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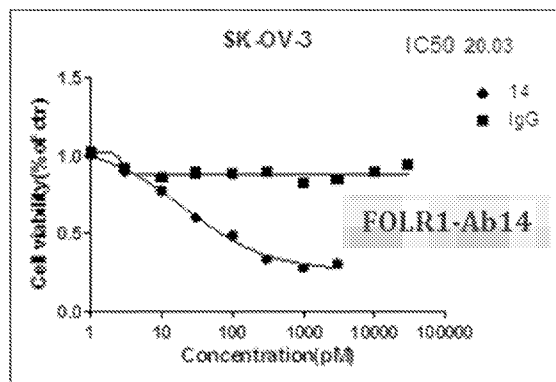
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(54) Title: ANTIBODIES SPECIFIC TO FOLATE RECEPTOR ALPHA

FIG. 2A



(57) Abstract: Antibodies specific to folate receptor alpha (a.k.a. folate receptor 1 or FOLR1) and uses thereof for therapeutic and diagnostic purposes. Also provided herein are chimeric antigen receptors (CARs) comprising an extracellular antigen-binding fragment that binds FOLR1 and immune cells expressing such.

ANTIBODIES SPECIFIC TO FOLATE RECEPTOR ALPHA

BACKGROUND OF THE INVENTION

Folate receptor alpha, also known as folate receptor 1 (FOLR1) belongs to the folate receptor family, members of which have high binding affinity to folic acid and/or derivatives thereof (*e.g.*, 5-methyltetrahydrofolate). FOLR1 has been reported to be overly expressed in a number of epithelial-derived tumors, such as ovarian, breast, renal, lung, colorectal, and brain. This receptor therefore can be a target for the treatment of such epithelial-derived tumors.

It is therefore of great importance to develop effective FOLR1 antagonists, such as anti-FOLR1 antibodies for use in both cancer treatment and diagnosis.

SUMMARY OF THE INVENTION

The present disclosure is based, at least in part, on the development of a number of antibodies specific to FOLR1. Such antibodies showed high binding affinity to the target FOLR1 antigen and/or high inhibitory activity against FOLR1⁺ cells.

Accordingly, one aspect of the present disclosure features an isolated antibody that binds to FOLR1 (anti-FOLR1 antibody), wherein the antibody binds the same epitope of human FOLR1 as a reference antibody, which is FOLR1-Ab1, FOLR1-Ab4, FOLR1-Ab14, FOLR1-Ab20, or FOLR1-Ab23, the structure features of each of which are provided herein.

In some embodiments, the anti-FOLR1 antibody described herein may comprise heavy chain variable region (V_H), which comprises one or more of the following:

(a) a heavy chain complementary determining region 1 (HC CDR 1) set forth as X₁YTFTX₂YX₃, in which X₁ is G or I, X₂ is D or S, and X₃ is W, N, or S;

(b) a heavy chain complementary determining region 2 (HC CDR2) set forth as INX₁X₂X₃X₄X₅X₆, in which X₁ is P or T, X₂ is N, Y, or E, X₃ is N, D, or T, X₄ is G or S, X₅ is G or E, and X₆ is T or P; and

(c) a heavy chain complementary determining region 3 (HC CDR3) set forth as ARX₁X₂X₃YX₄X₅X₆X₇X₈X₉X₁₀, in which X₁ is S, K or M, X₂ is G or P, X₃ is G

or Y, X₄ is G or absent, X₅ is P or absent, X₆ is A, R, or K, X₇ is W, Y or I, X₈ is F or M, X₉ is D or A, and X₁₀ is Y or V.

Such an anti-FOLR1 antibody may comprise a V_H , in which the HC CDR1, HC CDR2, and HC CDR3, collectively, are at least 85% (*e.g.*, at least 90%, at least 95%, at least 98% or more) identical to the HC CDR1, HC CDR2, and HC CDR3 of the reference antibody. In some instances, the antibody may comprise a V_H that includes the same HC CDR1, HC CDR2, and HC CDR3 as one of the reference antibodies noted above. In other embodiments, the anti-FOLR1 antibody described herein may comprise a V_H that comprises the HC CDR1, HC CDR2, and HC CDR3, which collectively contain up to 5, 4, 3, 2, or 1 mutation relative to the HC CDR1, HC CDR2, and HC CDR3 of the reference antibody.

Alternatively or in addition, the anti-FOLR1 antibody described herein may comprise a light chain variable region (V_L), which comprises one or more of the following:

- (a) a light chain complementary determining region 1 (LC CDR 1) set forth as ESVDNYGISF or QSLLYSSSQKNY;
- (b) a light chain complementary determining region 2 (LC CDR 2) set forth as X_1AS , in which X_1 is V, A, or W; and
- (c) a light chain complementary determining region 3 (LC CDR 3) set forth as $QQX_1X_2X_3X_4PX_5T$, in which X_1 is Y or S, X_2 is Y or K, X_3 is E or S, X_4 is Y or V, and X_5 is W, Y, or absent.

Such an antibody may comprise a V_L , in which the LC CDR1, LC CDR2, and LC CDR3, collectively, are at least 85% (*e.g.*, at least 90%, at least 95%, at least 98% or more) identical to the LC CDR1, LC CDR2, and LC CDR3 of the reference antibody. In some instances, the antibody may comprise the same LC CDR1, LC CDR2, and LC CDR3 as one of the reference antibodies noted above. In other embodiments, the anti-FOLR1 antibody described herein may comprise the LC CDR1, LC CDR2, and LC CDR3, which collectively contain up to 5, 4, 3, 2, or 1 mutation relative to the LC CDR1, LC CDR2, and LC CDR3 of the reference antibody.

In some examples, the anti-FOLR1 antibody described herein comprises the same heavy chain and/or light chain CDRs as one of the reference antibodies noted above. In some instances, such an anti-FOLR1 antibody may comprise the same V_H and/or V_L as the reference antibody.

Any of the anti-FOLR1 antibodies described herein may specifically bind to human

FOLR1. In some instances, the anti-FOLR1 antibody may cross-react with human FOLR1 and a non-human FOLR1, such as a rodent FOLR1 or a primate FOLR1. The antibody may be a human antibody or a humanized antibody. In some examples, it can be a chimeric antibody.

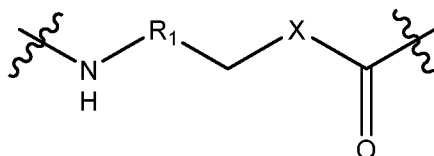
5 In some embodiments, the anti-FOLR1 antibody may be a full-length antibody (*e.g.*, an IgG molecule) or an antigen-binding fragment thereof. Alternatively, it can be a single-chain antibody.

In another aspect, the present disclosure features a nucleic acid or set of nucleic acids (*e.g.*, two nucleic acids), which collectively encodes any of the anti-FOLR1 antibodies
10 described herein, and a vector or set of vectors (*e.g.*, two vectors) comprising the nucleic acid(s) coding for the anti-FOLR1 antibodies. In some instances, the vector or vector set can be an expression vector(s). Also provided herein are host cells comprising the nucleic acid(s) or vector(s). Further, the present disclosure provides a method for making an anti-FOLR1 antibody described herein, comprising culturing the host cell that comprises the vector or
15 vector set comprising coding sequences for the antibody, wherein the coding sequences are in operably linkage to a suitable promoter, and harvesting the antibodies thus produced, for example, from the host cell or the culture medium.

In addition, the present disclosure provides an antibody-drug conjugate (ADC) comprising: any of the anti-FOLR1 antibodies described herein, and at least one therapeutic
20 agent, which is covalently conjugated to the antibody. In some examples, the therapeutic agent can be a cytotoxic agent, for example, monomethyl auristatin E.

In some embodiments, the antibody and the therapeutic agent may be conjugated through a linker. In some examples, the linker can be a cleavable linker, for example, a protease-sensitive linker, a pH-sensitive linker, or a glutathione-sensitive linker. In some
25 instances, the linker can be a protease-sensitive linker, which may comprise a peptide having 2-5 amino acids. The peptide may comprise naturally-occurring amino acid residues, non-naturally-occurring amino acid residues, or a combination thereof. In one example, the peptide may comprise valine-citrulline. In other examples, the linker can be a non-cleavable linker. Such a non-cleavable linker may comprise an optionally substituted alkane or a
30 thioether.

In some embodiments, the linker may comprise a functional group that forms a covalent bond between the antibody and the linker. Exemplary functional groups include, but are not limited to, a maleimide group, an iodoacetamide group, a vinyl sulfone group, an acrylate group, an acrylamide group, an acrylonitrile group, and a methacrylate group. In one example, the linker may further a molecular spacer of Formula I:



(Formula I),

in which

R1 is optionally substituted C1-6 alkyl, optionally substituted phenyl, optionally substituted C2-6 alkylene, optionally substituted C2-6 alkenylene, optionally substituted C2-6 alkynylene, or optionally substituted triazole; and X is O, S, or N.

Further, the present disclosure provides a chimeric antigen receptor (CAR), which may comprise: (i) an extracellular domain comprising an antigen binding fragment that binds FOLR1, (ii) a transmembrane domain, and (iii) one or more intracellular stimulatory domains. The antigen binding fragment may binds the same epitope of human FOLR1 as any of the reference antibodies described herein. In some examples, the antigen binding fragment may comprise the same HC CDRs and/or LC CDRs of any of the reference antibodies. Such an antigen binding fragment may comprise the same V_H and V_L as the reference antibody. In some examples, the antigen binding fragment can be a single chain antibody (scFv).

In any of the CARs described herein, the transmembrane domain may comprise a transmembrane domain derived from CD28 or CD8. Alternatively or in addition, the one or more intracellular stimulatory domains may comprise a signaling domain from CD3 ζ and optionally a co-stimulatory signaling domain, which may be from 4-1BB, CD7, CD27, CD28, CD40, OX40, ICOS, GITR, HVEM, TIM1, or LFA-1. Nucleic acids encoding any of the CAR described herein, vectors comprising such, and host cells expressing the CAR are also within the scope of the present disclosure. In some examples, the host cell expressing the CAR is an immune cell such as a T cell.

In yet another aspect, the present disclosure provides a pharmaceutical composition

DETAILED DESCRIPTION OF THE INVENTION

Disclosed herein are a number of anti-FOLR1 antibodies, which showed superior features, including high binding affinity to the target FOLR1 antigen, and/or high inhibitory activity against FOLR1⁺ cells.

Accordingly, provided herein are antibodies capable of binding to FOLR1, nucleic acids encoding such, antibody-drug conjugates (ADCs) and chimeric antigen receptors (CARs) comprising the anti-FOLR1 antibodies, and uses thereof for both therapeutic and diagnostic purposes. Also provided herein are kits for therapeutic and/or diagnostic use of the antibodies and/or ADCs and CARs comprising such, as well as methods for producing the anti-FOLR1 antibodies.

Antibodies binding to FOLR1

The present disclosure provides antibodies that bind folate receptor alpha, which is also known as folate receptor 1 (FOLR1). As a member of the folate receptor family, FOLR1 has a high binding affinity to folic acid and its derivatives. In humans, FOLR1 is encoded by the *FOLR1* gene. FOLR1 was found to be overly expressed on various epithelial tumors, for example, ovarian cancer. Thus this receptor can serve as a target and/or a biomarker for treatment and diagnosis of the target cancer. Accordingly, the anti-FOLR1 antibodies disclosed herein may be used in treating and/or diagnosing a target cancer as described herein, either by itself or being conjugated to other moieties, for example, being conjugated to a therapeutic agent to form an antibody-drug conjugate or being the extracellular antigen-binding domain in a chimeric antigen receptor.

An antibody (interchangeably used in plural form) is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term “antibody” encompasses not only intact (*i.e.*, full-length) polyclonal or monoclonal antibodies, but also antigen-binding fragments thereof (such as Fab, Fab', F(ab')₂, Fv), single chain (scFv), mutants thereof, fusion proteins comprising an antibody portion, humanized antibodies, chimeric antibodies, diabodies, nanobodies, linear antibodies, single chain antibodies, multispecific antibodies

(e.g., bispecific antibodies) and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity, including glycosylation variants of antibodies, amino acid sequence variants of antibodies, and covalently modified antibodies. An antibody includes an antibody of any class, such as IgD, IgE, IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant domain of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

A typical antibody molecule comprises a heavy chain variable region (V_H) and a light chain variable region (V_L), which are usually involved in antigen binding. The V_H and V_L regions can be further subdivided into regions of hypervariability, also known as “complementarity determining regions” (“CDR”), interspersed with regions that are more conserved, which are known as “framework regions” (“FR”). Each V_H and V_L is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The extent of the framework region and CDRs can be precisely identified using methodology known in the art, for example, by the Kabat definition, the Chothia definition, the AbM definition, and/or the contact definition, all of which are well known in the art. See, e.g., Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, Chothia et al., (1989) *Nature* 342:877; Chothia, C. et al. (1987) *J. Mol. Biol.* 196:901-917, Al-lazikani et al (1997) *J. Molec. Biol.* 273:927-948; and Almagro, J. *Mol. Recognit.* 17:132-143 (2004). See also hgmp.mrc.ac.uk and bioinf.org.uk/abs.

In some embodiments, the anti-FOLR1 antibody as described herein can bind and inhibit the activity of FOLR1 by at least 50% (e.g., 60%, 70%, 80%, 90%, 95% or greater). The apparent inhibition constant (K_i^{app} or $K_{i,app}$), which provides a measure of inhibitor

potency, is related to the concentration of inhibitor required to reduce enzyme activity and is not dependent on enzyme concentrations. The inhibitory activity of an anti-FOLR1 antibody described herein can be determined by routine methods known in the art.

The K_i^{app} value of an antibody may be determined by measuring the inhibitory effect of different concentrations of the antibody on the extent of the reaction (*e.g.*, enzyme activity); fitting the change in pseudo-first order rate constant (v) as a function of inhibitor concentration to the modified Morrison equation (Equation 1) yields an estimate of the apparent K_i value. For a competitive inhibitor, the K_i^{app} can be obtained from the y-intercept extracted from a linear regression analysis of a plot of K_i^{app} versus substrate concentration.

$$v = A \cdot \frac{([E] - [I] - K_i^{app}) + \sqrt{([E] - [I] - K_i^{app})^2 + 4[E] \cdot K_i^{app}}}{2} \quad (\text{Equation 1})$$

Where A is equivalent to v_o/E , the initial velocity (v_o) of the enzymatic reaction in the absence of inhibitor (I) divided by the total enzyme concentration (E).

In some embodiments, the anti-FOLR1 antibody described herein may have a K_i^{app} value of 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 50, 40, 30, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5 pM or less for a FOLR1 antigen or an antigenic epitope thereof. In some embodiments, the anti-FOLR1 antibody may have a lower K_i^{app} for a first target relative to a second target. Differences in K_i^{app} (*e.g.*, for specificity or other comparisons) can be at least 1.5, 2, 3, 4, 5, 10, 15, 20, 37.5, 50, 70, 80, 91, 100, 500, 1000, 10,000 or 10^5 fold.

The antibodies described herein can be murine, rat, human, or any other origin (including chimeric or humanized antibodies). Such antibodies are non-naturally occurring, *i.e.*, would not be produced in an animal without human act (*e.g.*, immunizing such an animal with a desired antigen or fragment thereof).

Any of the antibodies described herein can be either monoclonal or polyclonal. A “monoclonal antibody” refers to a homogenous antibody population and a “polyclonal antibody” refers to a heterogeneous antibody population. These two terms do not limit the source of an antibody or the manner in which it is made.

