), the polynucleotide sequences encoding a particular isolated polypeptide of interest will be inserted into an expression vector and operatively linked to an expression control sequence appropriate for expression of the protein in a desired host. Proper assembly can be confirmed by nucleotide sequencing, restriction mapping, and expression of a biologically active polypeptide in a suitable host. As is well known in the art, in order to obtain high expression levels of a transfected gene in a host, the gene must be operatively linked to transcriptional and translational expression control sequences that are functional in the chosen expression host.

[00106] In certain embodiments, recombinant expression vectors are used to amplify and express DNA encoding antibodies, or fragments thereof, against human FOLR1. Recombinant expression vectors are replicable DNA constructs which have synthetic or cDNA-derived DNA fragments encoding a polypeptide chain of an anti-FOLR1 antibody, or fragment thereof, operatively linked to suitable transcriptional or translational regulatory elements derived from mammalian, microbial, viral or insect genes. A transcriptional unit generally comprises an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, transcriptional promoters or enhancers, (2) a structural

or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences, as described in detail below. Such regulatory elements can include an operator sequence to control transcription. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants can additionally be incorporated. DNA regions are operatively linked when they are functionally related to each other. For example, DNA for a signal peptide (secretory leader) is operatively linked to DNA for a polypeptide if it is expressed as a precursor which participates in the secretion of the polypeptide; a promoter is operatively linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operatively linked to a coding sequence if it is positioned so as to permit translation. Structural elements intended for use in yeast expression systems include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant protein is expressed without a leader or transport sequence, it can include an N-terminal methionine residue. This residue can optionally be subsequently cleaved from the expressed recombinant protein to provide a final product.

[00107] The choice of expression control sequence and expression vector will depend upon the choice of host. A wide variety of expression host/vector combinations can be employed. Useful expression vectors for eukaryotic hosts, include, for example, vectors comprising expression control sequences from SV40, bovine papilloma virus, adenovirus and cytomegalovirus. Useful expression vectors for bacterial hosts include known bacterial plasmids, such as plasmids from *Escherichia coli*, including pCR 1, pBR322, pMB9 and their derivatives, wider host range plasmids, such as M13 and filamentous single-stranded DNA phages.

[00108] Suitable host cells for expression of a FOLR1-binding polypeptide or antibody (or a FOLR1 protein to use as an antigen) include prokaryotes, yeast, insect or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or bacilli. Higher eukaryotic cells include established cell lines of mammalian origin as described below. Cell-free translation systems could also be employed. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels et al. (Cloning Vectors: A Laboratory Manual, Elsevier, N.Y., 1985), the relevant disclosure of which is hereby incorporated by reference. Additional information regarding methods of protein production, including antibody production, can be found, e.g., in U.S. Patent Publication No. 2008/0187954, U.S. Patent Nos. 6,413,746 and 6,660,501, and International Patent Publication No. WO 04009823, each of which is hereby incorporated by reference herein in its entirety.

[00109] Various mammalian or insect cell culture systems are also advantageously employed to express recombinant protein. Expression of recombinant proteins in mammalian cells can be performed because such proteins are generally correctly folded, appropriately modified and completely functional.

Examples of suitable mammalian host cell lines include the COS-7 lines of monkey kidney cells, described by Gluzman (Cell 23:175, 1981), and other cell lines capable of expressing an appropriate vector including, for example, L cells, C127, 3T3, Chinese hamster ovary (CHO), HeLa and BHK cell lines. Mammalian expression vectors can comprise nontranscribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5' or 3' flanking nontranscribed sequences, and 5' or 3' nontranslated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, Bio/Technology 6:47 (1988).

[00110] The proteins produced by a transformed host can be purified according to any suitable method. Such standard methods include chromatography (e.g., ion exchange, affinity and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for protein purification. Affinity tags such as hexahistidine, maltose binding domain, influenza coat sequence and glutathione-S-transferase can be attached to the protein to allow easy purification by passage over an appropriate affinity column. Isolated proteins can also be physically characterized using such techniques as proteolysis, nuclear magnetic resonance and x-ray crystallography.

[00111] For example, supernatants from systems which secrete recombinant protein into culture media can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a suitable purification matrix. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a FOLR1-binding agent. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a homogeneous recombinant protein.

[00112] Recombinant protein produced in bacterial culture can be isolated, for example, by initial extraction from cell pellets, followed by one or more concentration, salting-out, aqueous ion exchange or size exclusion chromatography steps. High performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of a recombinant protein can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

[00113] Methods known in the art for purifying antibodies and other proteins also include, for example, those described in U.S. Patent Publication No. 2008/0312425, 2008/0177048, and 2009/0187005, each of which is hereby incorporated by reference herein in its entirety.

III. Immunoconjugates

[00114] Methods for administering conjugates comprising the anti-FOLR1 antibodies, antibody fragments, and their functional equivalents as disclosed herein, linked or conjugated to a drug or prodrug (also referred to herein as immunoconjugates) are also described herein. Suitable drugs or prodrugs are known in the art. The drugs or prodrugs can be cytotoxic agents. The cytotoxic agent used in the cytotoxic conjugate of the present invention can be any compound that results in the death of a cell, or induces cell death, or in some manner decreases cell viability, and includes, for example, maytansinoids and maytansinoid analogs. Other suitable cytotoxic agents are for example benzodiazepines, taxoids, CC-1065 and CC-1065 analogs, duocarmycins and duocarmycin analogs, enediynes, such as calicheamicins, dolastatin and dolastatin analogs including auristatins, tomaymycin derivatives, leptomyacin derivatives, methotrexate, cisplatin, carboplatin, daunorubicin, doxorubicin, vincristine, vinblastine, melphalan, mitomycin C, chlorambucil and morpholino doxorubicin.

[00115] Such conjugates can be prepared by using a linking group in order to link a drug or prodrug to the antibody or functional equivalent. Suitable linking groups are well known in the art and include, for example, disulfide groups, thioether groups, acid labile groups, photolabile groups, peptidase labile groups and esterase labile groups.

[00116] The drug or prodrug can, for example, be linked to the anti-FOLR1 antibody or fragment thereof through a disulfide bond. The linker molecule or crosslinking agent comprises a reactive chemical group that can react with the anti-FOLR1 antibody or fragment thereof. The reactive chemical groups for reaction with the cell-binding agent can be *N*-succinimidyl esters and *N*-sulfosuccinimidyl esters. Additionally the linker molecule comprises a reactive chemical group, which can be a dithiopyridyl group that can react with the drug to form a disulfide bond. Linker molecules include, for example, *N*-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) (see, e.g., Carlsson et al., *Biochem. J.*, 173: 723-737 (1978)), *N*-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB) (see, e.g., U.S. Patent No. 4,563,304), *N*-succinimidyl 4-(2-pyridyldithio)2-sulfobutanoate (sulfo-SPDB) (see US Publication No. 20090274713), *N*-succinimidyl 4-(2-pyridyldithio) pentanoate (SPP) (see, e.g., CAS Registry number 341498-08-6), 2-iminothiolane, or acetylsuccinic anhydride. For example, the antibody or cell binding agent can be modified with crosslinking reagents and the antibody or cell binding agent containing free or protected thiol groups thus derived is then reacted with a disulfide- or thiol-containing maytansinoid to produce conjugates. The conjugates can be purified by chromatography, including but not limited to HPLC, size-exclusion, adsorption, ion exchange and affinity capture, dialysis or tangential flow filtration.

[00117] In another aspect of the present invention, the anti-FOLR1 antibody is linked to cytotoxic drugs via disulfide bonds and a polyethylene glycol spacer in enhancing the potency, solubility or the efficacy of the immunoconjugate. Such cleavable hydrophilic linkers are described in WO2009/0134976. The additional benefit of this linker design is the desired high monomer ratio and the minimal aggregation of the antibody-drug conjugate. Specifically contemplated in this aspect are conjugates of cell-binding agents and drugs linked via disulfide group (-S-S-) bearing polyethylene glycol spacers $((\text{CH}_2\text{CH}_2\text{O})_{n=1-14})$ with a narrow range of drug load of 2-8 are described that show relatively high potent biological activity toward cancer cells and have the desired biochemical properties of high conjugation yield and high monomer ratio with minimal protein aggregation.

