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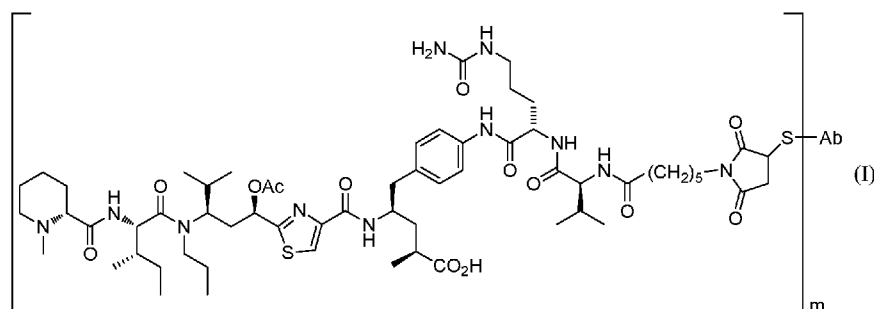
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(54) Title: ANTIBODY-DRUG CONJUGATE OF AN ANTI-GLYPICAN-3 ANTIBODY AND A TUBULYSIN ANALOG, PREPARATION AND USES



(57) Abstract: An antibody-drug conjugate having a structure represented by formula (I) wherein m is 1, 2, 3, or 4 and Ab is an anti-glypican-3 antibody having heavy and light chain CDRs as disclosed herein.

ANTIBODY-DRUG CONJUGATE OF AN ANTI-GLYPICAN-3 ANTIBODY AND A
TUBULYSIN ANALOG, PREPARATION AND USES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit under 35 U.S.C. §119(e) of US Provisional
5 Application Ser. No. 62/333,944, filed May 10, 2016; the disclosure of which is incorporated
herein by reference

BACKGROUND OF THE INVENTION

[0002] This disclosure relates to an antibody-drug conjugate of an anti-glypican-3 antibody
and a tubulysin analog, and its preparation and uses.

10 [0003] Antibody-drug conjugates (ADCs, also referred to as immunoconjugates) are anti-
cancer agents that are generating intense current interest. In an ADC, a therapeutic agent (also
referred to as the drug, warhead, or payload) is covalently linked, or conjugated, to an antibody
whose antigen is expressed by a cancer cell. The antibody, through its binding to the antigen,
directs the ADC to the cancer – that is, the antibody acts as a targeting agent specifically
15 delivering the ADC to the cancer cell. Once there, cleavage of the covalent link (referred to as
the linker) or degradation of the antibody results in the release of the therapeutic agent at the
cancer site. Conversely, while the ADC is circulating in the blood system, the therapeutic agent
is held inactive because of its covalent linkage to the antibody. Thus, the therapeutic agent in an
ADC can be much more potent (*i.e.*, cytotoxic) than ordinary chemotherapy agents because of its
20 localized release. Recently, two ADCs have received marketing approval: ADCETRIS™, in
which an anti-CD30 antibody is conjugated to an auristatin, and KADCYLA™, in which the
anti-Her2 antibody trastuzumab is conjugated to a maytansinoid. For a review on the mode of
action of ADCs, see Schrama *et al.* 2006. (The full bibliographic citation for this and other
documents cited herein by first author or inventor and year are listed at the end of this
25 specification.)

[0004] Glypican-3 is an oncofetal antigen that belongs to the glypican family of glycosyl-
phosphatidylinositol-anchored heparin sulfate proteoglycans. Glypicans are characterized by a
covalent linkage to complex polysaccharide chains called heparinsulphate glycosaminoglycans.
Glypicans are involved in cell signaling at the cellular-extracellular matrix interface
30 (Sasisekharan *et al.* 2002). To date, six distinct members of the human glypican family have

been identified. Cell membrane-bound glypican-3 is composed of two subunits, linked by one or more disulfide bonds.