In one example, the antibody used in the methods described herein is a humanized antibody. Humanized antibodies refer to forms of non-human (*e.g.*, murine) antibodies that are specific chimeric immunoglobulins, immunoglobulin chains, or antigen-binding fragments thereof that contain minimal sequence derived from non-human immunoglobulin.

5 For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-
10 human residues. Furthermore, the humanized antibody may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences, but are included to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human
15 immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin. Antibodies may have Fc regions modified as described in WO 99/58572. Other forms of humanized antibodies have one or more CDRs (one, two, three,
20 four, five, and/or six), which are altered with respect to the original antibody, which are also termed one or more CDRs “derived from” one or more CDRs from the original antibody. Humanized antibodies may also involve affinity maturation.

In another example, the antibody described herein can be a chimeric antibody, which can include a heavy constant region and a light constant region from a human antibody.

25 Chimeric antibodies refer to antibodies having a variable region or part of variable region from a first species and a constant region from a second species. Typically, in these chimeric antibodies, the variable region of both light and heavy chains mimics the variable regions of antibodies derived from one species of mammals (*e.g.*, a non-human mammal such as mouse, rabbit, and rat), while the constant portions are homologous to the sequences in antibodies

derived from another mammal such as human. In some embodiments, amino acid modifications can be made in the variable region and/or the constant region.

In some embodiments, the anti-FOLR1 antibodies described herein specifically bind to the corresponding target antigen or an epitope thereof. An antibody that “specifically binds” to an antigen or an epitope is a term well understood in the art. A molecule is said to exhibit “specific binding” if it reacts more frequently, more rapidly, with greater duration and/or with greater affinity with a particular target antigen than it does with alternative targets. An antibody “specifically binds” to a target antigen or epitope if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. For example, an antibody that specifically (or preferentially) binds to a FOLR1 antigen or an antigenic epitope therein is an antibody that binds this target antigen with greater affinity, avidity, more readily, and/or with greater duration than it binds to other antigens or other epitopes in the same antigen. It is also understood with this definition that, for example, an antibody that specifically binds to a first target antigen may or may not specifically or preferentially bind to a second target antigen. As such, “specific binding” or “preferential binding” does not necessarily require (although it can include) exclusive binding. In some examples, an antibody that “specifically binds” to a target antigen or an epitope thereof may not bind to other antigens or other epitopes in the same antigen. In some embodiments, the anti-FOLR1 antibody described herein specifically binds human FOLR1. In some examples, its binding activity to a non-human FOLR1 antigen is not detectable in a conventional assay or is very low such that it would have no significant biological significance as known to those skilled in the art. In other examples, the anti-FOLR1 antibody described herein may cross-react with FOLR1 from different species, for example, between human FOLR1 and a non-human FOLR1 (*e.g.*, FOLR1 from an experimental animal such as a non-human primate, mouse, or rat).

As used herein, the term “folate receptor alpha,” “folate receptor 1”, or “FOLR1” refers to a folate receptor alpha of any suitable species, *e.g.*, human, a non-human mammal such as a non-human primate, or a rodent (*e.g.*, mouse or rat). FOLR1 is a single chain membrane protein capable of binding to folic acid and its derivatives. The amino acid

sequence of an exemplary human FOLR1 is provided below (see also GenBank accession no. P15328):

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1      maqrmttqll lllvwvavvg eaqtriawar tellnvcma khkhekpge dklheqcrpw
61     rknaccstnt sqeahkdvsy lyrfnwnhcg emapackrhf iqdtclyecs pnlgpwiqqv
5  121    dqswrkervl nvplckedce qwwedcrtsy tcksnwhkgw nwtsgfnkca vgaacqpfhf
181    yfptptvlcn eiwthsykvs nysrgsgrci qmwfdpaqgn pneevarfya aamsgagpwa
241    awpfllslal mllwlls

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FOLR1 molecules from other species were well known in the art and the amino acid sequences thereof can be retrieved from a publically available database, for example, GenBank.

In some embodiments, an anti-FOLR1 antibody as described herein has a suitable binding affinity for the target antigen (*e.g.*, human FOLR1) or antigenic epitopes thereof. As used herein, “binding affinity” refers to the apparent association constant or K_A . The K_A is the reciprocal of the dissociation constant (K_D). The anti-FOLR1 antibody described herein may have a binding affinity (K_D) of at least 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} , 10^{-10} M, or lower for the target antigen or antigenic epitope. An increased binding affinity corresponds to a decreased K_D . Higher affinity binding of an antibody for a first antigen relative to a second antigen can be indicated by a higher K_A (or a smaller numerical value K_D) for binding the first antigen than the K_A (or numerical value K_D) for binding the second antigen. In such cases, the antibody has specificity for the first antigen (*e.g.*, a first protein in a first conformation or mimic thereof) relative to the second antigen (*e.g.*, the same first protein in a second conformation or mimic thereof; or a second protein). In some embodiments, the anti-FOLR1 antibodies described herein have a higher binding affinity (a higher K_A or smaller K_D) to human FOLR1 as compared to the binding affinity to FOLR1 of a different species. Differences in binding affinity (*e.g.*, for specificity or other comparisons) can be at least 1.5, 2, 3, 4, 5, 10, 15, 20, 37.5, 50, 70, 80, 91, 100, 500, 1000, 10,000 or 10^5 fold. In some embodiments, any of the anti-FOLR1 antibodies may be further affinity matured to increase the binding affinity of the antibody to the target antigen or antigenic epitope thereof.

Binding affinity (or binding specificity) can be determined by a variety of methods including equilibrium dialysis, equilibrium binding, gel filtration, ELISA, surface plasmon resonance, or spectroscopy (*e.g.*, using a fluorescence assay). Exemplary conditions for evaluating binding affinity are in HBS-P buffer (10 mM HEPES pH7.4, 150 mM NaCl,

0.005% (v/v) Surfactant P20). These techniques can be used to measure the concentration of bound binding protein as a function of target protein concentration. The concentration of bound binding protein ([Bound]) is generally related to the concentration of free target protein ([Free]) by the following equation:

5 $[Bound] = [Free]/(K_d+[Free])$

It is not always necessary to make an exact determination of K_A , though, since sometimes it is sufficient to obtain a quantitative measurement of affinity, e.g., determined using a method such as ELISA or FACS analysis, is proportional to K_A , and thus can be used for comparisons, such as determining whether a higher affinity is, e.g., 2-fold higher, to
10 obtain a qualitative measurement of affinity, or to obtain an inference of affinity, e.g., by activity in a functional assay, e.g., an in vitro or in vivo assay.

In some embodiments, the anti-FOLR1 antibodies described herein bind to the same epitope in a FOLR1 antigen (e.g., human FOLR1) as one of the reference antibodies provided herein or compete against the reference antibody from binding to the FOLR1 antigen.
15 Reference antibodies provided herein include FOLR1-Ab217, FOLR1-Ab218, FOLR1-Ab220, FOLR1-Ab221, and FOLR1-Ab222, the structural features of each of which are provided herein. An antibody that binds the same epitope as a reference antibody described herein may bind to exactly the same epitope or a substantially overlapping epitope (e.g., containing less than 3 non-overlapping amino acid residue, less than 2 non-overlapping
20 amino acid residues, or only 1 non-overlapping amino acid residue) as the reference antibody. Whether two antibodies compete against each other from binding to the cognate antigen can be determined by a competition assay, which is well known in the art. Such antibodies can be identified as known to those skilled in the art, e.g., those having substantially similar structural features (e.g., complementary determining regions), and/or those identified by
25 assays known in the art. For example, competition assays can be performed using one of the reference antibodies to determine whether a candidate antibody binds to the same epitope as the reference antibody or competes against its binding to the FOLR1 antigen.

The anti-FOLR1 antibodies described herein may comprise a heavy chain variable region (V_H), which may comprise (a) a heavy chain complementary determining region 1

(HC CDR 1) set forth as $X_1YTFTX_2YX_3$, in which X_1 is G or I, X_2 is D or S, and X_3 is W, N, or S; (b)

a heavy chain complementary determining region 2 (HC CDR2) set forth as

$INX_1X_2X_3X_4X_5X_6$, in which X_1 is P or T, X_2 is N, Y, or E, X_3 is N, D, or T, X_4 is G or S, X_5

is G or E, and X_6 is T or P; (c) a heavy chain complementary determining region 3 (HC

CDR3) set forth as $ARX_1X_2X_3YX_4X_5X_6X_7X_8X_9X_{10}$, in which X_1 is S, K or M, X_2 is G or P, X_3 is G or Y, X_4 is G or absent, X_5 is P or absent, X_6 is A, R, or K, X_7 is W, Y or I, X_8 is F or M, X_9 is D or A, and X_{10} is Y or V, or (d) a combination of any one of (a)-(c). In some instances, the antibody may comprise a HC CDR1 of (a), a HC CDR2 of (b), and a HC CDR3 of (c).

In some examples, the HC CDR1 motif $X_1YTFTX_2YX_3$ may contain G at position X_1 , D at position X_2 , and/or N at position X_3 . Alternatively or in addition, the HC CDR2 motif $INX_1X_2X_3X_4X_5X_6$ may contain P at position X_1 , N at position X_2 and/or position X_3 , G at position X_4 , G or S at position X_5 , and/or T at position X_6 . Alternatively or in addition, the HC CDR3 motif $ARX_1X_2X_3YX_4X_5X_6X_7X_8X_9X_{10}$ may include K at position X_1 , P or G at position X_2 , Y at position X_3 , G at position X_4 , P at position X_5 , K or R at position X_6 , Y at position X_7 , F at position X_8 , D at position X_9 , and/or Y or V at position X_{10} .

Table 1 provides the amino acid sequences of the heavy chain CDRs (by IMGT definitions) for exemplary anti-FOLR1 antibodies. Antibodies having the same heavy chain CDR1, CDR2, and CDR3 regions as those exemplary anti-FOLR1 antibodies are also within the scope of the present disclosure.

Table 1: Heavy chain CDR sequences of anti-FOLR1 antibodies

Exemplary Antibody	CDR1	CDR2	CDR3
FOLR1-Ab1	GYTFTSYW	INPYDSET	ARSGGYAWFAY
FOLR1-Ab4	IYTFDYS	INTETGEP	ARMGYYGPKIMDY
FOLR1-Ab14	GYTFDYN	INPNNGGT	ARKPYYGPRYFDV
FOLR1-Ab20	GYTFDYN	INPNNGGT	ARKPYYGPRYFDV
FOLR1-Ab23	GYTFDYN	INPNNGGT	ARKPYYGPRYFDV

Alternatively or in addition, the anti-FOLR1 antibodies described herein may comprise a light chain variable domain (V_L) that comprises a light chain variable region (V_L), which comprises (a) a light chain complementary determining region 1 (LC

CDR 1) set forth as ESVDNYGISF or QSLLYSSSQKNY; (b) a light chain complementary determining region 2 (LC CDR 2) set forth as X₁AS, in which X₁ is V, A, or W; (c) a light chain complementary determining region 3 (LC CDR 3) set forth as QQX₁X₂X₃X₄PX₅X₆, in which X₁ is Y or S, X₂ is Y or K, X₃ is E or S, X₄ is Y or V, X₅ is W, Y, or T, and X₆ is T or absent, or (d) any combination of (a)-(c).

In some examples, the LC CDR1 of the anti-FOLR1 antibody is ESVDNYGISF. In other examples, the LC CDR1 of the antibody is QSLLYSSSQKNY. Alternatively or in addition, the LC CDR2 motif X₁AS contains V at position X₁. Alternatively or in addition, the LC CDR3 motif QQX₁X₂X₃X₄PX₅T includes S at position X₁, K at position X₂, E at position X₃, V at position X₄, and/or no residue at position X₅.

Table 2 provides the amino acid sequences of the light chain CDRs for exemplary anti-FOLR1 antibodies. Antibodies having the same light chain CDR1, CDR2, and CDR3 regions as those exemplary anti-FOLR1 antibodies are also within the scope of the present disclosure.

Table 2: Light chain CDR sequences of anti-FOLR1 antibodies

Exemplary Antibody	CDR1	CDR2	CDR3
FOLR1-Ab217	QSLLYSSSQKNY	WAS	QQYYSYPWT
FOLR1-Ab218	ESVDNYGISF	AAS	QQSKEVPYT
FOLR1-Ab220	ESVDNYGISF	VAS	QQSKEVPPT
FOLR1-Ab221	ESVDNYGISF	VAS	QQSKEVPPT
FOLR1-Ab222	ESVDNYGISF	VAS	QQSKEVPPT

The heavy chain and light chain CDRs of the reference antibodies provided herein are determined based on the IMGT approach, which is well known in the art. In some instances, the anti-FOLR1 antibodies disclosed herein may comprise the same heavy chain and light chain CDRs of any of the reference antibodies disclosed herein. Two antibodies having the same V_H and/or V_L CDRs means that their CDRs are identical when determined by the same approach (*e.g.*, those described herein and/or known in the art).

In some examples, the anti-FOLR1 antibodies disclosed herein may comprise the same V_H and/or V_L sequence as one of the reference antibodies, which are provided below (CDRs in boldface):

FOLR1-Ab1:

V_H:

QVQLQQPGAELVRPGASVKLSCKAS**GYTF**TSYWMNWVKQRPEQGLEWIGR**INPYDSE**THSNQK
FKDKAIL
5 TVDKSSTAYMQLSSLTSEDSAVYYC**ARSGGYAWFAY**CARSGGYAWFAYWFFAYWGQGLVTVSAWGQG
TLVTVSA

V_L:

DIVMSQSPSSLAVSVGEKVTMSCKSS**QSLLYSSQ**KNYLAWYQQKPGQSPKLLI**YWAS**TRESG
10 VPDRTGS
GSGTDFTLTISSVKAEDLAVYYC**QQYYSYPWT**CQQYYSYPWTFWTFGGGKLEIKFGGGKLEIK

FOLR1-Ab4

V_H:

15 QIQLVQSGPELKKPGETVKISCKAS**IYTF**TDYSIQWVKQAPGKGLKWMGW**INTETGEP**TYADD
FKGRFAFS
LESSASTAFLQINNLKNETATYFC**ARMGYGPKIMDY**CARMGYGPKIMDYWMDYWGQTSVTVSSWG
20 QGTSVTVSS

V_L:

DIVLTQSPASLAVSLGQRATISCRASE**ESVDNYGISF**MNWFQQKPGQPPKLLI**YAS**NQSGVVP
25 ARFSDSGS
GTDFSLNIHPMEEDDTAMYFC**QQSKEVPY**TCQQSKEVPYTFYTFGGGKLEIKFGGGKLEIK

FOLR1-Ab14

V_H:

30 EVLLQQSGPELVKPGASVKIPCKAS**GYTF**TDYNMDWVKQSHGKSLEWIGD**INPNNGGT**IYNQK
FKGKATLT
VDKSSSTAYMELRSLTSEDTAVYYC**ARKPYYGPRYFDV**CARKPYYGPRYFDVWYFDVWGAGTTVTVSSW
GA GTTVTVSS

V_L:

35 DIVLTQSPASLAVSLGQRATISCRASE**ESVDNYGISF**MNWFQQKPGQPPKLLI**YVAS**NQSGVVP
ARFSGSGS GTDFSLNIHPMEEDDTAMYFC**QQSKEVPT**CQQSKEVPTFTFGGGKLEIKFGGGKLEIK

FOLR1-Ab20

V_H:

40 EVLLQQSGPELVKPGASVKIPCKAS**GYTF**TDYNMDWVKQSHGKSLEWIGD**INPNNGGT**IYNQK
FKGKATLT
45 VDKSSSTAYMELRSLTSEDTAVYYC**ARKPYYGPRYFDV**CARKPYYGPRYFDVWYFDVWGAGTTVTVSSW
GA GTTVTVSS

V_L:

50 DIVLTQFPASLAVSLGQRATISCRASE**ESVDNYGISF**MNWFQQKPGQPPKLLI**YVAS**NQSGVVP
ARFSGSGF GTDFSLNIHPMEEDDTAMYFC**QQSKEVPT**CQQSKEVPTFTFGGGKLEIKFGGGKLEIK

FOLR1-Ab23

VH:

EVLLQQSGPELVKPGASVKIPCKAS**GYTF**TDYNDMDWVKQSHGKSLEWIGD**INPNNGGT**IYNQK
 FK GKATLT
 VDKSSSTAYMELRSLTSEDTAVYYC**CARKPYGPRYFDV**CARKPYGPRYFDVWYFDVWGAGTTVTVSSW
 GA GTTVTVSS

VL:

DIVLTQFPAPFLAVFLGQRATISCRASE**ESVDNYGISF**MNWFQQKPGQPPKLLIY**VAS**NQGSQV
 ARFSGSGF GTEFSLNIHPMEEDDSAMYFC**QQSKEVPT**CQQSKEVPTFTFGGGTKLEIKFSGGGTKLEIK

Also within the scope of the present disclosure are functional variants of any of the reference anti-FOLR1 antibodies as disclosed herein (*e.g.*, those listed in Tables 1 and 2 above). A functional variant can comprise up to 5 (*e.g.*, 4, 3, 2, or 1) amino acid residue variations in one or more of the heavy chain and light chain CDR regions of the reference antibody and binds the same epitope of the FOLR1 antigen with substantially similar affinity (*e.g.*, having a K_D value in the same order). In some instances, each of the heavy chain and/or light chain CDR in a functional variant contain no more than 2 amino acid residue variations as relative to the counterpart CDR in the reference antibody. In some examples, each of the heavy chain and/or light chain CDR in a functional variant contain no more than 1 amino acid residue variations as relative to the counterpart CDR in the reference antibody.