[00118] Antibody-maytansinoid conjugates with non-cleavable linkers can also be prepared. Such crosslinkers are described in the art (see US Publication No. 20050169933) and include but are not limited to, *N*-succinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (SMCC). In some embodiments, the antibody is modified with crosslinking reagents such as succinimidyl 4-(*N*-maleimidomethyl)-cyclohexane-1-carboxylate (SMCC), sulfo-SMCC, maleimidobenzoyl-*N*-hydroxysuccinimide ester (MBS), sulfo-MBS or succinimidyl-iodoacetate, as described in the literature, to introduce 1-10 reactive groups (Yoshitake et al, Eur. J. Biochem., 101:395-399 (1979); Hashida et al, J. Applied Biochem., 56-63 (1984); and Liu et al, Biochem., 18:690-697 (1979)). The modified antibody is then reacted with the thiol-containing maytansinoid derivative to produce a conjugate. The conjugate can be purified by gel filtration through a Sephadex G25 column or by dialysis or tangential flow filtration. The modified antibodies are treated with the thiol-containing maytansinoid (1 to 2 molar equivalent/maleimido group) and antibody-maytansinoid conjugates are purified by gel filtration through a Sephadex G-25 column, chromatography on a ceramic hydroxyapatite column, dialysis or tangential flow filtration or a combination of methods thereof. Typically, an average of 1-10 maytansinoids per antibody are linked. One method is to modify antibodies with succinimidyl 4-(*N*-maleimidomethyl)-cyclohexane-1-carboxylate (SMCC) to introduce maleimido groups followed by reaction of the modified antibody with a thiol-containing maytansinoid to give a thioether-linked conjugate. Again conjugates with 1 to 10 drug molecules per antibody molecule result. Maytansinoid conjugates of antibodies, antibody fragments, and other proteins are made in the same way.

[00119] In another aspect of the invention, the FOLR1 antibody is linked to the drug via a non-cleavable bond through the intermediacy of a PEG spacer. Suitable crosslinking reagents comprising hydrophilic PEG chains that form linkers between a drug and the anti-FOLR1 antibody or fragment are also well known in the art, or are commercially available (for example from Quanta Biodesign, Powell, Ohio). Suitable PEG-containing crosslinkers can also be synthesized from commercially available PEGs themselves using standard synthetic chemistry techniques known to one skilled in the art. The drugs can be reacted with bifunctional PEG-containing cross linkers to give compounds of the following formula, Z

$-X_I-(CH_2-CH_2-O)_n-Y_p-D$, by methods described in detail in US Patent Publication 20090274713 and in WO2009/0134976, which can then react with the cell binding agent to provide a conjugate. Alternatively, the cell binding can be modified with the bifunctional PEG crosslinker to introduce a thiol-reactive group (such as a maleimide or haloacetamide) which can then be treated with a thiol-containing maytansinoid to provide a conjugate. In another method, the cell binding can be modified with the bifunctional PEG crosslinker to introduce a thiol moiety which can then be treated with a thiol-reactive maytansinoid (such as a maytansinoid bearing a maleimide or haloacetamide), to provide a conjugate.

[00120] Examples of suitable PEG-containing linkers include linkers having an *N*-succinimidyl ester or *N*-sulfosuccinimidyl ester moiety for reaction with the anti-FOLR1 antibody or fragment thereof, as well as a maleimido- or haloacetyl-based moiety for reaction with the compound. A PEG spacer can be incorporated into any crosslinker known in the art by the methods described herein.

[00121] In some embodiments, the linker is a linker containing at least one charged group as described, for example, in U.S. Patent Publication No. 2012/0282282, the contents of which are entirely incorporated herein by reference. In some embodiments, the charged or pro-charged cross-linkers are those containing sulfonate, phosphate, carboxyl or quaternary amine substituents that significantly increase the solubility of the modified cell-binding agent and the cell-binding agent-drug conjugates, especially for monoclonal antibody-drug conjugates with 2 to 20 drugs/antibody linked. Conjugates prepared from linkers containing a pro-charged moiety would produce one or more charged moieties after the conjugate is metabolized in a cell. In some embodiments, the linker is selected from the group consisting of: *N*-succinimidyl 4-(2-pyridyldithio)-2-sulfopentanoate (sulfo-SPP) and *N*-succinimidyl 4-(2-pyridyldithio)-2-sulfobutanoate (sulfo-SPDB).

[00122] Many of the linkers disclosed herein are described in detail in U.S. Patent Publication Nos. 2005/0169933, 2009/0274713, and 2012/0282282, and in WO2009/0134976; the contents of which are entirely incorporated herein by reference.

[00123] The present invention includes aspects wherein about 2 to about 8 drug molecules ("drug load"), for example, maytansinoid, are linked to an anti-FOLR1 antibody or fragment thereof. "Drug load", as used herein, refers to the number of drug molecules (e.g., a maytansinoid) that can be attached to a cell binding agent (e.g., an anti-FOLR1 antibody or fragment thereof). In one aspect, the number of drug molecules that can be attached to a cell binding agent can average from about 2 to about 8 (e.g., 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1). *N*2'-deacetyl-*N*2'-(3-mercapto-1-oxopropyl)-maytansine (DM1) and *N*2'-deacetyl-*N*2'-(4-mercapto-4-methyl-1-oxopentyl) maytansine (DM4) can be used.

[00124] Thus, in one aspect, an immunoconjugate comprises 1 maytansinoid per antibody. In another aspect, an immunoconjugate comprises 2 maytansinoids per antibody. In another aspect, an immunoconjugate comprises 3 maytansinoids per antibody. In another aspect, an immunoconjugate comprises 4 maytansinoids per antibody. In another aspect, an immunoconjugate comprises 5 maytansinoids per antibody. In another aspect, an immunoconjugate comprises 6 maytansinoids per antibody. In another aspect, an immunoconjugate comprises 7 maytansinoids per antibody. In another aspect, an immunoconjugate comprises 8 maytansinoids per antibody.

[00125] In one aspect, an immunoconjugate comprises about 1 to about 8 maytansinoids per antibody. In another aspect, an immunoconjugate comprises about 2 to about 7 maytansinoids per antibody. In another aspect, an immunoconjugate comprises about 2 to about 6 maytansinoids per antibody. In another aspect, an immunoconjugate comprises about 2 to about 5 maytansinoids per antibody. In another aspect, an immunoconjugate comprises about 3 to about 5 maytansinoids per antibody. In another aspect, an immunoconjugate comprises about 3 to about 4 maytansinoids per antibody.

[00126] In one aspect, a composition comprising immunoconjugates has an average of about 2 to about 8 (e.g., 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1) drug molecules (e.g., maytansinoids) attached per antibody. In one aspect, a composition comprising immunoconjugates has an average of about 1 to about 8 drug molecules (e.g., maytansinoids) per antibody. In one aspect, a composition comprising immunoconjugates has an average of about 2 to about 7 drug molecules (e.g., maytansinoids) per antibody. In one aspect, a composition comprising immunoconjugates has an average of about 2 to about 6 drug molecules (e.g., maytansinoids) per antibody. In one aspect, a composition comprising immunoconjugates has an average of about 2 to about 5 drug molecules (e.g., maytansinoids) per antibody. In one aspect, a composition comprising immunoconjugates has an average of about 3 to about 5 drug molecules (e.g., maytansinoids) per antibody. In one aspect, a composition comprising immunoconjugates has an average of about 3 to about 4 drug molecules (e.g., maytansinoids) per antibody.

[00127] In one aspect, a composition comprising immunoconjugates has an average of about 2 ± 0.5 , about 3 ± 0.5 , about 4 ± 0.5 , about 5 ± 0.5 , about 6 ± 0.5 , about 7 ± 0.5 , or about 8 ± 0.5 drug molecules (e.g., maytansinoids) attached per antibody. In one aspect, a composition comprising immunoconjugates has an average of about 3.5 ± 0.5 drug molecules (e.g., maytansinoids) per antibody.

[00128] The anti-FOLR1 antibody or fragment thereof can be modified by reacting a bifunctional crosslinking reagent with the anti-FOLR1 antibody or fragment thereof, thereby resulting in the covalent attachment of a linker molecule to the anti-FOLR1 antibody or fragment thereof. As used herein, a "bifunctional crosslinking reagent" is any chemical moiety that covalently links a cell-binding agent to a

drug, such as the drugs described herein. In another method, a portion of the linking moiety is provided by the drug. In this respect, the drug comprises a linking moiety that is part of a larger linker molecule that is used to join the cell-binding agent to the drug. For example, to form the maytansinoid DM1, the side chain at the C-3 hydroxyl group of maytansine is modified to have a free sulfhydryl group (SH). This thiolated form of maytansine can react with a modified cell-binding agent to form a conjugate. Therefore, the final linker is assembled from two components, one of which is provided by the crosslinking reagent, while the other is provided by the side chain from DM1.

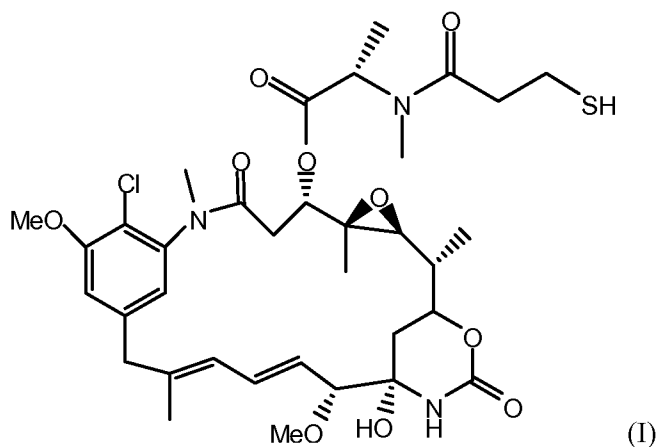
[00129] The drug molecules can also be linked to the antibody molecules through an intermediary carrier molecule such as serum albumin.