In one example, the amino acid residue variations are conservative amino acid residue substitutions. As used herein, a “conservative amino acid substitution” refers to an amino acid substitution that does not alter the relative charge or size characteristics of the protein in which the amino acid substitution is made. Variants can be prepared according to methods for altering polypeptide sequence known to one of ordinary skill in the art such as are found in references which compile such methods, *e.g.* Molecular Cloning: A Laboratory Manual, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or Current Protocols in Molecular Biology, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. Conservative substitutions of amino acids include substitutions made amongst amino acids within the following groups: (a) M, I, L, V; (b) F, Y, W; (c) K, R, H; (d) A, G; (e) S, T; (f) Q, N; and (g) E, D.

In some embodiments, the anti-FOLR1 antibody comprises heavy chain CDRs that, collectively, are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) identical to the heavy chain CDRs of a reference antibody, and/or light chain CDRs that, collectively, are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) identical to the light chain CDRs of the reference antibody.

In some embodiments, the anti-FOLR1 antibody comprises a heavy chain variable region (V_H) that is at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) identical to the heavy chain variable region of any of the reference antibody and/or a light chain variable region (V_L) that is at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) identical to the light chain variable region of the reference antibody.

The “percent identity” of two amino acid sequences is determined using the algorithm of Karlin and Altschul Proc. Natl. Acad. Sci. USA 87:2264-68, 1990, modified as in Karlin and Altschul Proc. Natl. Acad. Sci. USA 90:5873-77, 1993. Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. J. Mol. Biol. 215:403-10, 1990. BLAST protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to the protein molecules of interest. Where gaps exist between two sequences, Gapped BLAST can be utilized as described in Altschul et al., Nucleic Acids Res. 25(17):3389-3402, 1997. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used.

The present disclosure also provides germlined variants of any of the reference anti-FOLR1 antibodies disclosed herein. A germlined variant contains one or more mutations in the framework regions as relative to its parent antibody towards the corresponding germline sequence. To make a germline variant, the heavy or light chain variable region sequence of the parent antibody or a portion thereof (*e.g.*, a framework sequence) can be used as a query against an antibody germline sequence database (*e.g.*, bioinfo.org.uk/abs/, www.vbase2.org, or imgt.org) to identify the corresponding germline sequence used by the parent antibody and amino acid residue variations in one or more of the framework regions between the germline sequence and the parent antibody. One or more amino acid substitutions can then be introduced into the parent antibody based on the germline sequence to produce a germlined variant.

In some embodiments, the heavy chain of any of the anti-FOLR1 antibodies as described herein may further comprise a heavy chain constant region (CH) or a portion thereof (*e.g.*, CH1, CH2, CH3, or a combination thereof). The heavy chain constant region can of any suitable origin, *e.g.*, human, mouse, rat, or rabbit. In one specific example, the

heavy chain constant region is from a human IgG (a gamma heavy chain). When needed, the anti-FOLR1 antibody as described herein may comprise a modified constant region. For example, it may comprise a modified constant region that is immunologically inert, *e.g.*, does not trigger complement mediated lysis, or does not stimulate antibody-dependent cell mediated cytotoxicity (ADCC). ADCC activity can be assessed using methods disclosed in U.S. Pat. No. 5,500,362. In other embodiments, the constant region is modified as described in Eur. J. Immunol. (1999) 29:2613-2624; PCT Application No. PCT/GB99/01441; and/or UK Patent Application No. 9809951.8.

Any of the anti-FOLR1 antibodies described herein may further comprise a light chain that includes a light chain variable region and optionally, a light chain constant region, which can be any CL known in the art. In some examples, the CL is a kappa light chain. In other examples, the CL is a lambda light chain. Antibody heavy and light chain constant regions are well known in the art, *e.g.*, those provided in the IMGT database (www.imgt.org) or at www.vbase2.org/vbstat.php., both of which are incorporated by reference herein.

Preparation of anti-FOLR1 antibodies

Antibodies capable of binding FOLR1 as described herein can be made by any method known in the art. See, for example, Harlow and Lane, (1998) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York.

In some embodiments, antibodies specific to a target FOLR1 antigen (*e.g.*, human FOLR1) can be made by the conventional hybridoma technology. The full-length target antigen or a fragment thereof, optionally coupled to a carrier protein such as KLH, can be used to immunize a host animal for generating antibodies binding to that antigen. The route and schedule of immunization of the host animal are generally in keeping with established and conventional techniques for antibody stimulation and production, as further described herein. General techniques for production of mouse, humanized, and human antibodies are known in the art and are described herein. It is contemplated that any mammalian subject including humans or antibody producing cells therefrom can be manipulated to serve as the basis for production of mammalian, including human hybridoma cell lines. Typically, the host animal is inoculated intraperitoneally, intramuscularly, orally, subcutaneously,

intraplantar, and/or intradermally with an amount of immunogen, including as described herein.

Hybridomas can be prepared from the lymphocytes and immortalized myeloma cells using the general somatic cell hybridization technique of Kohler, B. and Milstein, C. (1975) Nature 256:495-497 or as modified by Buck, D. W., et al., In Vitro, 18:377-381 (1982).

Available myeloma lines, including but not limited to X63-Ag8.653 and those from the Salk Institute, Cell Distribution Center, San Diego, Calif., USA, may be used in the hybridization. Generally, the technique involves fusing myeloma cells and lymphoid cells using a fusogen such as polyethylene glycol, or by electrical means well known to those skilled in the art.

After the fusion, the cells are separated from the fusion medium and grown in a selective growth medium, such as hypoxanthine-aminopterin-thymidine (HAT) medium, to eliminate unhybridized parent cells. Any of the media described herein, supplemented with or without serum, can be used for culturing hybridomas that secrete monoclonal antibodies. As another alternative to the cell fusion technique, EBV immortalized B cells may be used to produce the anti-FOLR1 monoclonal antibodies described herein. The hybridomas are expanded and subcloned, if desired, and supernatants are assayed for anti-immunogen activity by conventional immunoassay procedures (*e.g.*, radioimmunoassay, enzyme immunoassay, or fluorescence immunoassay).

Hybridomas that may be used as source of antibodies encompass all derivatives, progeny cells of the parent hybridomas that produce monoclonal antibodies capable of interfering with the FOLR1 activity. Hybridomas that produce such antibodies may be grown in vitro or in vivo using known procedures. The monoclonal antibodies may be isolated from the culture media or body fluids, by conventional immunoglobulin purification procedures such as ammonium sulfate precipitation, gel electrophoresis, dialysis, chromatography, and ultrafiltration, if desired. Undesired activity if present, can be removed, for example, by running the preparation over adsorbents made of the immunogen attached to a solid phase and eluting or releasing the desired antibodies off the immunogen. Immunization of a host animal with a target antigen or a fragment containing the target amino acid sequence conjugated to a protein that is immunogenic in the species to be immunized, *e.g.*, keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using

a bifunctional or derivatizing agent, for example maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, SOCl₂, or R₁N=C=NR, where R and R₁ are different alkyl groups, can yield a population of antibodies (*e.g.*, monoclonal antibodies).

5 If desired, an antibody (monoclonal or polyclonal) of interest (*e.g.*, produced by a hybridoma) may be sequenced and the polynucleotide sequence may then be cloned into a vector for expression or propagation. The sequence encoding the antibody of interest may be maintained in vector in a host cell and the host cell can then be expanded and frozen for future use. In an alternative, the polynucleotide sequence may be used for genetic
10 manipulation to "humanize" the antibody or to improve the affinity (affinity maturation), or other characteristics of the antibody. For example, the constant region may be engineered to more resemble human constant regions to avoid immune response if the antibody is used in clinical trials and treatments in humans. It may be desirable to genetically manipulate the antibody sequence to obtain greater affinity to the target antigen and greater efficacy in
15 inhibiting the activity of FOLR1. It will be apparent to one of skill in the art that one or more polynucleotide changes can be made to the antibody and still maintain its binding specificity to the target antigen.

 In other embodiments, fully human antibodies can be obtained by using commercially available mice that have been engineered to express specific human immunoglobulin
20 proteins. Transgenic animals that are designed to produce a more desirable (*e.g.*, fully human antibodies) or more robust immune response may also be used for generation of humanized or human antibodies. Examples of such technology are Xenomouse^{RTM} from Amgen, Inc. (Fremont, Calif.) and HuMAb-Mouse^{RTM} and TC MouseTM from Medarex, Inc. (Princeton, N.J.). In another alternative, antibodies may be made recombinantly by phage display or
25 yeast technology. See, for example, U.S. Pat. Nos. 5,565,332; 5,580,717; 5,733,743; and 6,265,150; and Winter et al., (1994) *Annu. Rev. Immunol.* 12:433-455, and . Alternatively, the phage display technology (McCafferty et al., (1990) *Nature* 348:552-553) can be used to produce human antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors.

In some embodiments, antibodies capable of binding to a FOLR1 antigen can be isolated from an antibody library, for example, a phage display antibody library or a yeast display antibody library. In one example, the anti-FOLR1 antibody described herein can be isolated from a monoclonal antibody library, for example, following the methods disclosed in
5 US 2015/0153356, the relevant disclosures of which are incorporated by reference herein for the purposes or subject matter referenced herein.

Antigen-binding fragments of an intact antibody (full-length antibody) can be prepared via routine methods. For example, F(ab')₂ fragments can be produced by pepsin digestion of an antibody molecule, and Fab fragments that can be generated by reducing the
10 disulfide bridges of F(ab')₂ fragments.

Genetically engineered antibodies, such as humanized antibodies, chimeric antibodies, single-chain antibodies, and bi-specific antibodies, can be produced via, *e.g.*, conventional recombinant technology. In one example, DNA encoding a monoclonal antibodies specific to a target antigen can be readily isolated and sequenced using
15 conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into one or more expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do
20 not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. See, *e.g.*, PCT Publication No. WO 87/04462. The DNA can then be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences, Morrison et al., (1984) Proc. Nat. Acad. Sci. 81:6851, or by covalently joining to the
25 immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. In that manner, genetically engineered antibodies, such as “chimeric” or “hybrid” antibodies; can be prepared that have the binding specificity of a target antigen.

Techniques developed for the production of “chimeric antibodies” are well known in
30 the art. See, *e.g.*, Morrison et al. (1984) Proc. Natl. Acad. Sci. USA 81, 6851; Neuberger et

al. (1984) Nature 312, 604; and Takeda et al. (1984) Nature 314:452.

Methods for constructing humanized antibodies are also well known in the art. See, *e.g.*, Queen et al., Proc. Natl. Acad. Sci. USA, 86:10029-10033 (1989). In one example, variable regions of V_H and V_L of a parent non-human antibody are subjected to three-dimensional molecular modeling analysis following methods known in the art. Next, framework amino acid residues predicted to be important for the formation of the correct CDR structures are identified using the same molecular modeling analysis. In parallel, human V_H and V_L chains having amino acid sequences that are homologous to those of the parent non-human antibody are identified from any antibody gene database using the parent V_H and V_L sequences as search queries. Human V_H and V_L acceptor genes are then selected.

The CDR regions within the selected human acceptor genes can be replaced with the CDR regions from the parent non-human antibody or functional variants thereof. When necessary, residues within the framework regions of the parent chain that are predicted to be important in interacting with the CDR regions (see above description) can be used to substitute for the corresponding residues in the human acceptor genes.

A single-chain antibody can be prepared via recombinant technology by linking a nucleotide sequence coding for a heavy chain variable region and a nucleotide sequence coding for a light chain variable region. Preferably, a flexible linker is incorporated between the two variable regions. Alternatively, techniques described for the production of single chain antibodies (U.S. Patent Nos. 4,946,778 and 4,704,692) can be adapted to produce a phage or yeast scFv library and scFv clones specific to a FOLR1 can be identified from the library following routine procedures. Positive clones can be subjected to further screening to identify those that inhibit FOLR1 activity.

Antibodies obtained following a method known in the art and described herein can be characterized using methods well known in the art. For example, one method is to identify the epitope to which the antigen binds, or "epitope mapping." There are many methods known in the art for mapping and characterizing the location of epitopes on proteins, including solving the crystal structure of an antibody-antigen complex, competition assays, gene fragment expression assays, and synthetic peptide-based assays, as described, for example, in Chapter 11 of Harlow and Lane, Using Antibodies, a Laboratory Manual, Cold

Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1999. In an additional example, epitope mapping can be used to determine the sequence to which an antibody binds. The epitope can be a linear epitope, i.e., contained in a single stretch of amino acids, or a conformational epitope formed by a three-dimensional interaction of amino acids that may not necessarily be contained in a single stretch (primary structure linear sequence). Peptides of varying lengths (*e.g.*, at least 4-6 amino acids long) can be isolated or synthesized (*e.g.*, recombinantly) and used for binding assays with an antibody. In another example, the epitope to which the antibody binds can be determined in a systematic screening by using overlapping peptides derived from the target antigen sequence and determining binding by the antibody. According to the gene fragment expression assays, the open reading frame encoding the target antigen is fragmented either randomly or by specific genetic constructions and the reactivity of the expressed fragments of the antigen with the antibody to be tested is determined. The gene fragments may, for example, be produced by PCR and then transcribed and translated into protein *in vitro*, in the presence of radioactive amino acids. The binding of the antibody to the radioactively labeled antigen fragments is then determined by immunoprecipitation and gel electrophoresis. Certain epitopes can also be identified by using large libraries of random peptide sequences displayed on the surface of phage particles (phage libraries). Alternatively, a defined library of overlapping peptide fragments can be tested for binding to the test antibody in simple binding assays. In an additional example, mutagenesis of an antigen binding domain, domain swapping experiments and alanine scanning mutagenesis can be performed to identify residues required, sufficient, and/or necessary for epitope binding. For example, domain swapping experiments can be performed using a mutant of a target antigen in which various fragments of the FOLR1 polypeptide have been replaced (swapped) with sequences from a closely related, but antigenically distinct protein. By assessing binding of the antibody to the mutant FOLR1, the importance of the particular antigen fragment to antibody binding can be assessed.