[00130] As used herein, the expression "linked to a cell-binding agent" or "linked to an anti-FOLR1 antibody or fragment" refers to the conjugate molecule comprising at least one drug derivative bound to a cell-binding agent anti-FOLR1 antibody or fragment via a suitable linking group, or a precursor thereof. Exemplary linking groups are SPDB or sulfo-SPDB.

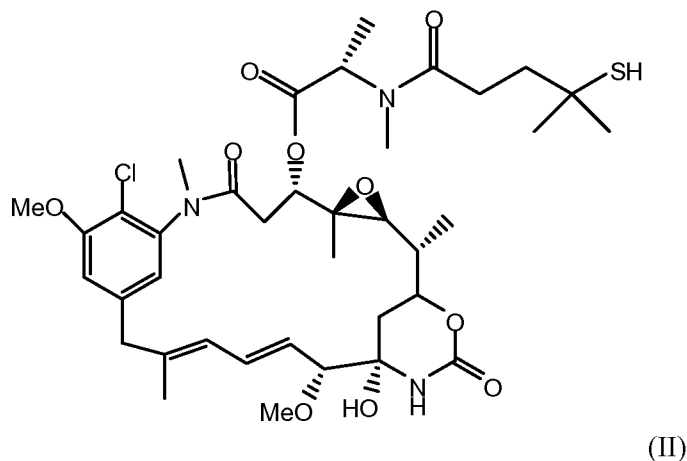
[00131] In certain embodiments, cytotoxic agents useful in the present invention are maytansinoids and maytansinoid analogs. Examples of suitable maytansinoids include esters of maytansinol and maytansinol analogs. Included are any drugs that inhibit microtubule formation and that are highly toxic to mammalian cells, as are maytansinol and maytansinol analogs.

[00132] Examples of suitable maytansinol esters include those having a modified aromatic ring and those having modifications at other positions. Such suitable maytansinoids are disclosed in U.S. Patent Nos. 4,424,219; 4,256,746; 4,294,757; 4,307,016; 4,313,946; 4,315,929; 4,331,598; 4,361,650; 4,362,663; 4,364,866; 4,450,254; 4,322,348; 4,371,533; 5,208,020; 5,416,064; 5,475,092; 5,585,499; 5,846,545; 6,333,410; 7,276,497 and 7,473,796.

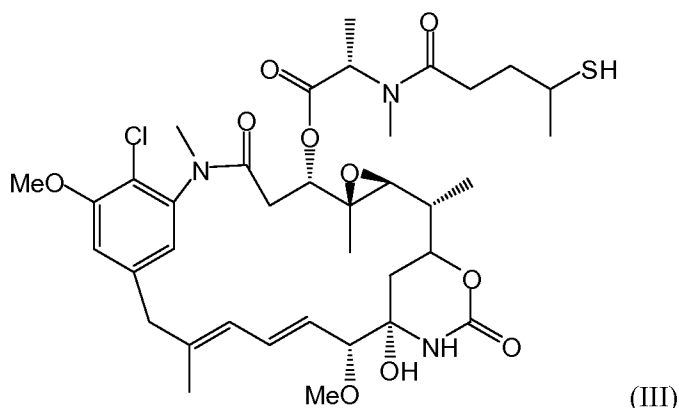
[00133] In a certain embodiment, the immunoconjugates of the invention utilize the thiol-containing maytansinoid (DM1), formally termed *N*²-deacetyl-*N*²-(3-mercapto-1-oxopropyl)-maytansine, as the cytotoxic agent. DM1 is represented by the following structural formula (I):



[00134] In another embodiment, the conjugates of the present invention utilize the thiol-containing maytansinoid *N*^{2'}-deacetyl-*N*^{2'}-(4-methyl-4-mercapto-1-oxopentyl)-maytansine (e.g., DM4) as the cytotoxic agent. DM4 is represented by the following structural formula (II):



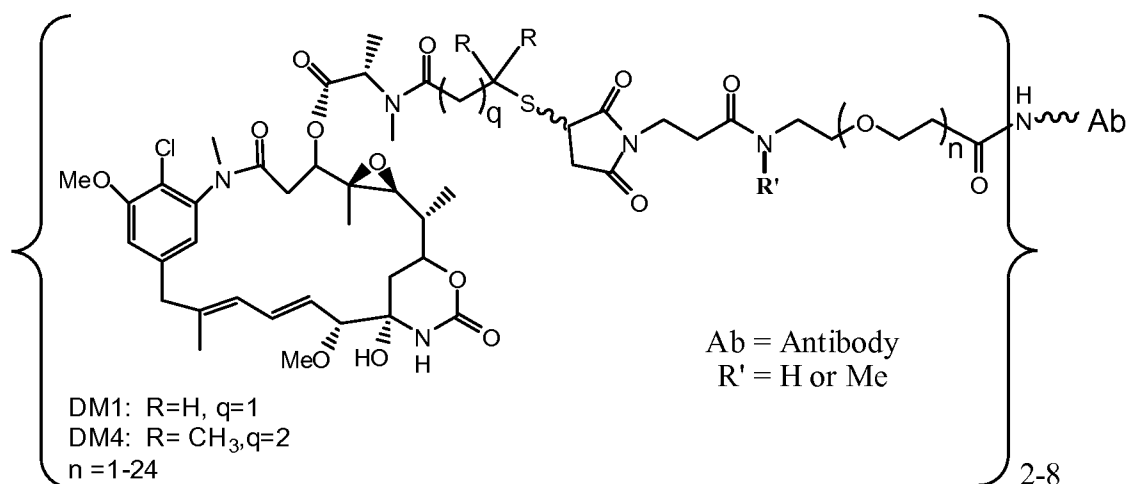
[00135] Another maytansinoid comprising a side chain that contains a sterically hindered thiol bond is *N*^{2'}-deacetyl-*N*^{2'}-(4-mercapto-1-oxopentyl)-maytansine (termed DM3), represented by the following structural formula (III):



[00136] Each of the maytansinoids taught in US Patent No. 5,208,020 and 7,276,497, can also be used in the conjugate of the present invention. In this regard, the entire disclosure of 5,208,020 and 7,276,697 is incorporated herein by reference.

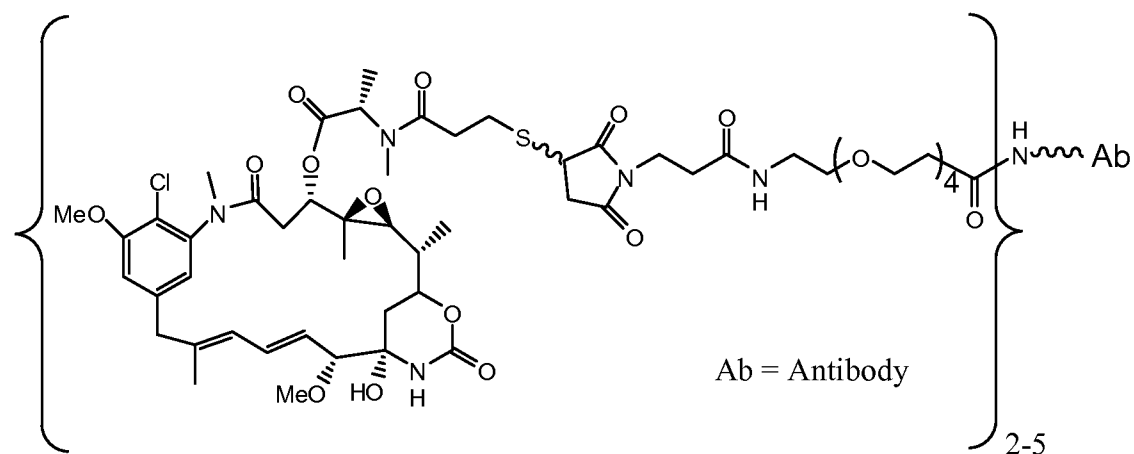
[00137] Many positions on maytansinoids can serve as the position to chemically link the linking moiety. For example, the C-3 position having a hydroxyl group, the C-14 position modified with hydroxymethyl, the C-15 position modified with hydroxy and the C-20 position having a hydroxy group are all expected to be useful. In some embodiments, the C-3 position serves as the position to chemically link the linking moiety, and in some particular embodiments, the C-3 position of maytansinol serves as the position to chemically link the linking moiety.

[00138] Structural representations of some conjugates are shown below:



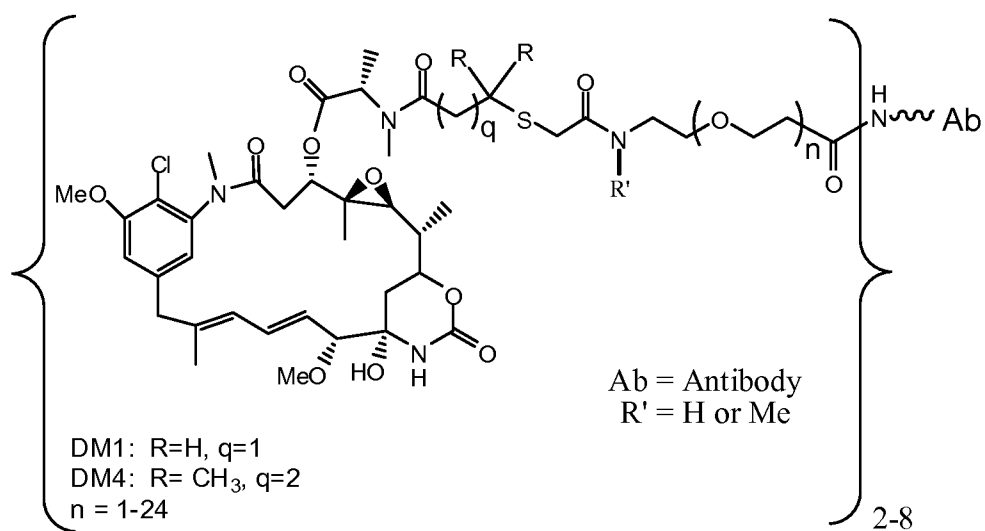
Ab-PEG-Mal-DM1/DM4

(IV)



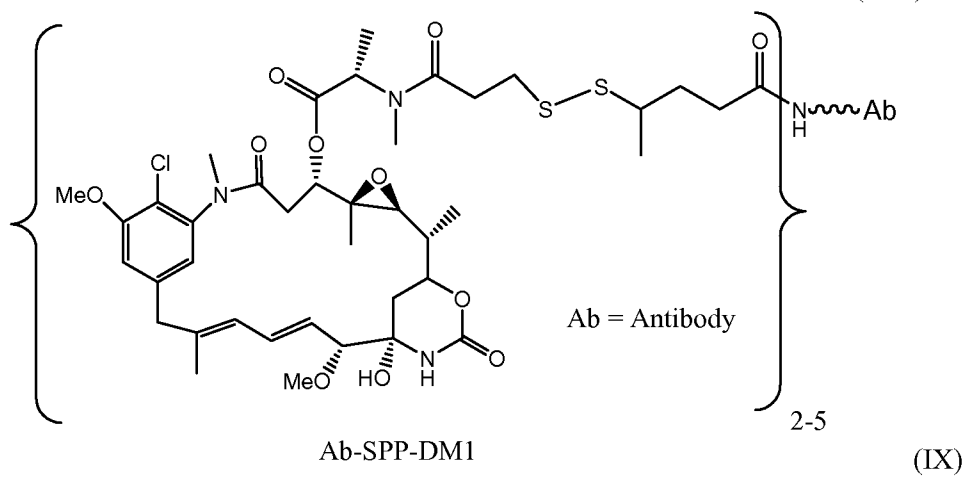
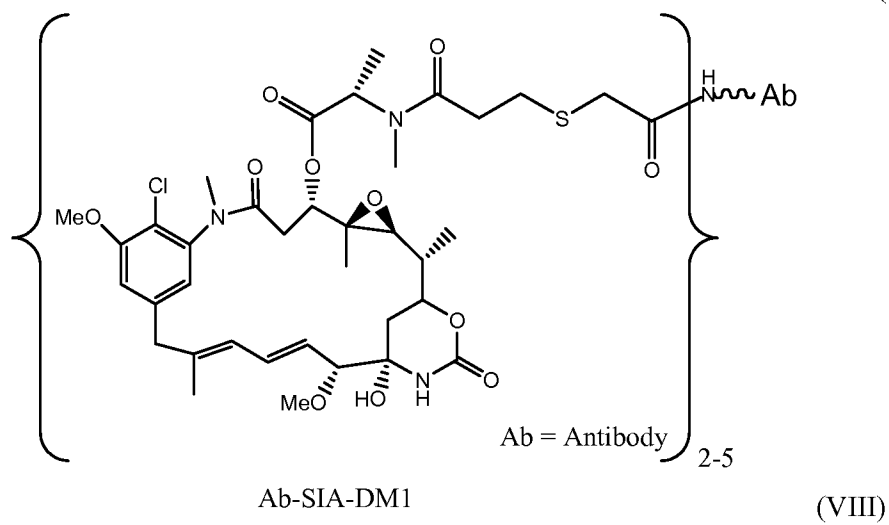
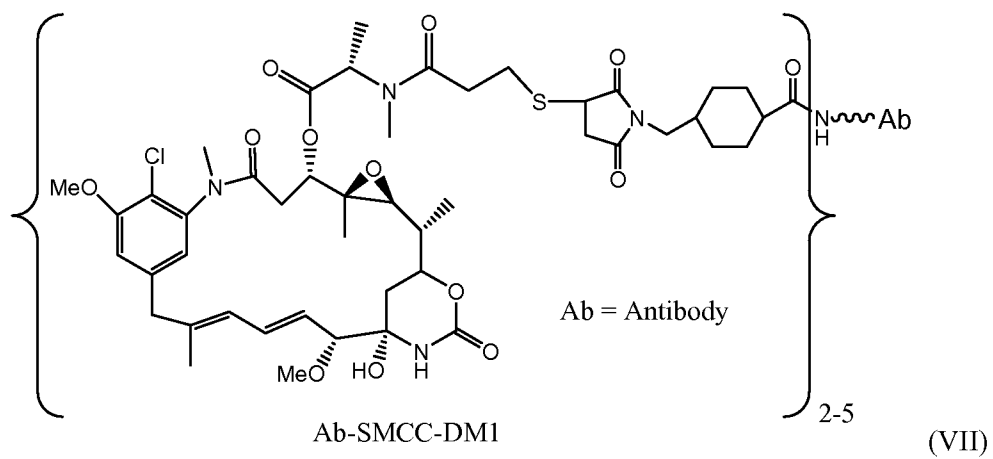
Ab-PEG4-Mal-DM1

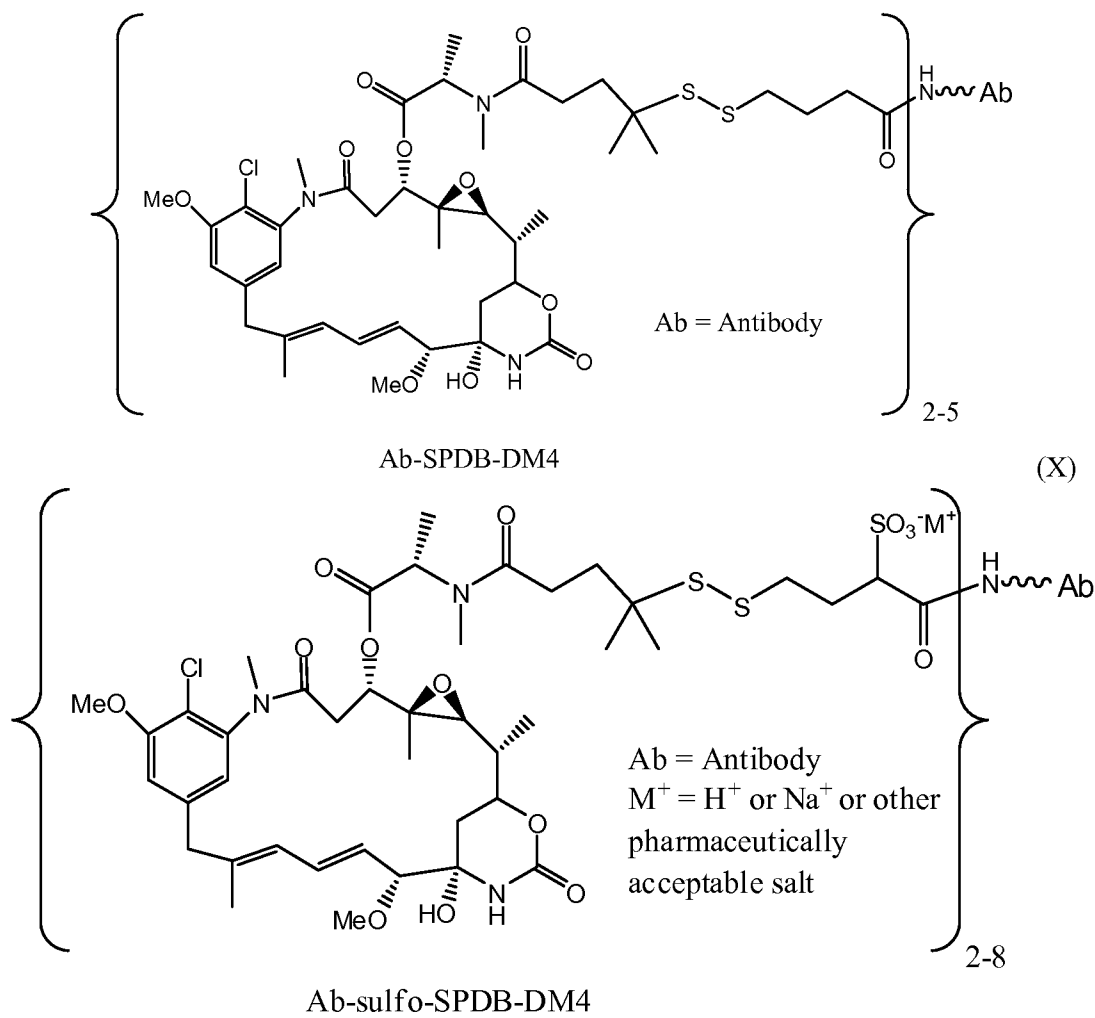
(V)



Ab-PEG-SIA-DM1/DM4

(VI)





[00139] Also included in the present invention are any stereoisomers and mixtures thereof for any compounds or conjugates depicted by any structures above.