Alternatively, competition assays can be performed using other antibodies known to bind to the same antigen to determine whether an antibody binds to the same epitope as the other antibodies. Competition assays are well known to those of skill in the art.

In some examples, an anti-FOLR1 antibody is prepared by recombinant technology as

exemplified below.

Nucleic acids encoding the heavy and light chain of an anti-FOLR1 antibody as described herein can be cloned into one expression vector, each nucleotide sequence being in operable linkage to a suitable promoter. In one example, each of the nucleotide sequences encoding the heavy chain and light chain is in operable linkage to a distinct promoter.
5 Alternatively, the nucleotide sequences encoding the heavy chain and the light chain can be in operable linkage with a single promoter, such that both heavy and light chains are expressed from the same promoter. When necessary, an internal ribosomal entry site (IRES) can be inserted between the heavy chain and light chain encoding sequences.

10 In some examples, the nucleotide sequences encoding the two chains of the antibody are cloned into two vectors, which can be introduced into the same or different cells. When the two chains are expressed in different cells, each of them can be isolated from the host cells expressing such and the isolated heavy chains and light chains can be mixed and incubated under suitable conditions allowing for the formation of the antibody.

15 Generally, a nucleic acid sequence encoding one or all chains of an antibody can be cloned into a suitable expression vector in operable linkage with a suitable promoter using methods known in the art. For example, the nucleotide sequence and vector can be contacted, under suitable conditions, with a restriction enzyme to create complementary ends on each molecule that can pair with each other and be joined together with a ligase. Alternatively,
20 synthetic nucleic acid linkers can be ligated to the termini of a gene. These synthetic linkers contain nucleic acid sequences that correspond to a particular restriction site in the vector. The selection of expression vectors/promoter would depend on the type of host cells for use in producing the antibodies.

A variety of promoters can be used for expression of the antibodies described herein,
25 including, but not limited to, cytomegalovirus (CMV) intermediate early promoter, a viral LTR such as the *Rous sarcoma* virus LTR, HIV-LTR, HTLV-1 LTR, the simian virus 40 (SV40) early promoter, *E. coli* lac UV5 promoter, and the herpes simplex tk virus promoter.

Regulatable promoters can also be used. Such regulatable promoters include those using the lac repressor from *E. coli* as a transcription modulator to regulate transcription from
30 lac operator-bearing mammalian cell promoters [Brown, M. et al., Cell, 49:603-612 (1987)],

those using the tetracycline repressor (tetR) [Gossen, M., and Bujard, H., Proc. Natl. Acad. Sci. USA 89:5547-5551 (1992); Yao, F. et al., Human Gene Therapy, 9:1939-1950 (1998); Shockelt, P., et al., Proc. Natl. Acad. Sci. USA, 92:6522-6526 (1995)]. Other systems include FK506 dimer, VP16 or p65 using astradiol, RU486, diphenol murislerone, or rapamycin. Inducible systems are available from Invitrogen, Clontech and Ariad.

Regulatable promoters that include a repressor with the operon can be used. In one embodiment, the lac repressor from *E. coli* can function as a transcriptional modulator to regulate transcription from lac operator-bearing mammalian cell promoters [M. Brown et al., Cell, 49:603-612 (1987)]; Gossen and Bujard (1992); [M. Gossen et al., Natl. Acad. Sci. USA, 89:5547-5551 (1992)] combined the tetracycline repressor (tetR) with the transcription activator (VP 16) to create a tetR-mammalian cell transcription activator fusion protein, tTa (tetR-VP 16), with the tetO-bearing minimal promoter derived from the human cytomegalovirus (hCMV) major immediate-early promoter to create a tetR-tet operator system to control gene expression in mammalian cells. In one embodiment, a tetracycline inducible switch is used. The tetracycline repressor (tetR) alone, rather than the tetR-mammalian cell transcription factor fusion derivatives can function as potent trans-modulator to regulate gene expression in mammalian cells when the tetracycline operator is properly positioned downstream for the TATA element of the CMVIE promoter (Yao et al., Human Gene Therapy). One particular advantage of this tetracycline inducible switch is that it does not require the use of a tetracycline repressor-mammalian cells transactivator or repressor fusion protein, which in some instances can be toxic to cells (Gossen et al., Natl. Acad. Sci. USA, 89:5547-5551 (1992); Shockett et al., Proc. Natl. Acad. Sci. USA, 92:6522-6526 (1995)), to achieve its regulatable effects.

Additionally, the vector can contain, for example, some or all of the following: a selectable marker gene, such as the neomycin gene for selection of stable or transient transfectants in mammalian cells; enhancer/promoter sequences from the immediate early gene of human CMV for high levels of transcription; transcription termination and RNA processing signals from SV40 for mRNA stability; SV40 polyoma origins of replication and ColE1 for proper episomal replication; internal ribosome binding sites (IRESes), versatile multiple cloning sites; and T7 and SP6 RNA promoters for in vitro transcription of sense and

antisense RNA. Suitable vectors and methods for producing vectors containing transgenes are well known and available in the art.

Examples of polyadenylation signals useful to practice the methods described herein include, but are not limited to, human collagen I polyadenylation signal, human collagen II polyadenylation signal, and SV40 polyadenylation signal.

One or more vectors (*e.g.*, expression vectors) comprising nucleic acids encoding any of the antibodies may be introduced into suitable host cells for producing the antibodies. The host cells can be cultured under suitable conditions for expression of the antibody or any polypeptide chain thereof. Such antibodies or polypeptide chains thereof can be recovered by the cultured cells (*e.g.*, from the cells or the culture supernatant) via a conventional method, *e.g.*, affinity purification. If necessary, polypeptide chains of the antibody can be incubated under suitable conditions for a suitable period of time allowing for production of the antibody.

In some embodiments, methods for preparing an antibody described herein involve a recombinant expression vector that encodes both the heavy chain and the light chain of an anti-FOLR1 antibody, as also described herein. The recombinant expression vector can be introduced into a suitable host cell (*e.g.*, a dhfr- CHO cell) by a conventional method, *e.g.*, calcium phosphate-mediated transfection. Positive transformant host cells can be selected and cultured under suitable conditions allowing for the expression of the two polypeptide chains that form the antibody, which can be recovered from the cells or from the culture medium. When necessary, the two chains recovered from the host cells can be incubated under suitable conditions allowing for the formation of the antibody.

In one example, two recombinant expression vectors are provided, one encoding the heavy chain of the anti-FOLR1 antibody and the other encoding the light chain of the anti-FOLR1 antibody. Both of the two recombinant expression vectors can be introduced into a suitable host cell (*e.g.*, dhfr- CHO cell) by a conventional method, *e.g.*, calcium phosphate-mediated transfection. Alternatively, each of the expression vectors can be introduced into a suitable host cells. Positive transformants can be selected and cultured under suitable conditions allowing for the expression of the polypeptide chains of the antibody. When the two expression vectors are introduced into the same host cells, the antibody produced therein

can be recovered from the host cells or from the culture medium. If necessary, the polypeptide chains can be recovered from the host cells or from the culture medium and then incubated under suitable conditions allowing for formation of the antibody. When the two expression vectors are introduced into different host cells, each of them can be recovered
5 from the corresponding host cells or from the corresponding culture media. The two polypeptide chains can then be incubated under suitable conditions for formation of the antibody.

Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and
10 recovery of the antibodies from the culture medium. For example, some antibodies can be isolated by affinity chromatography with a Protein A or Protein G coupled matrix.

Any of the nucleic acids encoding the heavy chain, the light chain, or both of an anti-FOLR1 antibody as described herein, vectors (e.g., expression vectors) containing such; and host cells comprising the vectors are within the scope of the present disclosure.

15 *Antibody-Drug Conjugate*

The present disclosure also provides antibody-drug conjugates comprising any of the anti-FOLR1 antibodies described herein, which is in covalent linkage to a therapeutic agent. The term “antibody-drug conjugate” or “ADC” used herein refers to a conjugate wherein the
20 anti-FOLR1 antibody described herein and a therapeutic agent are covalently linked. Generally, this antibody-drug conjugate may include the anti-FOLR1 antibody, the therapeutic agent, and optionally a linker between the antibody and the therapeutic agent. The ADS may increase therapeutic effects by delivering the therapeutic agent to a FOLR1⁺ cell, which is targeted by the antibody, in particular, a FOLR1⁺ cancer cell. The antibody-
25 drug conjugate may be prepared by various methods of preparing antibody-drug conjugates, which are known in the art.

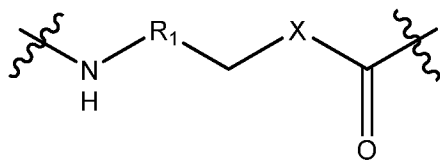
The therapeutic agent in the ADC described herein may be a toxin, a chemotherapeutic agent, an antibiotic, ADP-ribosyl transferase, a radioactive isotope or a nucleolytic enzyme. In some instances, the therapeutic agent is a cytotoxic agent. Examples
30 include, but are not limited to, anthracycline, an auristatin (e.g., auristatin E), a camptothecin, a combretastain, a dolastatin, a duocarmycin, an enediyne, a geldanamycin, an indolino-

benzodiazepine dimer, a maytansine, a puromycin, a pyrrolobenzodiazepine dimer, a taxane, a vinca alkaloid, a tubulysin, a hemisterlin, a spliceostatin, a pladienolide, and calicheamicin.

In some embodiments, the anti-FOLR1 antibody and the therapeutic agent are connected via a linker. Such a linker may be a cleavable linker, for example, cleavable under a certain pH condition (a pH-sensitive linker), cleavable by a protease (a protease-sensitive linker), or cleavable in the presence of glutathione (a glutathione-sensitive linker). In some examples, the linker comprises a protease cleavage site, which may contain 2-5 amino acid residues that are recognizable and/or cleavable by a suitable protease. Such a peptide may comprise naturally-occurring amino acid residues, non-naturally occurring amino acid residues, or a combination thereof. In one example, the peptide linker can be a dipeptide linker. Examples include a valine-citrulline (val-cit) linker, a phenylalanine-lysine (phe-lys) linker, or maleimidocapronic-valine-citruline-p-aminobenzyloxycarbonyl (vc) linker. Alternatively, the linker may be non-cleavable, *e.g.*, a linker comprising optionally substituted alkane or thioether.

In some examples, the linker may comprise a functional group that can form a covalent bond with the antibody. Exemplary functional groups include, but are not limited to, a maleimide group, an iodoacetamide group, a vinyl sulfone group, an acrylate group, an acrylamide group, an acrylonitrile group, or a methacrylate group. In some instances, the linker can contain one or more reactive amines include, but are not limited to, acetyl-lysine-valine-citrulline-p-aminobenzyloxycarbonyl (AcLys-VC-PABC) or amino PEG6-propionyl. See, *e.g.*, WO2012/059882. Other exemplary linkers include Sulfosuccinimidyl-4-[Nmaleimidomethyl]cyclohexane-1-carboxylate (smcc). Sulfo-smcc conjugation occurs via a maleimide group which reacts with sulfhydryls (thiols, --SH), while its Sulfo-NHS ester is reactive toward primary amines (as found in Lysine and the protein or peptide N terminus).

In some examples, the linker may comprise a molecular spacer, for example, a moiety



of Formula I: $\text{---N(H)---R}_1\text{---CH}_2\text{---CH}_2\text{---X---C(=O)---}$, in which R_1 can be optionally substituted C_{1-6} alkyl (*e.g.*, C_{1-3} alkyl), optionally substituted phenyl, optionally substituted C_{2-6} alkylene,

optionally substituted C₂₋₆ alkenylene, optionally substituted C₂₋₆ alkynylene, or optionally substituted triazole; and/or X can be O, S, or N.

Methods for conjugating cytotoxic agent or other therapeutic agents to antibodies were known in the art and have been described in various publications. For example, chemical modification can be made in the antibodies either through lysine side chain amines or through cysteine sulfhydryl groups activated by reducing interchain disulfide bonds for the conjugation reaction to occur. See, *e.g.*, Tanaka et al., FEBS Letters 579:2092-2096, (2005), and Gentle et al., Bioconjug. Chem. 15:658-663, (2004). Reactive cysteine residues engineered at specific sites of antibodies for specific drug conjugation with defined stoichiometry have also been described. See, *e.g.*, Junutula et al., Nature Biotechnology, 26:925-932, (2008). Conjugation using an acyl donor glutamine-containing tag and/or an endogenous glutamine made reactive by polypeptide engineering in the presence of transglutaminase and an amine, for example, a cytotoxic agent modified with a reactive amine, is also described in WO2012/059882, Strop et al., Chem. Biol. 20(2):161-167 (2013), and Farias et al., Bioconjug. Chem. 25(2):245-250 (2014). The relevant disclosures of such publications are herein incorporated by reference for the purpose and subject matter referenced therein.

Chimeric Antigen Receptor (CAR) and Immune Cells Expressing Such

The present disclosure also features chimeric antigen receptors targeting FOLR1 and immune cells expressing such. Chimeric antigen receptors (CARs) as disclosed herein are artificial cell-surface receptors that redirect binding specificity of immune cells (*e.g.*, T cells) expressing such to FOLR1⁺ cells, such as epithelium-derived cancer cells, thereby eliminating the target disease cells *via, e.g.*, the effector activity of the immune cells. A CAR construct often comprises an extracellular antigen binding domain fused to at least an intracellular signaling domain. Cartellieri et al., J Biomed Biotechnol 2010:956304, 2010. The extracellular antigen binding domain, which can be a single-chain antibody fragment (scFv), is specific to a FOLR1 antigen and the intracellular signaling domain can mediate a cell signaling that lead to activation of immune cells. As such, immune cells expressing a CAR construct specific to FOLR1 can bind to diseased cells (*e.g.*, tumor cells) expressing FOLR1, leading to activation of the immune cells and elimination of the diseased cells.

Any of the anti-FOLR1 antibodies described herein can be used to produce the CAR constructs also described herein. For example, the V_H and V_L domains of an anti-FOLR1 antibody can be fused to the intracellular signaling domain(s) to produce a CAR construct using the conventional recombinant technology. In some examples, the V_H and V_L domains of an anti-FOLR1 are connected *via* a peptide linker to form a scFv fragment.

The CAR construct disclosed herein may comprise one or more intracellular signaling domains. In some examples, CAR comprises an intracellular signaling domain that includes an immunoreceptor tyrosine-based activation motif (ITAM). Such an intracellular signaling domain may be from CD3 ζ . In addition, the CAR construct may further comprise one or more co-stimulatory signaling domains, which may be from a co-stimulatory receptor, for example, from 4-1BB (CD137), CD7, CD27, CD28, CD40, OX40, ICOS, GITR, HVEM, TIM1, or LFA-1.

The CAR construct disclosed herein may further comprise a transmembrane-hinge domain, which can be obtained from a suitable cell-surface receptor, for example, CD28 or CD8.

Also provided are isolated nucleic acid molecules and vectors encoding any of the anti-FOLR1 CARs as disclosed herein, and host cells, such as host immune cells (*e.g.*, T cells and natural killer cells), comprising the nucleic acid molecules or vectors. Immune cells expressing anti-FOLR1 CARs, which comprises a FOLR1-specific antibody binding fragment, can be used for the treatment of cancers that express FOLR1. Thus, also provided herein are methods of treating a subject with FOLR1⁺ cancer by selecting a subject with a cancer that expresses FOLR1, and administering to the subject a therapeutically effective amount of the immune cells expressing the FOLR1-targeted CARs.