[00140] Several descriptions for producing such antibody-maytansinoid conjugates are provided in U.S. Patent Nos. 6,333,410, 6,441,163, 6,716,821, and 7,368,565, each of which is incorporated herein in its entirety.

[00141] In general, a solution of an antibody in aqueous buffer can be incubated with a molar excess of maytansinoids having a disulfide moiety that bears a reactive group. The reaction mixture can be quenched by addition of excess amine (such as ethanolamine, taurine, etc.). The maytansinoid-antibody conjugate can then be purified by gel filtration.

[00142] The number of maytansinoid molecules bound per antibody molecule can be determined by measuring spectrophotometrically the ratio of the absorbance at 252 nm and 280 nm. The average number of maytansinoid molecules/antibody can be, for example, 1-10 or 2-5. The average number of maytansinoid molecules/antibody can be, for example about 3 to about 4. The average number of maytansinoid molecules/antibody can be about 3.5.

[00143] Conjugates of antibodies with maytansinoid or other drugs can be evaluated for their ability to suppress proliferation of various unwanted cell lines *in vitro*. For example, cell lines such as the human lymphoma cell line Daudi and the human lymphoma cell line Ramos, can easily be used for the assessment of cytotoxicity of these compounds. Cells to be evaluated can be exposed to the compounds for 4 to 5 days and the surviving fractions of cells measured in direct assays by known methods. IC_{50} values can then be calculated from the results of the assays.

[00144] The immunoconjugates can, according to some embodiments described herein, be internalized into cells. The immunoconjugate, therefore, can exert a therapeutic effect when it is taken up by, or internalized, by a FOLR1-expressing cell. In some particular embodiments, the immunoconjugate comprises an antibody, antibody fragment, or polypeptide, linked to a cytotoxic agent by a cleavable linker, and the cytotoxic agent is cleaved from the antibody, antibody fragment, or polypeptide, wherein it is internalized by a FOLR1-expressing cell.

[00145] In some embodiments, the immunoconjugates are capable of reducing tumor volume. For example, in some embodiments, treatment with an immunoconjugate results in a %T/C value that is less than about 50%, less than about 45%, less than about 40%, less than about 35%, less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, or less than about 5%. In some particular embodiments, the immunoconjugates can reduce tumor size in a KB, OVCAR-3, IGROV-1, and/or OV-90 xenograft model. In some embodiments, the immunoconjugates are capable of inhibiting metastases.

III. Methods of administering FOLR1-binding agents

[00146] The FOLR1-binding agents (including antibodies, immunoconjugates, and polypeptides) of the invention are useful in a variety of applications including, but not limited to, therapeutic treatment methods, such as the treatment of cancer. In certain embodiments, the agents are useful for inhibiting tumor growth, inducing differentiation, inhibiting metastases, reducing tumor volume, and/or reducing the tumorigenicity of a tumor. The methods of use can be *in vivo* methods.

[00147] According to the methods described herein, the FOLR1-binding agents can be administered at particular dosages. For example, the FOLR1-binding agents (e.g., IMGN853) can be administered at a dose of about 0.15 mg/kg to about 7 mg/kg. In some embodiments, the FOLR1-binding agents (e.g., IMGN853) are administered at a dose of about 3.0 mg/kg to about 6.0 mg/kg. In some embodiments, the FOLR1-binding agents (e.g., IMGN853) are administered at a dose of about 3.3 mg/kg to about 6.0 mg/kg. In some embodiments, the FOLR1-binding agents (e.g., IMGN853) are administered at about 0.15 mg/kg. Thus, in some embodiments, the FOLR1-binding agents (e.g., IMGN853) are administered at about 0.5 mg/kg. In some embodiments, the FOLR1-binding agents (e.g., IMGN853) are administered at about 1.0 mg/kg. In some embodiments, the FOLR1-binding agents (e.g., IMGN853) are administered

at about 2.0 mg/kg. In some embodiments, the FOLR1-binding agents (e.g., IMGN853) are administered at about 3.0 mg/kg. In some embodiments, the FOLR1-binding agents (e.g., IMGN853) are administered at about 3.3 mg/kg. In some embodiments, the FOLR1-binding agents (e.g., IMGN853) are administered at about 5.0 mg/kg. In some embodiments, the FOLR1-binding agents (e.g., IMGN853) are administered at about 5.5 mg/kg. In some embodiments, the FOLR1-binding agents (e.g., IMGN853) are administered at about 6.0 mg/kg. In some embodiments, the FOLR1-binding agents (e.g., IMGN853) are administered at about 6.5 mg/kg. In some embodiments, the FOLR1-binding agents (e.g., IMGN853) are administered at about 7.0 mg/kg.

[00148] Furthermore, the FOLR1-binding agents can be administered at particular dose interval. For example, the the FOLR1-binding agents can be administered from about four times a week to about once every four weeks. Thus, in some embodiments, the FOLR1-binding agents are administered about once every three weeks. In some embodiments, the FOLR1-binding agents are administered about once every two and a half weeks. In some embodiments, the FOLR1-binding agents are administered about once every two weeks. In some embodiments, the FOLR1-binding agents are administered about once every ten days. In some embodiments, the FOLR1-binding agents are administered about once every week. In some embodiments, the FOLR1-binding agents are administered about once every five days. In some embodiments, the FOLR1-binding agents are administered about once every four days. In some embodiments, the FOLR1-binding agents are administered about once every three days. In some embodiments, the FOLR1-binding agents are administered about once every two days. In some embodiments, the FOLR1-binding agents are administered about twice a week. In some embodiments, the FOLR1-binding agents are administered about three times a week.

[00149] The FOLR1-binding agents can also be administered in an about 3-week (i.e. about 21-day) cycle. For example, the FOLR1-binding agents can be administered twice in about 3 weeks. Thus, in some embodiments, the FOLR1-binding agents can be administered at about days 1 and 8 of a 21-day cycle. In other embodiments, the FOLR1-binding agents can be administered three times in about 3 weeks. Thus, in some embodiments, the FOLR1-binding agents can be administered at about days 1, 8, and 15 of a 21-day cycle.

[00150] The FOLR1-binding agents can also be administered in an about 4-week (i.e. about 28-day) cycle. For example, the FOLR1-binding agents can be administered three times in about 4 weeks. Thus, in some embodiments, the FOLR1-binding agents can be administered at about days 1, 8, and 15 of a 28-day cycle.

[00151] In some embodiments, the FOLR1-binding agents can be administered at a dose that results in a particular C_{max}. For example, the FOLR1-binding agents can be administered at a dose that results in a C_{max} of about 0.5 to about 250 µg/mL. Thus, in some embodiments, the FOLR1-binding agents are administered at a dose that results in a C_{max} of about 50 to about 250 µg/mL. In some embodiments, the

FOLR1-binding agents are administered at a dose that results in a C_{max} of about 50 to about 200 µg/mL. Thus, in some embodiments, the FOLR1-binding agents are administered at a dose that results in a C_{max} of about 50 to about 175 µg/mL. In some embodiments, the FOLR1-binding agents are administered at a dose that results in a C_{max} of about 50 to about 150 µg/mL. Thus, in some embodiments, the FOLR1-binding agents are administered at a dose that results in a C_{max} of about 100 to about 175 µg/mL. In some embodiments, the FOLR1-binding agents are administered at a dose that results in a C_{max} of about 100 to about 150 µg/mL.

[00152] In certain embodiments, the FOLR1-binding agents can be administered at a dose that results in a particular AUC. For example, the FOLR1-binding agents can be administered at a dose that results in an AUC of about 50 hr•µg/mL to about 18,000 hr•µg/mL. In some embodiments, the FOLR1-binding agents can be administered at a dose that results in an AUC of about 10,000 hr•µg/mL to about 18,000 hr•µg/mL. In some embodiments, the FOLR1-binding agents can be administered at a dose that results in an AUC of about 10,000 hr•µg/mL to about 17,500 hr•µg/mL. In some embodiments, the FOLR1-binding agents can be administered at a dose that results in an AUC of about 10,000 hr•µg/mL to about 17,000 hr•µg/mL. In some embodiments, the FOLR1-binding agents can be administered at a dose that results in an AUC of about 10,000 hr•µg/mL to about 16,000 hr•µg/mL. In some embodiments, the FOLR1-binding agents can be administered at a dose that results in an AUC of about 10,000 hr•µg/mL to about 15,000 hr•µg/mL.

[00153] In certain embodiments, the disease treated with the FOLR1-binding agent or antagonist (e.g., an anti-FOLR1 antibody) is a cancer. In certain embodiments, the cancer is characterized by FOLR1 expressing cells to which the FOLR1-binding agent (e.g., antibody) binds. In certain embodiments, a tumor overexpresses the human FOLR1.

[00154] The present invention provides for methods of treating cancer comprising administering a therapeutically effective amount of a FOLR1-binding agent to a subject (e.g., a subject in need of treatment). Cancers that can be treated by the methods encompassed by the invention include, but are not limited to, neoplasms, tumors, metastases, or any disease or disorder characterized by uncontrolled cell growth. The cancer can be a primary or metastatic cancer. Specific examples of cancers that can be treated by the methods encompassed by the invention include, but are not limited to ovarian cancer, lung cancer, colorectal cancer, pancreatic cancer, liver cancer, breast cancer, brain cancer, kidney cancer, prostate cancer, gastrointestinal cancer, melanoma, cervical cancer, bladder cancer, glioblastoma, and head and neck cancer. In certain embodiments, the cancer is ovarian cancer. In certain embodiments, the cancer is lung cancer.

[00155] In some embodiments, the cancer is a cancer that expresses FOLR1 (polypeptide or nucleic acid). In some embodiments, the FOLR1-binding agent is administered to a patient with an increased expression level of FOLR1, for example, as described in U.S. Published Application No. 2012/0282175 or

International Published Application No. WO 2012/135675, both of which are incorporated by reference herein in their entireties. Thus, in some embodiments, the FOLR1 expression is measured by immunohistochemistry (IHC) and given a staining intensity score and/or a staining uniformity score by comparison to controls (e.g., calibrated controls) exhibiting defined scores (e.g. an intensity score of 3 is given to the test sample if the intensity is comparable to the level 3 calibrated control or an intensity of 2 is given to the test sample if the intensity is comparable to the level 2 calibrated control). A staining uniformity that is heterogeneous or homogeneous is also indicative of increased FOLR1 expression. The staining intensity and staining uniformity scores can be used alone or in combination (e.g., 2 homo, 2 hetero, 3 homo, 3 hetero, etc.). In another example, an increase in FOLR1 expression can be determined by detection of an increase of at least 2-fold, at least 3-fold, or at least 5-fold) relative to control values (e.g., expression level in a tissue or cell from a subject without cancer or with a cancer that does not have elevated FOLR1 values).

[00156] In some embodiments, the cancer is a cancer that express FOLR1 at a level of 2 hetero or higher by IHC. In some embodiments, the cancer is a cancer that express FOLR1 at a level of 3 hetero or higher by IHC. In some embodiments, the cancer is a lung cancer that expresses FOLR1 at a level of 2 hetero or higher by IHC. In some embodiments, the cancer is a lung cancer that expresses FOLR1 at a level of 3 hetero or higher by IHC.

[00157] In certain embodiments, the method of inhibiting tumor growth comprises administering to a subject a therapeutically effective amount of a FOLR1-binding agent. In certain embodiments, the subject is a human. In certain embodiments, the subject has a tumor or has had a tumor removed.

[00158] In addition, the invention provides a method of reducing the tumorigenicity of a tumor in a subject, comprising administering a therapeutically effective amount of a FOLR1-binding agent to the subject. In certain embodiments, the tumor comprises cancer stem cells. In certain embodiments, the frequency of cancer stem cells in the tumor is reduced by administration of the agent.

[00159] The present invention further provides pharmaceutical compositions comprising one or more of the FOLR1-binding agents described herein. In certain embodiments, the pharmaceutical compositions further comprise a pharmaceutically acceptable vehicle. These pharmaceutical compositions find use in inhibiting tumor growth and treating cancer in human patients.

[00160] In certain embodiments, formulations are prepared for storage and use by combining a purified antibody or agent of the present invention with a pharmaceutically acceptable vehicle (e.g. carrier, excipient) (Remington, The Science and Practice of Pharmacy 20th Edition Mack Publishing, 2000). Suitable pharmaceutically acceptable vehicles include, but are not limited to, nontoxic buffers such as phosphate, citrate, and other organic acids; salts such as sodium chloride; antioxidants including ascorbic acid and methionine; preservatives (e.g. octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl

alcohol; alkyl parabens, such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight polypeptides (e.g. less than about 10 amino acid residues); proteins such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; carbohydrates such as monosaccharides, disaccharides, glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and non-ionic surfactants such as TWEEN or polyethylene glycol (PEG).

[00161] The pharmaceutical compositions described herein can be administered in any number of ways for either local or systemic treatment. Administration can be topical (such as to mucous membranes including vaginal and rectal delivery) such as transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders; pulmonary (e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal); oral; or parenteral including intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial (e.g., intrathecal or intraventricular) administration. In some particular embodiments, the administration is intravenous.

[00162] An antibody or immunoconjugate can be combined in a pharmaceutical combination formulation, or dosing regimen as combination therapy, with a second compound. In some embodiments, the second compound is a steroid. In some embodiments, the methods encompass administration of a steroid and an immunoconjugate that results in a reduction of headaches as compared to administration of the immunoconjugate alone.

[00163] The steroid can be administered at the same time as the immunoconjugate, prior to the administration of the immunoconjugate, and/or after the administration of the immunoconjugate. In some embodiments, the steroid is administered within about a week, about five days, about three days, about two days, or about one day or 24 hours prior to the administration of the immunoconjugate. In some embodiments, the steroid is administered within one day of the administration of the immunoconjugate. In some embodiments, the steroid is administered multiple times. In some embodiments, the steroid is administered about one day prior to the administration of the immunoconjugate and on the same day as the administration of the immunoconjugate. The steroid can be administered via any number of ways, including for example, topical, pulmonary, oral, parenteral, or intracranial administration. In some embodiments, the administration is oral. In some embodiments, the administration is intravenous. In some embodiments, the administration is both oral and intravenous.

[00164] An antibody or immunoconjugate can also be combined in a pharmaceutical combination formulation, or dosing regimen as combination therapy, with an analgesic, or other medications that prevent or treat headaches. For example, acetaminophen and/or dephenhydramine can be administered in

addition to the administration of the antibody or immunoconjugate. The analgesic can be administered prior to, at the same time, or after the administration of the immunoconjugate and can be via any appropriate administration route. In some embodiments, the analgesic is administered orally.

[00165] In some embodiments, the methods comprise administration of a first compound that is an antibody or immunoconjugate, a second compound that is a steroid, and a third compound that is an analgesic. In some embodiments, the methods comprise administration of a first compound that is IMGN388, a second compound that is dexamethasone, and a third compound that is acetaminophin and/or diphenhydramine.

[00166] An antibody or immunoconjugate can be combined in a pharmaceutical combination formulation, or dosing regimen as combination therapy, with a second compound having anti-cancer properties. The second compound of the pharmaceutical combination formulation or dosing regimen can have complementary activities to the ADC of the combination such that they do not adversely affect each other. Pharmaceutical compositions comprising the FOLR1-binding agent and the second anti-cancer agent are also provided.

[00167]

* * *

[00168] Embodiments of the present disclosure can be further defined by reference to the following non-limiting examples, which describe in detail preparation of certain antibodies of the present disclosure and methods for using antibodies of the present disclosure. It will be apparent to those skilled in the art that many modifications, both to materials and methods, can be practiced without departing from the scope of the present disclosure.

Examples

[00169] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application

Example 1

IMGN853 Dosing Trial in Human Cancer Patients

[00170] IMGN853 is an antibody-drug conjugate (ADC) comprising a folate receptor 1 (FOLR1)-binding antibody and the potent maytansinoid, DM4. IMGN853 has been previously described in International Published Application Nos. WO 2011/106528, WO 2012/135675, and WO 2012/138749, and U.S. Published Application Nos. 2012/0009181, 2012/0282175, and 2012/0282282, each of which is

incorporated by reference herein in its entirety. IMGN853 is huMov19-sSPDB-DM4, and the huMov19 antibody contains a variable heavy chain with the amino acid sequence of SEQ ID NO:3 and a variable light chain with the amino acid sequence of SEQ ID NO: 5. FOLR1 protein is expressed at elevated levels on many solid tumors, particularly epithelial ovarian cancer (EOC), endometrial cancer, non-small cell lung cancer (NSCLC), and clear-cell renal cell cancer.

[00171] A study to determine the maximum tolerated dose (MTD) and recommended phase 2 dose (RP2D) as well as to evaluate the safety, pharmacokinetics (PK), pharmacodynamics (PD), and efficacy of IMGN853 was initiated. The study includes two components: an accelerated dose titration component, where the IMGN853 immunoconjugate was administered to patients with any type of FOLR1-expressing refractory solid tumors including epithelial ovarian cancer (EOC) and other FOLR1-positive solid tumors, and a dose expansion component.

[00172] For the accelerated titration portion of the study, IMGN853 was given intravenously (IV) on Day 1 of each 21-day (3 week) cycle. Eighteen patients were enrolled across seven dose levels ranging from 0.15 to 7.0 mg/kg IMGN853: 11 patients with EOC, 5 patients with endometrial cancer, and 2 patients with clear cell renal cell cancer (see Table 1). Among these 18 patients, 8 patients reported adverse events (AEs) considered study-drug related. Most of the AEs were mild or moderate.