Pharmaceutical Compositions

The anti-FOLR1 antibodies, the encoding nucleic acids or nucleic acid sets, vectors comprising such, or host cells comprising the vectors, as described herein, as well as the ADCs comprising the anti-FOLR1 antibodies and/or immune cells expressing FOLR1-targeting CARs, can be mixed with a pharmaceutically acceptable carrier (excipient) to form a pharmaceutical composition for use in treating a target disease. "Acceptable" means that the carrier must be compatible with the active ingredient of the composition (and preferably,

capable of stabilizing the active ingredient) and not deleterious to the subject to be treated. Pharmaceutically acceptable excipients (carriers) including buffers, which are well known in the art. See, e.g., Remington: The Science and Practice of Pharmacy 20th Ed. (2000) Lippincott Williams and Wilkins, Ed. K. E. Hoover.

5 The pharmaceutical compositions to be used in the present methods can comprise pharmaceutically acceptable carriers, excipients, or stabilizers in the form of lyophilized formulations or aqueous solutions. (Remington: The Science and Practice of Pharmacy 20th Ed. (2000) Lippincott Williams and Wilkins, Ed. K. E. Hoover). Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations used,
10 and may comprise buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as 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 (less
15 than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such
20 as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

In some examples, the pharmaceutical composition described herein comprises liposomes containing the antibodies (or the encoding nucleic acids, or the ADCs), which can be prepared by methods known in the art, such as described in Epstein, et al., Proc. Natl.
25 Acad. Sci. USA 82:3688 (1985); Hwang, et al., Proc. Natl. Acad. Sci. USA 77:4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Pat. No. 5,013,556. Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are
30 extruded through filters of defined pore size to yield liposomes with the desired diameter.

The antibodies, the encoding nucleic acid(s), or the ADCs may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are known in the art, see, *e.g.*, Remington, The Science and Practice of Pharmacy 20th Ed. Mack Publishing (2000).

In other examples, the pharmaceutical composition described herein can be formulated in sustained-release format. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethylmethacrylate), or poly(v nylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and 7 ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOTTM (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), sucrose acetate isobutyrate, and poly-D-(-)-3-hydroxybutyric acid.

The pharmaceutical compositions to be used for *in vivo* administration must be sterile. This is readily accomplished by, for example, filtration through sterile filtration membranes. Therapeutic antibody compositions are generally placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The pharmaceutical compositions described herein can be in unit dosage forms such as tablets, pills, capsules, powders, granules, solutions or suspensions, or suppositories, for oral, parenteral or rectal administration, or administration by inhalation or insufflation.

For preparing solid compositions such as tablets, the principal active ingredient can be mixed with a pharmaceutical carrier, *e.g.*, conventional tableting ingredients such as corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate or gums, and other pharmaceutical diluents, *e.g.*, water, to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention, or a

non-toxic pharmaceutically acceptable salt thereof. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation composition is then subdivided into unit dosage forms of the type described above containing from 0.1 to about 500 mg of the active ingredient of the present invention. The tablets or pills of the novel composition can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer that serves to resist disintegration in the stomach and permits the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol and cellulose acetate.

Suitable surface-active agents include, in particular, non-ionic agents, such as polyoxyethylenesorbitans (*e.g.*, TweenTM 20, 40, 60, 80 or 85) and other sorbitans (*e.g.*, SpanTM 20, 40, 60, 80 or 85). Compositions with a surface-active agent will conveniently comprise between 0.05 and 5% surface-active agent, and can be between 0.1 and 2.5%. It will be appreciated that other ingredients may be added, for example mannitol or other pharmaceutically acceptable vehicles, if necessary.

Suitable emulsions may be prepared using commercially available fat emulsions, such as IntralipidTM, LiposynTM, InfonutrolTM, LipofundinTM and LipiphysanTM. The active ingredient may be either dissolved in a pre-mixed emulsion composition or alternatively it may be dissolved in an oil (*e.g.*, soybean oil, safflower oil, cottonseed oil, sesame oil, corn oil or almond oil) and an emulsion formed upon mixing with a phospholipid (*e.g.* egg phospholipids, soybean phospholipids or soybean lecithin) and water. It will be appreciated that other ingredients may be added, for example glycerol or glucose, to adjust the tonicity of the emulsion. Suitable emulsions will typically contain up to 20% oil, for example, between 5 and 20%.

The emulsion compositions can be those prepared by mixing an antibody with Intralipid™ or the components thereof (soybean oil, egg phospholipids, glycerol and water).

Pharmaceutical compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as set out above. In some embodiments, the compositions are administered by the oral or nasal respiratory route for local or systemic effect.

Compositions in preferably sterile pharmaceutically acceptable solvents may be nebulised by use of gases. Nebulised solutions may be breathed directly from the nebulising device or the nebulising device may be attached to a face mask, tent or intermittent positive pressure breathing machine. Solution, suspension or powder compositions may be administered, preferably orally or nasally, from devices which deliver the formulation in an appropriate manner.

Methods of Treatment and Diagnosis

Any of the anti-FOLR1 antibodies, the encoding nucleic acids or nucleic acid sets, vectors comprising such, the ADCs comprising the anti-FOLR1 antibodies, and immune cells (*e.g.*, T cells or NK cells) expressing FOLR1-targeting CARs, as described herein, are useful for inhibiting and/or eliminating FOLR1⁺ disease cells, such as FOLR1⁺ cancer cells, thereby benefiting treatment of a disease or disorder associated with the FOLR1⁺ disease cells.

To practice the method disclosed herein, an effective amount of the pharmaceutical composition described herein can be administered to a subject (*e.g.*, a human) in need of the treatment via a suitable route, such as intravenous administration, *e.g.*, as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, inhalation or topical routes. Commercially available nebulizers for liquid formulations, including jet nebulizers and ultrasonic nebulizers are useful for administration. Liquid formulations can be directly nebulized and lyophilized powder can be nebulized after reconstitution.

Alternatively, the antibodies as described herein can be aerosolized using a fluorocarbon formulation and a metered dose inhaler, or inhaled as a lyophilized and milled powder.

The subject to be treated by the methods described herein can be a mammal, more preferably a human. Mammals include, but are not limited to, farm animals, sport animals, pets, primates, horses, dogs, cats, mice and rats. A human subject who needs the treatment may be a human patient having, at risk for, or suspected of having a target disease/disorder associated with FOLR1⁺ disease cells. In some embodiments, the FOLR1⁺ disease cells are cancer cells, for example, epithelial cancer cells (*i.e.*, derived from epithelial cells). Examples include, but are not limited to, ovarian cancer cells, breast cancer cells, renal cancer cells, lung cancer cells, colorectal cancer cells, and brain cancer cells. A subject having a target disease or disorder can be identified by routine medical examination, *e.g.*, laboratory tests, organ functional tests, CT scans, or ultrasounds. A subject suspected of having any of such target disease/disorder might show one or more symptoms of the disease/disorder. A subject at risk for the disease/disorder can be a subject having one or more of the risk factors for that disease/disorder.

As used herein, “an effective amount” refers to the amount of each active agent required to confer therapeutic effect on the subject, either alone or in combination with one or more other active agents. In some embodiments, the therapeutic effect is reduced FOLR1 activity or the activity of FOLR1⁺ cells. Determination of whether an amount of the antibody or other therapeutic agents comprising such (*e.g.*, ADC or CAR-T cells) achieved the therapeutic effect would be evident to one of skill in the art. Effective amounts vary, as recognized by those skilled in the art, depending on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition, size, gender and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is generally preferred that a maximum dose of the individual components or combinations thereof be used, that is, the highest safe dose according to sound medical judgment.

Empirical considerations, such as the half-life, generally will contribute to the determination of the dosage. For example, antibodies that are compatible with the human immune system, such as humanized antibodies or fully human antibodies, may be used to

prolong half-life of the antibody and to prevent the antibody being attacked by the host's immune system. Frequency of administration may be determined and adjusted over the course of therapy, and is generally, but not necessarily, based on treatment and/or suppression and/or amelioration and/or delay of a target disease/disorder. Alternatively, sustained
5 continuous release formulations of an antibody may be appropriate. Various formulations and devices for achieving sustained release are known in the art.

In one example, dosages for an antibody as described herein may be determined empirically in individuals who have been given one or more administration(s) of the antibody. Individuals are given incremental dosages of the antagonist. To assess efficacy of
10 the antagonist, an indicator of the disease/disorder can be followed.

Generally, for administration of any of the anti-FOLR1 antibodies or ADCs comprising such as described herein, an initial candidate dosage can be about 2 mg/kg. For the purpose of the present disclosure, a typical daily dosage might range from about any of 0.1 $\mu\text{g}/\text{kg}$ to 3 $\mu\text{g}/\text{kg}$ to 30 $\mu\text{g}/\text{kg}$ to 300 $\mu\text{g}/\text{kg}$ to 3 mg/kg, to 30 mg/kg to 100 mg/kg or
15 more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of symptoms occurs or until sufficient therapeutic levels are achieved to alleviate a target disease or disorder, or a symptom thereof. An exemplary dosing regimen comprises administering an initial dose of about 2 mg/kg, followed by a weekly maintenance dose of
20 about 1 mg/kg of the antibody, or followed by a maintenance dose of about 1 mg/kg every other week. However, other dosage regimens may be useful, depending on the pattern of pharmacokinetic decay that the practitioner wishes to achieve. For example, dosing from one-four times a week is contemplated. In some embodiments, dosing ranging from about 3
25 $\mu\text{g}/\text{mg}$ to about 2 mg/kg (such as about 3 $\mu\text{g}/\text{mg}$, about 10 $\mu\text{g}/\text{mg}$, about 30 $\mu\text{g}/\text{mg}$, about 100 $\mu\text{g}/\text{mg}$, about 300 $\mu\text{g}/\text{mg}$, about 1 mg/kg, and about 2 mg/kg) may be used. In some embodiments, dosing frequency is once every week, every 2 weeks, every 4 weeks, every 5 weeks, every 6 weeks, every 7 weeks, every 8 weeks, every 9 weeks, or every 10 weeks; or once every month, every 2 months, or every 3 months, or longer. The progress of this therapy is easily monitored by conventional techniques and assays. The dosing regimen
30 (including the antibody used) can vary over time.

For the purpose of the present disclosure, the appropriate dosage of an antibody as described herein will depend on the specific antibody, antibodies, and/or non-antibody peptide (or compositions thereof) employed, the type and severity of the disease/disorder, whether the antibody is administered for preventive or therapeutic purposes, previous
5 therapy, the patient's clinical history and response to the antagonist, and the discretion of the attending physician. Typically the clinician will administer an antibody, until a dosage is reached that achieves the desired result. In some embodiments, the desired result is a decrease in thrombosis. Methods of determining whether a dosage resulted in the desired result would be evident to one of skill in the art. Administration of one or more antibodies
10 can be continuous or intermittent, depending, for example, upon the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of an antibody may be essentially continuous over a preselected period of time or may be in a series of spaced dose, *e.g.*, either before, during, or after developing a target disease or disorder.

15 As used herein, the term "treating" refers to the application or administration of a composition including one or more active agents to a subject, who has a target disease or disorder, a symptom of the disease/disorder, or a predisposition toward the disease/disorder, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disorder, the symptom of the disease, or the predisposition toward the disease or disorder.

20 Alleviating a target disease/disorder includes delaying the development or progression of the disease, or reducing disease severity. Alleviating the disease does not necessarily require curative results. As used therein, "delaying" the development of a target disease or disorder means to defer, hinder, slow, retard, stabilize, and/or postpone progression of the disease. This delay can be of varying lengths of time, depending on the history of the disease
25 and/or individuals being treated. A method that "delays" or alleviates the development of a disease, or delays the onset of the disease, is a method that reduces probability of developing one or more symptoms of the disease in a given time frame and/or reduces extent of the symptoms in a given time frame, when compared to not using the method. Such comparisons are typically based on clinical studies, using a number of subjects sufficient to give a
30 statistically significant result.

“Development” or “progression” of a disease means initial manifestations and/or ensuing progression of the disease. Development of the disease can be detectable and assessed using standard clinical techniques as well known in the art. However, development also refers to progression that may be undetectable. For purpose of this disclosure, 5 development or progression refers to the biological course of the symptoms. “Development” includes occurrence, recurrence, and onset. As used herein “onset” or “occurrence” of a target disease or disorder includes initial onset and/or recurrence.

In some embodiments, the antibodies described herein are administered to a subject in need of the treatment at an amount sufficient to inhibit the activity of one or both of the target 10 antigen by at least 20% (*e.g.*, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater) *in vivo*. In other embodiments, the antibodies are administered in an amount effective in reducing the activity level of a target antigens by at least 20% (*e.g.*, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater).

Conventional methods, known to those of ordinary skill in the art of medicine, can be 15 used to administer the pharmaceutical composition to the subject, depending upon the type of disease to be treated or the site of the disease. This composition can also be administered via other conventional routes, *e.g.*, administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term “parenteral” as used herein includes subcutaneous, intracutaneous, intravenous, 20 intramuscular, intraarticular, intraarterial, intrasynovial, intrasternal, intrathecal, intralesional, and intracranial injection or infusion techniques. In addition, it can be administered to the subject via injectable depot routes of administration such as using 1-, 3-, or 6-month depot injectable or biodegradable materials and methods. In some examples, the pharmaceutical composition is administered intraocularly or intravitreally.

25 Injectable compositions may contain various carriers such as vegetable oils, dimethylactamide, dimethylformamide, ethyl lactate, ethyl carbonate, isopropyl myristate, ethanol, and polyols (glycerol, propylene glycol, liquid polyethylene glycol, and the like). For intravenous injection, water soluble antibodies can be administered by the drip method, whereby a pharmaceutical formulation containing the antibody and a physiologically 30 acceptable excipient is infused. Physiologically acceptable excipients may include, for

example, 5% dextrose, 0.9% saline, Ringer's solution or other suitable excipients.

Intramuscular preparations, e.g., a sterile formulation of a suitable soluble salt form of the antibody, can be dissolved and administered in a pharmaceutical excipient such as Water-for-Injection, 0.9% saline, or 5% glucose solution.

5 In one embodiment, an antibody is administered via site-specific or targeted local delivery techniques. Examples of site-specific or targeted local delivery techniques include various implantable depot sources of the antibody or local delivery catheters, such as infusion catheters, an indwelling catheter, or a needle catheter, synthetic grafts, adventitial wraps, shunts and stents or other implantable devices, site specific carriers, direct injection, or direct
10 application. See, e.g., PCT Publication No. WO 00/53211 and U.S. Pat. No. 5,981,568.

Targeted delivery of therapeutic compositions containing an antisense polynucleotide, expression vector, or subgenomic polynucleotides can also be used. Receptor-mediated DNA delivery techniques are described in, for example, Findeis et al., Trends Biotechnol. (1993) 11:202; Chiou et al., Gene Therapeutics: Methods And Applications Of Direct Gene Transfer
15 (J. A. Wolff, ed.) (1994); Wu et al., J. Biol. Chem. (1988) 263:621; Wu et al., J. Biol. Chem. (1994) 269:542; Zenke et al., Proc. Natl. Acad. Sci. USA (1990) 87:3655; Wu et al., J. Biol. Chem. (1991) 266:338.

Therapeutic compositions containing a polynucleotide (e.g., those encoding the antibodies described herein) are administered in a range of about 100 ng to about 200 mg of
20 DNA for local administration in a gene therapy protocol. In some embodiments, concentration ranges of about 500 ng to about 50 mg, about 1 µg to about 2 mg, about 5 µg to about 500 µg, and about 20 µg to about 100 µg of DNA or more can also be used during a gene therapy protocol.

The therapeutic polynucleotides and polypeptides described herein can be delivered
25 using gene delivery vehicles. The gene delivery vehicle can be of viral or non-viral origin (see generally, Jolly, Cancer Gene Therapy (1994) 1:51; Kimura, Human Gene Therapy (1994) 5:845; Connelly, Human Gene Therapy (1995) 1:185; and Kaplitt, Nature Genetics (1994) 6:148). Expression of such coding sequences can be induced using endogenous mammalian or heterologous promoters and/or enhancers. Expression of the coding sequence
30 can be either constitutive or regulated.

Viral-based vectors for delivery of a desired polynucleotide and expression in a desired cell are well known in the art. Exemplary viral-based vehicles include, but are not limited to, recombinant retroviruses (see, *e.g.*, PCT Publication Nos. WO 90/07936; WO 94/03622; WO 93/25698; WO 93/25234; WO 93/11230; WO 93/10218; WO 91/02805; U.S. Pat. Nos. 5,219,740 and 4,777,127; GB Patent No. 2,200,651; and EP Patent No. 0 345 242),
5 alphavirus-based vectors (*e.g.*, Sindbis virus vectors, Semliki forest virus (ATCC VR-67; ATCC VR-1247), Ross River virus (ATCC VR-373; ATCC VR-1246) and Venezuelan equine encephalitis virus (ATCC VR-923; ATCC VR-1250; ATCC VR 1249; ATCC VR-532)), and adeno-associated virus (AAV) vectors (see, *e.g.*, PCT Publication Nos. WO
10 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655). Administration of DNA linked to killed adenovirus as described in Curiel, *Hum. Gene Ther.* (1992) 3:147 can also be employed.

Non-viral delivery vehicles and methods can also be employed, including, but not limited to, polycationic condensed DNA linked or unlinked to killed adenovirus alone (see,
15 *e.g.*, Curiel, *Hum. Gene Ther.* (1992) 3:147); ligand-linked DNA (see, *e.g.*, Wu, *J. Biol. Chem.* (1989) 264:16985); eukaryotic cell delivery vehicles cells (see, *e.g.*, U.S. Pat. No. 5,814,482; PCT Publication Nos. WO 95/07994; WO 96/17072; WO 95/30763; and WO 97/42338) and nucleic charge neutralization or fusion with cell membranes. Naked DNA can also be employed. Exemplary naked DNA introduction methods are described in PCT
20 Publication No. WO 90/11092 and U.S. Pat. No. 5,580,859. Liposomes that can act as gene delivery vehicles are described in U.S. Pat. No. 5,422,120; PCT Publication Nos. WO 95/13796; WO 94/23697; WO 91/14445; and EP Patent No. 0524968. Additional approaches are described in Philip, *Mol. Cell. Biol.* (1994) 14:2411, and in Woffendin, *Proc. Natl. Acad. Sci.* (1994) 91:1581.

25 The particular dosage regimen, *i.e.*, dose, timing and repetition, used in the method described herein will depend on the particular subject and that subject's medical history.

When immune cells expressing a FOLR1-targeting CAR are used for disease treatment, patients can be treated by infusing therapeutically effective doses of such immune cells such as T lymphocytes or NK cells in the range of about 10^5 to 10^{10} or more cells per
30 kilogram of body weight (cells/Kg). The infusion can be repeated as often and as many times

as the patient can tolerate until the desired response is achieved. The appropriate infusion dose and schedule will vary from patient to patient, but can be determined by the treating physician for a particular patient. Typically, initial doses of approximately 10^6 cells/Kg will be infused, escalating to 10^8 or more cells/Kg. IL-2 can be co-administered to expand
5 infused cells. The amount of IL-2 can about $1-5 \times 10^6$ international units per square meter of body surface.

In some embodiments, more than one antibody, or a combination of an antibody and another suitable therapeutic agent, may be administered to a subject in need of the treatment. The antibody, ADCs and/or CAR-T cells comprising such, can also be used in conjunction
10 with other agents that serve to enhance and/or complement the effectiveness of the agents.

Treatment efficacy for a target disease/disorder can be assessed by methods well-known in the art.

Any of the anti-FOLR1 antibodies described herein may also be used for detecting the presence or level of FOLR1⁺ cells in a sample. Such a diagnostic assay may be performed in
15 *vitro* or *in vivo*.

For diagnostic uses, an anti-FOLR1 antibody as described herein may be conjugated with a detectable label (*e.g.*, an imaging agent such as a contrast agent) for diagnostic purposes, either *in vivo* or *in vitro*. As used herein, "conjugated" or "attached" means two entities are associated, preferably with sufficient affinity that the therapeutic/diagnostic
20 benefit of the association between the two entities is realized. The association between the two entities can be either direct or *via* a linker, such as a polymer linker. Conjugated or attached can include covalent or noncovalent bonding as well as other forms of association, such as entrapment, *e.g.*, of one entity on or within the other, or of either or both entities on or within a third entity, such as a micelle.

In one example, an anti-FOLR1 antibody as described herein can be attached to a detectable label, which is a compound that is capable of releasing a detectable signal, either directly or indirectly, such that the aptamer can be detected, measured, and/or qualified, *in vitro* or *in vivo*. Examples of such "detectable labels" are intended to include, but are not limited to, fluorescent labels, chemiluminescent labels, colorimetric labels, enzymatic
25 markers, radioactive isotopes, and affinity tags such as biotin. Such labels can be conjugated
30

to the aptamer, directly or indirectly, by conventional methods.

In some embodiments, the detectable label is an agent suitable for imaging FOLR1⁺

cells *in vivo*, which can be a radioactive molecule, a radiopharmaceutical, or an iron oxide

particle. Radioactive molecules suitable for *in vivo* imaging include, but are not limited to,

5 ¹²²I, ¹²³I, ¹²⁴I, ¹²⁵I, ¹³¹I, ¹⁸F, ⁷⁵Br, ⁷⁶Br, ⁷⁶Br, ⁷⁷Br, ²¹¹At, ²²⁵Ac, ¹⁷⁷Lu, ¹⁵³Sm, ¹⁸⁶Re, ¹⁸⁸Re, ⁶⁷Cu,

213Bi, ²¹²Bi, ²¹²Pb, and ⁶⁷Ga. Exemplary radiopharmaceuticals suitable for *in vivo* imaging

include ¹¹¹In Oxyquinoline, ¹³¹I Sodium iodide, ^{99m}Tc Mebrofenin, and ^{99m}Tc Red Blood

Cells, ¹²³I Sodium iodide, ^{99m}Tc Exametazime, ^{99m}Tc Macroaggregate Albumin, ^{99m}Tc

Medronate, ^{99m}Tc Mertiatide, ^{99m}Tc Oxidronate, ^{99m}Tc Pentetate, ^{99m}Tc Pertechnetate, ^{99m}Tc

10 Sestamibi, ^{99m}Tc Sulfur Colloid, ^{99m}Tc Tetrofosmin, Thallium-201, and Xenon-133. The

reporting agent can also be a dye, *e.g.*, a fluorophore, which is useful in detecting a disease

mediated by FOLR1⁺ cells in tissue samples.

To perform a diagnostic assay *in vitro*, an anti-FOLR1 antibody can be brought in

contact with a sample suspected of containing FOLR1⁺ cells. The antibody and the sample

15 may be incubated under suitable conditions for a suitable period to allow for binding of the

antibody to the FOLR1 antigen. Such an interaction can then be detected *via* routine

methods, *e.g.*, ELISA or FACS. To perform a diagnostic assay *in vivo*, a suitable amount of

anti-FOLR1 antibodies, conjugated with a label, can be administered to a subject in need of

the examination. Presence of the labeled antibody can be detected based on the signal

20 released from the label by routine methods.

Kits for Use in Treatment and Diagnosis

The present disclosure also provides kits for use in inhibiting and/or eliminating

FOLR1⁺ disease cells and thus alleviating diseases/disorders associated with such disease

25 cells. Such kits can include one or more containers comprising an anti-FOLR1 antibody, an

ADC comprising such, or immune cells expressing FOLR1-targeting CAR polypeptide, *e.g.*,

any of those described herein.

In some embodiments, the kit can comprise instructions for use in accordance with

any of the methods described herein. The included instructions can comprise a description of

30 administration of the anti-FOLR1 antibody, the ADC, or the immune cells to treat, delay the

onset, or alleviate a target disease as those described herein. The kit may further comprise a

description of selecting an individual suitable for treatment based on identifying whether that individual has the target disease. In still other embodiments, the instructions comprise a description of administering an antibody, an ADC, or immune cells, to an individual at risk of the target disease.

5 The instructions relating to the use of an anti-FOLR1 antibody, an ADC comprising such, or immune cells expressing FOLR1-targeting CAR, generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (*e.g.*, multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the invention are typically written instructions on a label or
10 package insert (*e.g.*, a paper sheet included in the kit), but machine-readable instructions (*e.g.*, instructions carried on a magnetic or optical storage disk) are also acceptable.

 The label or package insert indicates that the composition is used for treating, delaying the onset and/or alleviating a disease or disorder associated with FOLR1⁺ cells, such as epithelial cancer. Instructions may be provided for practicing any of the methods
15 described herein.

 The kits of this invention are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (*e.g.*, sealed Mylar or plastic bags), and the like. Also contemplated are packages for use in combination with a specific device, such as an inhaler, nasal administration device (*e.g.*, an atomizer) or an infusion device such
20 as a minipump. A kit may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The container may also have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an anti-FOLR1 antibody, an ADC
25 comprising such, or immune cells expressing FOLR1-targeting CAR as those described herein.

 Kits may optionally provide additional components such as buffers and interpretive information. Normally, the kit comprises a container and a label or package insert(s) on or associated with the container. In some embodiments, the invention provides articles of
30 manufacture comprising contents of the kits described above.

Also provided herein are kits for use in detecting FOLR1⁺ cells in a sample. Such a kit may comprise any of the anti-FOLR1 antibodies described herein. In some instances, the anti-FOLR1 antibody can be conjugated with a detectable label as those described herein. As used herein, “conjugated” or “attached” means two entities are associated, preferably with sufficient affinity that the therapeutic/diagnostic benefit of the association between the two entities is realized. The association between the two entities can be either direct or via a linker, such as a polymer linker. Conjugated or attached can include covalent or noncovalent bonding as well as other forms of association, such as entrapment, *e.g.*, of one entity on or within the other, or of either or both entities on or within a third entity, such as a micelle.

Alternatively or in addition, the kit may comprise a secondary antibody capable of binding to anti-FOLR1 antibody. The kit may further comprise instructions for using the anti-FOLR1 antibody for detecting FOLR1⁺.

General techniques

The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as *Molecular Cloning: A Laboratory Manual*, second edition (Sambrook, et al., 1989) Cold Spring Harbor Press; *Oligonucleotide Synthesis* (M. J. Gait, ed. 1984); *Methods in Molecular Biology*, Humana Press; *Cell Biology: A Laboratory Notebook* (J. E. Cellis, ed., 1989) Academic Press; *Animal Cell Culture* (R. I. Freshney, ed. 1987); *Introduction to Cell and Tissue Culture* (J. P. Mather and P. E. Roberts, 1998) Plenum Press; *Cell and Tissue Culture: Laboratory Procedures* (A. Doyle, J. B. Griffiths, and D. G. Newell, eds. 1993-8) J. Wiley and Sons; *Methods in Enzymology* (Academic Press, Inc.); *Handbook of Experimental Immunology* (D. M. Weir and C. C. Blackwell, eds.): *Gene Transfer Vectors for Mammalian Cells* (J. M. Miller and M. P. Calos, eds., 1987); *Current Protocols in Molecular Biology* (F. M. Ausubel, et al. eds. 1987); *PCR: The Polymerase Chain Reaction*, (Mullis, et al., eds. 1994); *Current Protocols in Immunology* (J. E. Coligan et al., eds., 1991); *Short Protocols in Molecular Biology* (Wiley and Sons, 1999); *Immunobiology* (C. A. Janeway and P. Travers, 1997); *Antibodies* (P. Finch, 1997); *Antibodies: a practice approach* (D. Catty.,

ed., IRL Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Using antibodies: a laboratory manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanetti and J. D. Capra, eds. Harwood Academic Publishers, 1995); *DNA Cloning: A practical Approach*, Volumes I and II (D.N. Glover ed. 1985); *Nucleic Acid Hybridization* (B.D. Hames & S.J. Higgins eds.(1985»; *Transcription and Translation* (B.D. Hames & S.J. Higgins, eds. (1984»; *Animal Cell Culture* (R.I. Freshney, ed. (1986»; *Immobilized Cells and Enzymes* (IRL Press, (1986»; and B. Perbal, *A practical Guide To Molecular Cloning* (1984); F.M. Ausubel *et al.* (eds.).

Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present invention to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All publications cited herein are incorporated by reference for the purposes or subject matter referenced herein.

EXAMPLES

Example 1: Generation of Anti-FOLR1 Antibodies

Reagents and General Methods

Hybridoma cell culture medium (PFHM-II Protein-Free Hybridoma Medium; #12040077) was purchased from Thermo Fisher.

RNA was isolated using standard protocols and the TRIzol Reagent from Thermo Fisher (#15596018). Resultant cDNA molecules were generated using the cDNA synthesis kit (PrimeScript II 1st strand cDNA synthesis kit; #6210A) from Takara. Antibody V-region amplification was performed using Premix Taq (#RR901A) from Takara. Standard PCR primer sets (Ig-Primer Sets #TB326) were obtained from Novagen. Genes were cloned into pET28a (Novagen; #69864) using standard techniques, including use of EcoRI, HindIII, Sall, and T4 Ligase (all from NEB). QIAEX II Gel Extraction Kit (QIAGEN #20021) was utilized to purify some, but not all, oligonucleotide molecules.

SK-OV-3 and Daudi cell cultures were maintained *in vitro* as a monolayer culture at 37°C in an atmosphere of 5% CO₂. The tumor cells were passaged regularly, as needed.

Screening of antibody library to discover anti-FOLR1 antibodies

As previously described in US 2015/0153356, a large library of monoclonal antibodies (>100,000 in total) was generated using a mixture of proteome and peptide antigens. The library was segmented into a series of high density antibody arrays before being screened against cancer tumor samples and the FDA Normal Tissue Panel.

A number of antibodies isolated from the library, including FOLR1-Ab1, FOLR1-Ab4, FOLR1-Ab14, FOLR1-Ab20, and FOLR1-Ab23, were found to differentially target SK-OV-3 cell lines. FOLR1 was identified as the target antigen via immunoprecipitation with the antibodies followed by mass spectroscopy. Subsequent knockdown of FOLR1 using standard antisense oligonucleotide techniques and overexpression of FOLR1 confirmed that FOLR1 is the target antigen to which the antibodies bind.