Table 1: Enrollment by Tumor Type

Results						
TABLE 1: Enrollment by Tumor Type						
N = 18						
Diagnosis	Fra Expression					
	2 Hetero	2 Homo	3 Hetero	3 Homo	Other	Totals
Ovarian Cancer	3	1	5	2	0	11
Serous	1		4 ¹	2		7
Transitional			1 ²			1
Cell	2	1				2
Clear Cell						1
Carcinosarcoma						
Endometrial	1	0	3	1	0	5
Serous			2 ²	1		3
Endometrioid	1					1
Adenosquamous			1			1
Renal Cell	0	1	0	0	1	2

Clear Cell		1			Negative	2
¹ CA125 Response and SD lasting 6 cycles in 1 patient						
² Unconfirmed PR (confirmations pending)						

[00173] At the 7.0 mg/kg dose, there have been 4 patients who have experienced ocular toxicity. One patient was reported with Grade 3, dose-limiting punctate keratitis and Grade 2 blurred vision that were deemed definitely related to study treatment. Additionally, there was one patient each with Grade 3, Grade 2, and Grade 1 blurred vision; all events were deemed possibly or definitely related to IMGN853 treatment. As a result, the maximum tolerated dose on this schedule of administration (i.e., once every three weeks) was deemed to have been exceeded at the 7.0 mg/kg dose level, and all patients remaining at the 7.0 mg/kg dose level were dose reduced to the previous dose level (5.0 mg/kg).

[00174] Drug exposure was measured in 14 patients and found to generally increase linearly, with a half-life at doses ≥ 3.3 mg/kg of approximately 4 days. Two patients have reported confirmed CA125 response: one patient with serous ovarian and one with serous endometrial cancer. Additionally, the patient with endometrial cancer achieved an unconfirmed partial response. Patients receiving IMGN853 at doses greater than or equal to 5.0 mg/kg received dexamethasone, 10 mg IV (or similar steroid equivalent), 30 to 60 minutes prior to anti-FOLR1 immunoconjugate (e.g., IMGN853) administration.

[00175] The pharmacokinetic (PK) parameters are reported for Cycle 1 (first cycle of dosing for each patient only) of the IMGN853 Phase 1 trial. (Figure 1) The clearance of IMGN853 is shown to be rapid at low doses (CL= 1.1 mL/hr/kg) with a half life of approximately 35.4 hours or 1.5 days. The clearance decreases (CL= 0.4 mL/hr/kg) at the higher doses, and the half-life increases to about 4 days at 7.0 mg/kg. The exposure (AUC) and the Cmax are shown to generally increase at the higher doses as well.

[00176] The dose titration study demonstrated that IMGN853 is well tolerated at doses up to 5.0 mg/kg. Enrollment continues at the 5.0 mg/kg dose level. All patients who were previously treated at 7.0 mg/kg, who continue on study, have had their dose reduced to 5.0 mg/kg. Additional patients are also being enrolled to the 5.0 mg/kg to further confirm the safety profile seen with the 3 patients originally assigned to this dose.

[00177] Once the MTD is defined, the study will proceed to the dose expansion phase. Three expansion cohorts will evaluate patients with FOLR1 protein positive (1) platinum resistant epithelial ovarian cancer; (2) relapsed or refractory epithelial ovarian cancer, and (3) relapsed or refractory non small cell lung cancer (NSCLC). Cohorts 2 and 3 will have IMGN853 PD assessment by pre-and post-dose tumor biopsy and/or by FLT-PET imaging, respectively. IMGN853 will be administered at a dose of at least 3.3 mg/kg and may include doses of 5.0 mg/kg or as high as 6.0 mg/kg. Initially IMGN853 should be administered at a rate of 1 mg/min; after 30 minutes, the rate can be increased to 3 mg/min if well

tolerated. If well tolerated after 30 minutes at 3 mg/min, the rate may be increased to 5 mg/min. Subsequent infusions can be delivered at the tolerated rate.

[00178] For all IMGN853 dosing at 3.3 mg/kg or higher, prophylactic steroid treatment will be included using the protocols described in Example 2 (e.g., steroid treatment is included at 10 mg dexamethasone IV (or similar steroid equivalent) 30 to 60 minutes prior to IMGN853 administration is required and prophylactic diphenhydramine HCl and acetaminophen is recommended prior to IMGN853 administration). Cycles are repeated until (i) the patient's disease worsens, (ii) the patient experiences unacceptable toxicity, (iii) the patient withdraws consent, (iv) the patient develops a comorbid condition that would preclude further study treatment or (v) the patient is discontinued due to non-compliance or administrative reasons.

[00179] Responses are assessed using RECIST and Gynecologic Cancer Intergroup (GCIG) criteria (as appropriate).

Example 2

IMGN853 Steroid-Based Prophylaxis for Infusion Reaction

[00180] In order to decrease the likelihood of infusion reaction, any of the following steroid-based prophylaxis protocols can be used.

[00181] (1) Patients receive dexamethasone, 10 mg IV (or similar steroid equivalent), 30 to 60 minutes prior to anti-FOLR1 immunoconjugate (e.g., IMGN853) administration.

[00182] (2) Patients receive dexamethasone, 10 mg IV (or similar steroid equivalent) and diphenhydramine HCl (25-50 mg IV or PO), with or without acetaminophen (325-650 mg IV or PO), 30 to 60 minutes prior to anti-FOLR1 immunoconjugate (e.g., IMGN853) administration. This prophylactic protocol is recommended and at the discretion of each investigator.

[00183] (3) Patients receive dexamethasone 8 mg (or similar steroid equivalent) by mouth BID on the day prior to administration of anti-FOLR1 immunoconjugate (e.g., IMGN853). On the day of administration of anti-FOLR1 immunoconjugate (e.g., IMGN853), 30-60 mins prior to anti-FOLR1 immunoconjugate (e.g., IMGN853) administration, patients receive dexamethasone, 10 mg IV (or similar steroid equivalent), diphenhydramine HCl (25-50 mg IV or PO), with or without acetaminophen (325-650 mg IV or PO)

(4) Within 24 hours prior to infusion steroids (e.g., dexamethasone) are administered orally.

[00184] It is to be appreciated that the Detailed Description section, and not the Summary and Abstract sections, is intended to be used to interpret the claims. The Summary and Abstract sections sets forth one or more, but not all, exemplary embodiments of the present invention as contemplated by the inventor(s), and thus, are not intended to limit the present invention and the appended claims in any way.

[00185] The present invention has been described above with the aid of functional building blocks illustrating the implementation of specified functions and relationships thereof. The boundaries of these functional building blocks have been arbitrarily defined herein for the convenience of the description. Alternate boundaries can be defined so long as the specified functions and relationships thereof are appropriately performed.

[00186] The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art, readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance.

[00187] The breadth and scope of the present invention should not be limited by any of the above-described exemplary embodiments, but should be defined only in accordance with the following claims and their equivalents.

SEQUENCES

SEQ ID NO:1 - human folate receptor 1

MAQRMTTQLLLLLVWVAVVGEAQTRIAWARTELLNVCMNAKHHKEKPGPEDKLHEQ
CRPWRKNACCSTNTSQEAHKDVSYLRFNWNHCGEMAPACKRHFQIDTCLYECSPNLG
PWIQQVDQSWRKERVNLNPLCKEDCEQWWEDCRTSYTCKSNWHKGWNWTSGFNKCA
VGAACQPFHFYFPTPTVLCNEIWTHSYKVSNNYSRGSGRCIQMWFDPAQGNPNEEVARFY
AAAMSGAGPWAAWPFLLSLALMLLWLLS

SEQ ID NO:2 - human folate receptor 1 nucleic acid sequence

atggctcagcggatgacaacacagctgctgctccttctagtgtgggtggctgtagtaggggaggctcagacaaggattgcatgggccagga
ctgagcttctcaatgtctgcatgaacgccaagcaccacaaggaaaaggcagccccgaggacaagttgcatgagcagtgctgacctgga
ggaagaatgctgtgttctaccaacaccagccaggaagcccataaggatgtttctacctatagattcaactggaaccactgtggagag
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cccacacccactgttctgtgcaatgaaatctggactcactcctacaaggtcagcaactacagccgagggagtgccgctgcatccagatgtg
gttcgaccagcccagggcaacccaatgaggaggtggcgaggttctatgctgcagccatgagtggggctgggcccctgggcagcctggc
ctttctgcttagcctggcctaagtgtgctgtggctgctcagc