Production of Antibody Clones

Individual hybridoma clones were cultured in a T25 flask with 10 mL hybridoma cell culture medium (PFHM-II Protein-Free Hybridoma Medium). Cells were grown at 37°C until 80% confluent. Culture medium was then removed and cells were twice washed with 1x PBS. TRIzol reagent (1 mL vol.) was added directly to the flask and cells were lysed by pipette-mixing. The cell lysate was then recovered from the T25 flask and total RNA was isolated using standard methods. RNA concentrations were subsequently measured with a Nanodrop 2000 (Thermo Fisher). Strand cDNA was then generated from the isolated RNA according to the Takara PrimeScript II 1st strand cDNA synthesis kit protocol. Amplification of the hybridoma V-region of the resultant cDNA was then performed following Novagen user protocol TB326. Primer pairs, as shown in Table 3 below, were used for amplification:

Table 3. Primers for amplifying nucleic acids encoding anti-FOLR1 antibodies

	Primer	Sequence (5' to 3')
1	MuIgVH5'-A	GGGAATTCATGRASTTSKGGYTMARCTKGRTTT
2	MuIgVH5'-B	GGGAATTCATGRAATGSASCTGGGTYWTYCTCTT
3	MuIgVH5'-C1	ACTAGTCGACATGGACTCCAGGCTCAATTTAGTTTTTCCT
4	MuIgVH5'-C2	ACTAGTCGACATGGCTGTCYTRGBGCTGYTCYCTCG
5	MuIgVH5'-C3	ACTAGTCGACATGGVTTGGSTGTGGAMCTTGCYATTTCCT

6	MuIgVH5'-D1	ACTAGTCGACATGAAATGCAGCTGGRTYATSTTCTT
7	MuIgVH5'-D2	ACTAGTCGACATGGRCAGRCTTACWYYTCATTCCT
8	MuIgVH5'-D3	ACTAGTCGACATGATGGTGTAAAGTCTTCTGTACCT
9	MuIgVH5'-E1	ACTAGTCGACATGGGATGGAGCTRATCATSYTCTT
10	MuIgVH5'-E2	ACTAGTCGACATGAAGWTGTGGBTRAACTGGRT
11	MuIgVH5'-E3	ACTAGTCGACATGGRATGGASCKKIRTCTTTMTCT
12	MuIgVH5'-F1	ACTAGTCGACATGAACTTYGGGYTSAGMTTGRTT
13	MuIgVH5'-F2	ACTAGTCGACATGTACTTGGGACTGAGCTGTGTAT
14	MuIgVH5'-F3	ACTAGTCGACATGAGAGTGCTGATTCCTTTGTG
15	MuIgVH5'-F4	ACTAGTCGACATGGATTTTGGGCTGATTTTTTTTATTG
16	MuIgMVH3'-1	CCCAAGCTTACGAGGGGGAAGACATTTGGGAA
17	MuIgGVH3'-2	CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG
18	MuIgL5'-A	GGGAATTCATGRAGWCACAKWCYCAGGTCTTT
19	MuIgL5'-B	GGGAATTCATGGAGACAGACACACTCCTGCTAT
20	MuIgL5'-C	ACTAGTCGACATGGAGWCAGACACACTSCTGYTATGGGT
21	MuIgL5'-D1	ACTAGTCGACATGAGGRCCCCTGCTCAGWTTYTTGGIWTCTT
22	MuIgL5'-D2	ACTAGTCGACATGGGCWTCAAGATGRAGTCACAKWYYCWGG
23	MuIgL5'-E1	ACTAGTCGACATGAGTGTGCYCACTCAGGTCTGGSGTT
24	MuIgL5'-E2	ACTAGTCGACATGTGGGGAYCGKTTYAMMCTTTCAATTG
25	MuIgL5'-E3	ACTAGTCGACATGGAAGCCCCAGCTCAGCTTCTCTCC
26	MuIgL5'-F1	ACTAGTCGACATGAGIMMKTCTCMTTCAITTCYTGGG
27	MuIgL5'-F2	ACTAGTCGACATGAKGTHCYCIGCTCAGYTYCTIRG
28	MuIgL5'-F3	ACTAGTCGACATGGTRTCCWCASCTCAGTTCCTTG
29	MuIgL5'-F4	ACTAGTCGACATGTATATATGTTTGTGCTATTCT
30	MuIgL5'-G1	ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCT
31	MuIgL5'-G2	ACTAGTCGACATGGATTTWCARGTGCAGATTWTCAGCTT
32	MuIgL5'-G3	ACTAGTCGACATGGTYCTYATVTCCTTGCTGTTCTGG
33	MuIgL5'-G4	ACTAGTCGACATGGTYCTYATVTRCTGCTGCTATGG
34	MuIgL3'-1	CCCAAGCTTACTGGATGGTGGGAAGATGGA
35	MuIgL5'-A	GGGAATTCATGGCCTGGAYTYCWCTYWTMYTCT
36	MuIgL3'-1	CCCAAGCTTAGCTCYTCWGWGGAIGGYGGRAA

PCR products were checked with 1% Agarose gel. Positive PCR products were recovered using QIAgen gel extraction kits and subsequently cloned into pET28a vector using restriction enzymes (from NEB) corresponding to the primer sequence. pET28a vectors with PCR product insertion were transformed into DH5 α bacterial cells and cultured on Ampicillin-positive agar plates. Each bacterial clone was sent for Sanger sequencing using

the MuIgGVH3'-2, MuIgkVL3'-1 or MuIglVL3'-1 primers. Obtained sequences were compared for consistence to confirm target V_H and V_L sequences, respectively. V_H and V_L sequences were then analyzed on the IGMT database (<http://www.imgt.org/>) to provide the V-region, Frame and CDR elements of V_H and V_L.

5

Example 2: Evaluation of anti-FOLR1 antibodies

SK-OV-3 cells over-expressing FOLR1 (FOLR1⁺ SK-OV-3) and Daudi cells negative for expression of FOLR1 (FOLR1⁻ Daudi) were harvested using trypsin-EDTA partial digestion followed by centrifugation at 1000 rpm for 5 minutes. The cells were re-suspended in cold PBS and aliquoted. The anti-FOLR1 antibodies were diluted in PBS and added to either the FOLR1⁺ SK-OV-3 cells or the FOLR1⁻ Daudi cells. The cell solutions were mixed, incubated at 4°C in the dark and washed with PBS prior to addition of secondary antibody conjugates (for detection purposes). After incubation, the cells were washed with PBS, fixed with a fixative, and then subjected to FACS analysis. As shown in FIGs 1A-1E, these antibodies exhibited saturable binding to the FOLR1⁺ SK-OV-3. These antibodies did not exhibit saturable binding to the FOLR1⁻ Daudi.

Antibodies were tested in an antigen binding assay using ELISA titration experiments. Antibodies were incubated with varying concentrations of recombinant FOLR1 protein (rProtein) All antibodies tested bound with 0.39-12.5 nM affinity, as shown in Table 4 below.

SK-OV-3 cells were dosed with four of the anti-FOLR1 antibody clones. For the purposes of this experiment, dosing with an IgG alone functioned as a control experiment. After dosing, cell viability was determined to assess the indirect cytotoxicity of all antibody clones. In both cell lines, the anti-FOLR1 antibodies caused a decrease in cellular viability down to 26-32% total viability, with IC₅₀ values between 26-33.73 pM, as shown in Table 4 below and in FIGs 2A-2D. The IgG control antibody led to no significant losses in cellular viability.

30

Table 4. Characteristics of Anti-FOLR1 Antibodies

	Indirect Cytotoxicity		rProtein ELISA
	IC ₅₀ (pM)	Cell viability %	Affinity (nM)
FOLR1-Ab14	20.03	28	3.125
FOLR1-Ab4	33.73	32	0.78
FOLR1-Ab20	27	27	12.5
FOLR1-Ab23	26	26	6.25
FOLR1-Ab1	X	X	0.39

OTHER EMBODIMENTS

5 All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

10 From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, other embodiments are also within the claims.

EQUIVALENTS

15 While several inventive embodiments have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the function and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the inventive embodiments described herein. More generally, those
20 skilled in the art will readily appreciate that all parameters, dimensions, materials, and

configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the inventive teachings is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many
5 equivalents to the specific inventive embodiments described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, inventive embodiments may be practiced otherwise than as specifically described and claimed. Inventive embodiments of the present disclosure are directed to each individual feature, system, article, material, kit,
10 and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the inventive scope of the present disclosure.

All definitions, as defined and used herein, should be understood to control over
15 dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

20 The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple
25 elements listed with “and/or” should be construed in the same fashion, i.e., “one or more” of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to “A and/or B”, when used in conjunction with open-ended language such as “comprising” can refer, in one
30 embodiment, to A only (optionally including elements other than B); in another embodiment,

to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

As used herein in the specification and in the claims, “or” should be understood to have the same meaning as “and/or” as defined above. For example, when separating items in a list, “or” or “and/or” shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly one of,” or, when used in the claims, “consisting of,” will refer to the inclusion of exactly one element of a number or list of elements. In general, the term “or” as used herein shall only be interpreted as indicating exclusive alternatives (i.e. “one or the other but not both”) when preceded by terms of exclusivity, such as “either,” “one of,” “only one of,” or “exactly one of.” “Consisting essentially of,” when used in the claims, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the claims, the phrase “at least one,” in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase “at least one” refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, “at least one of A and B” (or, equivalently, “at least one of A or B,” or, equivalently “at least one of A and/or B”) can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts

of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

What Is Claimed Is:

1. An isolated antibody that binds to FOLR1, wherein the antibody binds the same epitope of human FOLR1 as a reference antibody, which is selected from the group
5 consisting of FOLR1-Ab1, FOLR1-Ab4, FOLR1-Ab14, FOLR1-Ab20, and FOLR1-Ab23.

2. The isolated antibody of claim 1, wherein the antibody comprises a heavy chain variable region (V_H), which comprises one or more of the following:

(d) a heavy chain complementary determining region 1 (HC CDR 1) set forth as
10 $X_1YTFX_2YX_3$, in which X_1 is G or I, X_2 is D or S, and X_3 is W, N, or S;

(e) a heavy chain complementary determining region 2 (HC CDR2) set forth as
 $INX_1X_2X_3X_4X_5X_6$, in which X_1 is P or T, X_2 is N, Y, or E, X_3 is N, D, or T, X_4 is G or S, X_5 is G or E, and X_6 is T or P; and

(f) a heavy chain complementary determining region 3 (HC CDR3) set forth as
15 $ARX_1X_2X_3YX_4X_5X_6X_7X_8X_9X_{10}$, in which X_1 is S, K or M, X_2 is G or P, X_3 is G or Y, X_4 is G or absent, X_5 is P or absent, X_6 is A, R, or K, X_7 is W, Y or I, X_8 is F or M, X_9 is D or A, and X_{10} is Y or V.

3. The isolated antibody of claim 1 or claim 2, wherein the antibody comprises a V_H ,
20 in which the HC CDR1, HC CDR2, and HC CDR3, collectively, are at least 85% identical to the HC CDR1, HC CDR2, and HC CDR3 of the reference antibody.

4. The isolated antibody of claim 3, wherein the V_H comprises the same HC CDR1, HC CDR2, and HC CDR3 as the reference antibody.

25 5. The isolated antibody of claim 3, wherein the V_H comprises the HC CDR1, HC CDR2, and HC CDR3, which collective contain up to 5, 4, 3, 2, or 1 mutation relative to the HC CDR1, HC CDR2, and HC CDR3 of the reference antibody.

30 6. The isolated antibody of any one of claims 1-5, wherein the antibody comprises a light chain variable region (V_L), which comprises one or more of the following:

- (d) a light chain complementary determining region 1 (LC CDR 1) set forth as ESVDNYGISF or QSLLYSSSQKNY;
- (e) a light chain complementary determining region 2 (LC CDR 2) set forth as X₁AS, in which X₁ is V, A, or W; and
- 5 (f) a light chain complementary determining region 3 (LC CDR 3) set forth as QQX₁X₂X₃X₄PX₅T, in which X₁ is Y or S, X₂ is Y or K, X₃ is E or S, X₄ is Y or V, and X₅ is W, Y, or absent.

7. The isolated antibody of any one of claims 1-6, wherein the antibody
10 comprises a V_L, in which the LC CDR1, LC CDR2, and LC CDR3, collectively, are at least 85% identical to the LC CDR1, LC CDR2, and LC CDR3 of the reference antibody.

8. The isolated antibody of claim 7 wherein the V_L comprises the same LC CDR1, LC CDR2, and LC CDR3 as the reference antibody.

15 9. The isolated antibody of claim 7, wherein the V_L comprises the LC CDR1, LC CDR2, and LC CDR3, which collective contain up to 5, 4, 3, 2, or 1 mutation relative to the LC CDR1, LC CDR2, and LC CDR3 of the reference antibody.

20 10. The antibody of any one of claims 1-9, wherein the antibody specifically binds to human FOLR1.

11. The antibody of any one of claims 1-9, wherein the antibody cross-reacts with human FOLR1 and a non-human FOLR1.

25 12. The antibody of claim 11, wherein the non-human FOLR1 is a rodent FOLR1 or a primate FOLR1.

13. The antibody of any one of claims 1-12, wherein the antibody is a full-length
30 antibody or an antigen-binding fragment thereof, or a single-chain antibody.

14. The antibody of any one of claims 13, wherein the antibody is an IgG molecule.

15. The antibody of any one of claims 1-14, wherein the antibody is a human antibody, a humanized antibody, or a chimeric antibody.

16. The antibody of claim 1, wherein the antibody comprises the same V_H and/or V_L as the reference antibody.

17. A nucleic acid or set of nucleic acids, which collectively encodes an anti-FOLR1 antibody of any one of claims 1-16.

18. A vector or set of vectors comprising the nucleic acid(s) of claim 17.

19. The vector or vector set of claim 18, wherein the vector(s) is an expression vector(s).

20. A host cell comprising the vector or vector set of claims 18 or 19.

21. An antibody-drug conjugate (ADC) comprising:
i. an antibody of any one of claims 1-16; and
ii. at least one therapeutic agent;

wherein the antibody is covalently conjugated to the at least one therapeutic agent.

22. The antibody-drug conjugate of claim 21, wherein the antibody and the therapeutic agent are conjugated through a linker.

23. The antibody-drug conjugate of claim 21, wherein the linker is a cleavable or non-cleavable linker.

24. The antibody-drug conjugate of claim 23, wherein the cleavable linker is a

protease-sensitive linker, a pH-sensitive linker, or a glutathione-sensitive linker.

25. The antibody-drug conjugate of claim 24, wherein the linker is a protease-sensitive linker comprising a peptide sequence of 2-5 amino acids.

5

26. The antibody-drug conjugate of claim 25, wherein the 2-5 amino acids comprise naturally-occurring amino acid residues, non-naturally-occurring amino acid residues, or a combination thereof.

10

27. The antibody-drug conjugate of claim 25, wherein the peptide sequence comprises valine-citrulline.

28. The antibody-drug conjugate of claim 23, wherein the linker is a non-cleavable linker comprising an optionally substituted alkane or a thioether.

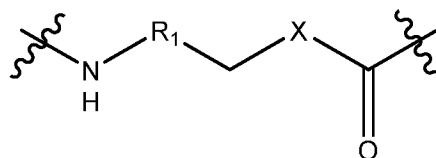
15

29. The antibody-drug conjugate of any one of claims 23-28, wherein the linker comprises a functional group that forms a covalent bond with the antibody and the linker.

30. The antibody-drug conjugate of claim 29, wherein the functional group comprises a maleimide group, an iodoacetamide group, a vinyl sulfone group, an acrylate group, an acrylamide group, an acrylonitrile group, or a methacrylate group.

20

31. The antibody-drug conjugate of any one of claims 24-30, wherein the linker further comprises a molecular spacer of Formula I:



25

(Formula I),

wherein

R₁ is optionally substituted C1-6 alkyl, optionally substituted phenyl, optionally

substituted C2-6 alkylene, optionally substituted C2-6 alkenylene, optionally substituted C2-6 alkynylene, or optionally substituted triazole; and

X is O, S, or N.

5 32. The antibody-drug conjugate of any of claims 21-31, wherein the at least one therapeutic agent is a cytotoxic agent.

 33. The antibody-drug conjugate of claim 32, wherein the cytotoxic agent is monomethyl auristatin E.

10

 34. A chimeric antigen receptor comprising:

(i) an extracellular domain comprising an antigen binding fragment that binds FOLR1,

(ii) a transmembrane domain, and

15

(iii) one or more intracellular stimulatory domains.