SEQ ID NO:3 - huMov19 vHC

QVQLVQSGAEVVKPGASVKISCKASGYTFTGYFMNWVKQSPGQSLEWIGRIHPYDGD
TFYNQKFQGKATLTVDKSSNTAHMELLSLTSEDFAVYYCTRYDGSRAMDYWGQGTTVT
VSS

SEQ ID NO:4 - huMov19 vLCv1.00

DIVLTQSPLSLAVSLGQPAIISCKASQSVSFAGTSLMHWHYHQPQGPRLLIYRASNLEA
GVPRDFSGSGSKTDFTLNISPVEAEDAATYYCQQSREYPYTFGGGTKLEIKR

SEQ ID NO:5 - huMov19 vLCv1.60

DIVLTQSPLSLAVSLGQPAIISCKASQSVSFAGTSLMHWHYHQPQGPRLLIYRASNLEA
GVPRDFSGSGSKTDFTLTISPVEAEDAATYYCQQSREYPYTFGGGTKLEIKR

SEQ ID NO:6 - huMov19 vLC CDR1

KASQSVSFAGTSLMH

SEQ ID NO:7 - huMov19 vLC CDR2

RASNLEA

SEQ ID NO:8 - huMov19 vLC CDR3

QQSREYPYT

SEQ ID NO:9 - huMov19 vHC CDR1

GYFMN

SEQ ID NO:10 - huMov19 vHC CDR2 – Kabat Defined

RIHPYDGDTFYNQKFQG

SEQ ID NO:11 – huMov19 vHC CDR2 – Abm Defined
RIHPYDGDTF

SEQ ID NO:12 - huMov19 vHC CDR3
YDGSRAMDY

SEQ ID NO:13 - huMov19 HC amino acid sequence
QVQLVQSGAEVVKPGASVKISCKASGYTFTGYFMNWVKQSPGQSLEWIGRIHPYDGD
FYNQKFQGKATLTVDKSSNTAHMELLSLTSEDFAVYYCTRYDGSRAMDYWGQGTTVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV
DKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:14 - huMov19 LCv1.00
DIVLTQSPLSLAVSLGQPAISCKASQSVSFAGTSLMHWYHQKPGQQPRLLIYRASNLEA
GVPDRFSGSGSKTDFTLNISPVEAEDAATYYCQQSREYPYTFGGGGTKLEIKRTVAAPSVFI
FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS
STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:15 - huMov19 LCv1.60
DIVLTQSPLSLAVSLGQPAISCKASQSVSFAGTSLMHWYHQKPGQQPRLLIYRASNLEA
GVPDRFSGSGSKTDFTLTISPVEAEDAATYYCQQSREYPYTFGGGGTKLEIKRTVAAPSVFI
FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS
STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:16 – muMov19 vHC CDR2 – Kabat Defined
RIHPYDGDTFYNQNFKD

WHAT IS CLAIMED IS:

1. A method for treating a human patient having cancer comprising administering to the patient an effective dose of an immunoconjugate that binds to FOLR1 polypeptide, wherein the administration produces a Cmax of about 100-150 $\mu\text{g/mL}$.
2. The method of claim 1, wherein the immunoconjugate is administered at a dose of about 3 mg/kg to about 6 mg/kg.
3. The method of claim 2, wherein the immunoconjugate is administered at a dose of about 3.0 mg/kg.
4. The method of claim 2, wherein the immunoconjugate is administered at a dose of about 3.3 mg/kg.
5. The method of claim 2, wherein the immunoconjugate is administered at a dose of about 5.0 mg/kg.
6. The method of claim 2, wherein the immunoconjugate is administered at a dose of about 5.5 mg/kg.
7. The method of claim 2, wherein the immunoconjugate is administered at a dose of about 6.0 mg/kg.
8. The method of claim 1, wherein the immunoconjugate is administered at a dose of about 6.5 mg/kg.
9. The method of any one of claims 1-8, wherein the immunoconjugate is administered about once every week.
10. A method for treating a human patient having cancer comprising administering to the patient an effective dose of an immunoconjugate that binds to FOLR1 polypeptide, wherein the immunoconjugate is administered at a dose of about 6 mg/kg about once every week.
11. A method for treating a human patient having cancer comprising administering to the patient an effective dose of an immunoconjugate that binds to FOLR1 polypeptide, wherein the immunoconjugate is administered at a dose of about 6.5 mg/kg about once every week.
12. The method of any one of claims 1-11, wherein the immunoconjugate comprises an antibody comprising a light chain CDR1 comprising SEQ ID NO:6, a light chain CDR2 comprising SEQ ID NO:7, a light chain CDR3 comprising SEQ ID NO:8, a heavy chain

- CDR1 comprising SEQ ID NO:9, a heavy chain CDR2 comprising SEQ ID NO: 10 or 11, and a heavy chain CDR3 comprising SEQ ID NO:12.
13. The method of claim 12, wherein the immunoconjugate comprises an antibody comprising a variable light chain comprising SEQ ID NO:5 and a variable heavy chain comprising SEQ ID NO:3.
 14. The method of claim 13, wherein the immunoconjugate is IMG853.
 15. The method of any one of claims 1-14, wherein the immunoconjugate is administered intravenously.
 16. The method of any one of claims 1-15, wherein cancer is selected from the group consisting of ovarian, brain, breast, uterine, endometrial, pancreatic, renal, and lung cancer.
 17. The method of claim 16, wherein the lung cancer is non small cell lung cancer or bronchioloalveolar carcinoma.
 18. The method of claim 16, wherein the ovarian cancer is epithelial ovarian cancer.
 19. The method of claim 18, wherein the ovarian cancer is platinum resistant, relapsed, or refractory.
 20. The method of any one of claims 1-19, wherein the cancer expresses FOLR1 polypeptide or nucleic acid.
 21. The method of claim 20, wherein the FOLR1 expression levels are measured by immunohistochemistry (IHC).
 22. The method of any one of claims 1-21, further comprising administering a steroid to the patient.
 23. The method of claim 22, wherein the steroid is dexamethasone.
 24. The method of any one of claims 1-22, wherein the administration results in a decrease in tumor size.
 25. The method of any one of claims 1-16 and 18-25, wherein the cancer is ovarian cancer and wherein the administration results in a decrease in CA125.
 26. The method of any one of claims 1-25, wherein the administration results in a decrease in adverse effects.

Figure 1

IMG853 Pharmacokinetic Results						
Dose (mg/kg)	0.15	0.5	1.0	2.0	3.3	7.0
	(n=2)	(n=1)	(n=1)	(n=1)	(n=3)	(n=4)
C _{max} (ug/mL)	2.9	10.5	22.1	65.7	96.6 (16.4)	179 (21.8)
Half-life (hr)	36.4	41.2	70.1	69.9	99.5 (15.7)	87.6 (11.5)
Half-life (d)	1.5	1.7	2.9	2.9	4.1 (0.65)	3.6 (0.5)
AUC _(0-∞) (hr*ug/mL)	150.9	596.6	1779	6505	12188 (2581)	17559(2850)
AUC ₍₀₋₁₆₈₎ (hr*ug/mL)	104.6	496.6	1678	5330	8178 (1129)	12177(1621)
CL (mL/hr/kg)	1.1	0.8	0.6	0.3	0.3 (0.06)	0.4 (0.06)
V _{ss} (mL/kg)	51.8	46.7	54.9	28.2	38.6 (5.2)	52.8 (8.3)

Exposure and C_{max} generally increase with increasing dose with half-life at approximately 4.0 days



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(54) Title: ANTI-FOLR1 IMMUNOCONJUGATE DOSING REGIMENS

(57) Abstract: Methods of administering immunoconjugates that bind to FOLR1 are provided. The methods comprise administering an anti-FOLR1 immunoconjugate to a person in need thereof, for example, a cancer patient, at a therapeutically effective dosing regimen that results in minimal adverse effects.



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2014/037911

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 16/32 (2014.01)

CPC - C07K 16/28 (2014.09)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - A61K 31/537, 38/00, 39/44, 39/395, 47/48, 49/00, 51/00, 51/10; A61P 35/00; C07K 16/32; C12Q 1/68; G01N 33/48, 33/53, 33/536, 33/574 (2014.01)

USPC - 424/174.1; 435/7.1, 7.92, 69.6, 252.3, 252.31, 252.33, 254.11, 254.2, 320.1, 334; 530/387.3, 389.7; 536/23.53

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

CPC - A61K 47/48407, 47/48561; C07K16/28; G01N 33/57492 (2014.09)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatBase, Google Patents, PubMed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2012/0009181 A1 (AB et al) 12 January 2012 (12.01.2012) entire document	1-11
A	US 2012/0282175 A1 (CARRIGAN et al) 08 November 2012 (08.11.2012) entire document	1-11
A	US 2012/0282282 A1 (LUTZ et al) 08 November 2012 (08.11.2012) entire document	1-11
A	WO 2005/080431 A2 (GRASSO et al) 01 September 2005 (01.09.2005) entire document	1-11
A	FARRELL et al. "Population pharmacokinetics of farletuzumab, a humanized monoclonal antibody against folate receptor alpha, in epithelial ovarian cancer," Cancer Chemother Pharmacol, 07 September 2012 (07.09.2012), Vol. 70, Pgs. 727-734. entire document	1-11

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

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Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2014/037911

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 12-26
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

摘要

本发明提供了施用结合到 FOLR1 的免疫缀合物的方法。所述方法包括向对其有需要的人，例如癌症患者，以导致最低不良反应的治疗有效的给药方案施用抗 FOLR1 免疫缀合物。