 35. The chimeric antigen receptor of claim 29, wherein the antigen binding fragment binds the same epitope of human FOLR1 as a reference antibody selected from the group consisting of FOLR1-Ab217, FOLR1-Ab218, FOLR1-Ab220, FOLR1-Ab221, and
20 FOLR1-Ab222

 36. The chimeric antigen receptor of claim 35, wherein the antigen binding fragment comprises the HC CDRs and/or LC CDRs as set forth in any one of claims 2-9.

25

 37. The chimeric antigen receptor of claim 35, wherein the antigen binding fragment comprises the same V_H and V_L as the reference antibody.

 38. The chimeric antigen receptor of any one of claims 34-37, wherein the antigen binding fragment is a single chain antibody (scFv).

30

39. The chimeric antigen receptor of any one of claims 33-38, wherein the transmembrane domain comprises a transmembrane domain derived from a CD28, or CD8 receptor.

5 40. The chimeric antigen receptor of any one of claims 33-39, wherein (iii) comprises a signaling domain from CD3 ζ .

41. The chimeric antigen receptor of any one of claims 33-40, wherein (iii) comprises a co-stimulatory signaling domain.

10 42. The chimeric antigen receptor of claim 41, wherein the co-stimulatory signaling domain is from a 4-1BB, CD7, CD27, CD28, CD40, OX40, ICOS, GITR, HVEM, TIM1, or LFA-1 receptor.

15 43. A nucleic acid, which comprises a nucleotide sequence encoding a chimeric antigen receptor of any one of claims 33-42.

44. A vector comprising the nucleic acid of claim 43.

20 45. A host cell, which expresses a chimeric receptor of any one of claims 33-42.

46. The host cell of claim 45, which is an immune cell.

47. The host cell of claim 46, wherein the immune cell is a T cell.

25 48. A pharmaceutical composition comprising (i) an antibody of any one of claims 1-16, a nucleic acid or set of nucleic acids of any one of claims 17-19, an antibody-drug conjugate of any one of claims 21-33, or a host cell of claim 46 or claim 47; and (ii) a pharmaceutically acceptable carrier.

30

49. A method of reducing the number of FOLR1⁺ cells, the method comprising administering to a subject in need thereof an effective amount of the pharmaceutical composition of claim 48.

5 50. The method of claim 49, wherein the subject has or is suspected of having cancer.

51. A method of detecting presence of FOLR1⁺ cells, the method comprising:
i. contacting a sample suspected of having FOLR1⁺ cells with an antibody of
10 any one of claims 1-16, wherein the antibody is conjugated with a labeling agent; and
ii. detecting presence FOLR1⁺ cells in the sample based on binding of the
antibody to cells in the sample.

15

FIG. 1A

FOLR1-Ab14

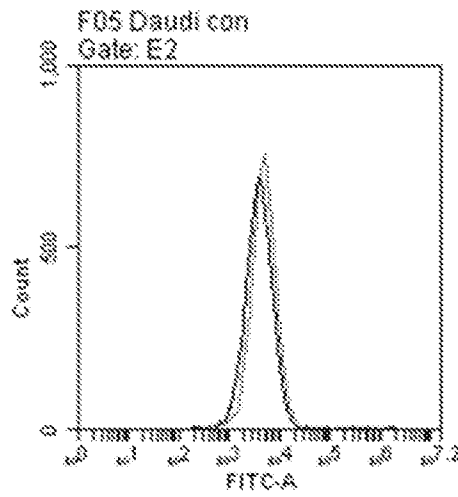
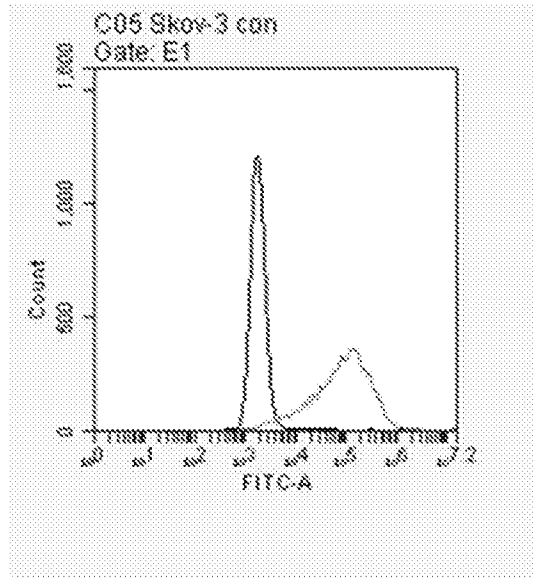


FIG. 1B

FOLR1-Ab4

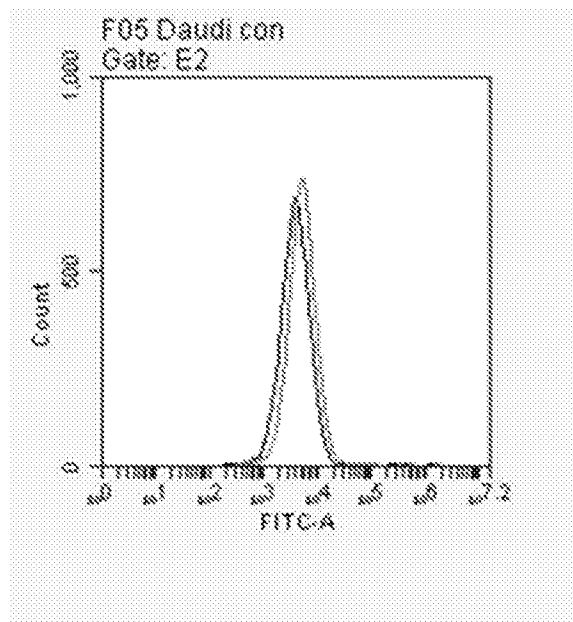
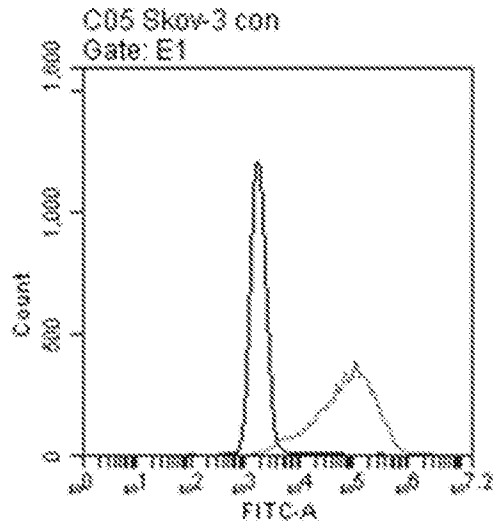


FIG. 1C

FOLR1-Ab20

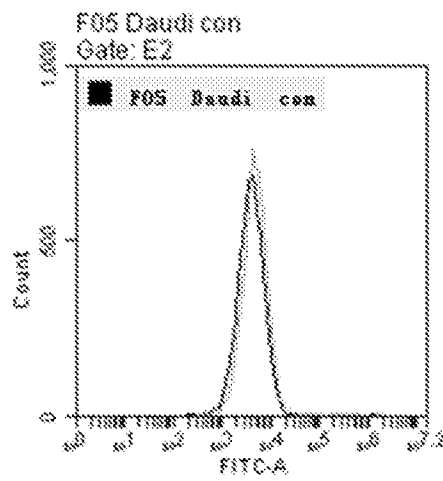
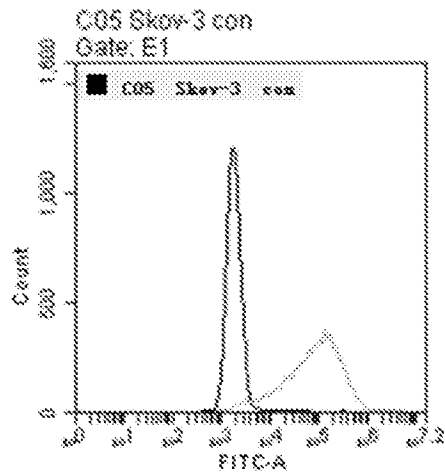


FIG. 1D

FOLR1-Ab23

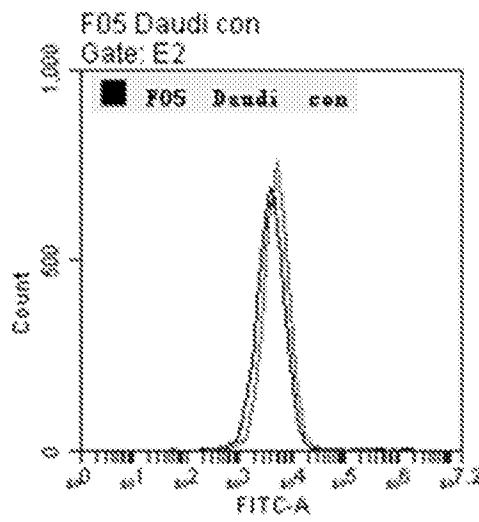
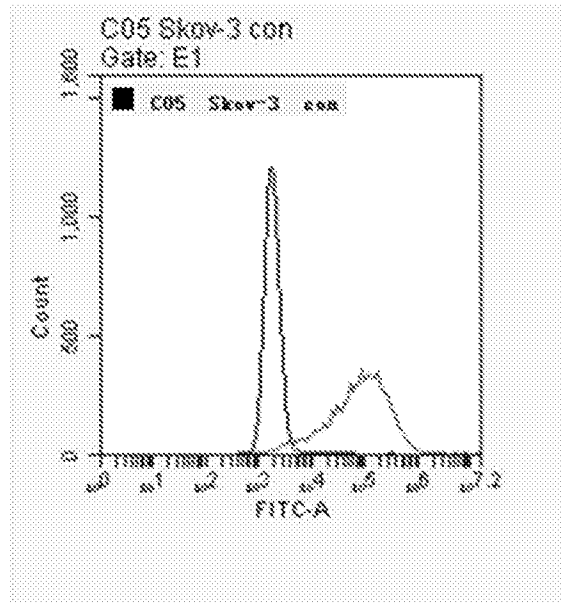


FIG. 1E

FOLR1-Ab1

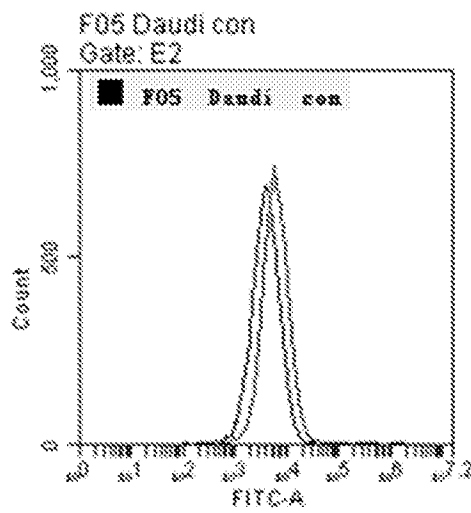
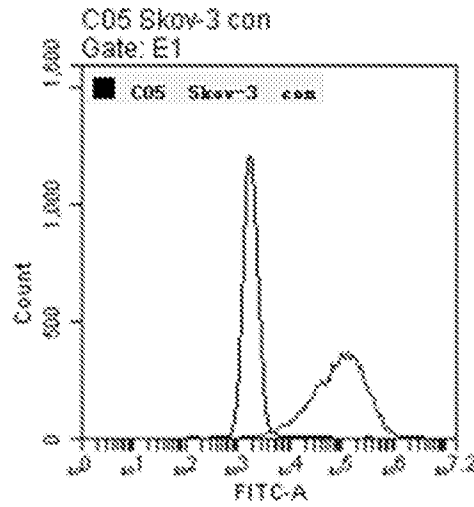


FIG. 2A

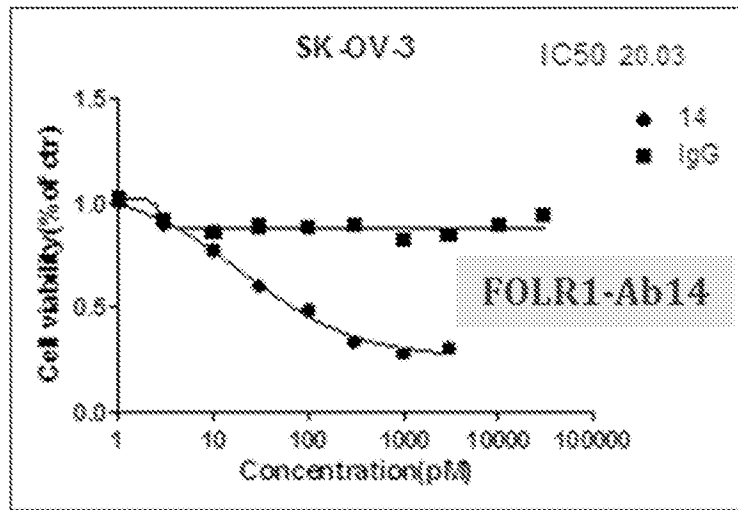


FIG. 2B

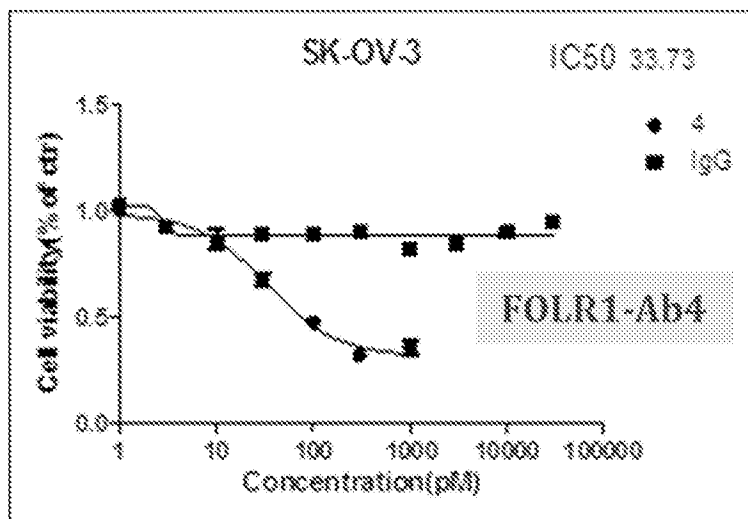


FIG. 2C

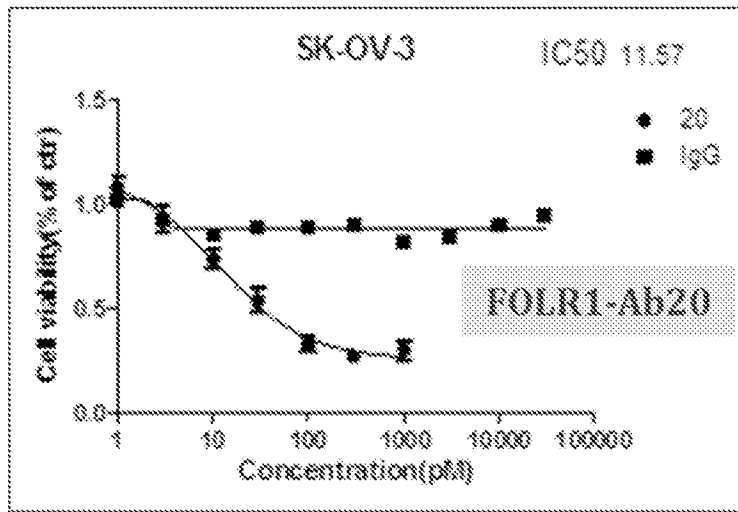


FIG. 2D