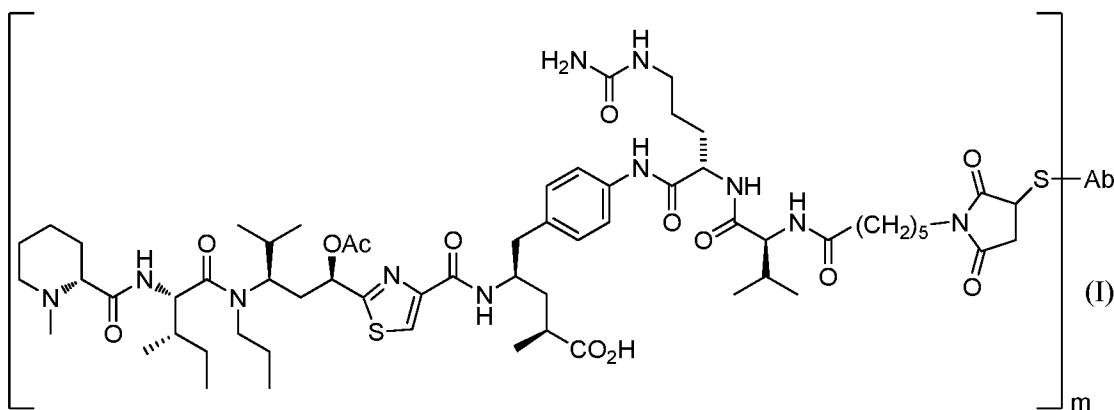
[0005] Glypican-3 is expressed in fetal liver and placenta during development and is down-regulated or silenced in normal adult tissues. Mutations and depletions in the glypican-3 gene are responsible for the Simpson-Golabi-Behmel or Simpson dysmorphia syndrome in humans. Glypican-3 is expressed in various cancers and, in particular, hepatocellular carcinoma (HCC, the most common form of liver cancer), melanoma, Wilm's tumor, and hepatoblastoma (Jakubovic and Jothy 2007; Nakatsura and Nishimura 2005).

[0006] HCC is the third leading cause of cancer-related deaths worldwide. Each year, HCC accounts for about 1 million deaths (Nakatsura and Nishimura 2005). Hepatitis B virus, hepatitis C virus, and chronic heavy alcohol use leading to cirrhosis of the liver remain the most common causes of HCC. Its incidence has increased dramatically in the United States because of the spread of hepatitis C virus infection and is expected to increase for the next 2 decades. HCC is treated primarily by liver transplantation or tumor resection. Patient prognosis is dependent on both the underlying liver function and the stage at which the tumor is diagnosed (Parikh and Hyman 2007). Thus, effective HCC treatment strategies are needed.

[0007] There are various disclosures of the uses of anti-glypican-3 antibodies in cancer therapy, either as a therapeutic antibody or in a conjugate. Smith *et al.* 2007 disclose conjugates of an anti-glypican-3 antibody and an auristatin. Zhang *et al.* 2014 disclose ADCs of an anti-glypican-3 antibody and a DNA minor groove binder-alkylator of the cyclopropabenzindole (CBI) type. Terrett *et al.* 2014 disclose anti-glypican-3 antibodies and their use for treating glypican-3 related conditions, including HCC and, generically, their use in immunoconjugates. Other disclosures relating to immunoconjugates of anti-glypican-3 antibodies include Ho *et al.*, 2015 and 2015.

BRIEF SUMMARY OF THE INVENTION

[0008] This disclosure provides an antibody-drug conjugate (ADC) comprising an anti-glypican-3 antibody as the targeting agent and a tubulysin analog, which ADC has an unexpectedly desirable combination of potency, therapeutic index, and pharmacokinetic properties and which can be used to treat a variety of cancers, including HCC, lung cancer, and ovarian cancer. The antibody-drug conjugate has a structure represented by formula I



wherein

m is 1, 2, 3, or 4 and

Ab is an anti-glypican-3 antibody having

- 5 (a) a heavy chain variable region CDR1 comprising SEQ ID NO:1;
- (b) a heavy chain variable region CDR2 comprising SEQ ID NO:2;
- (c) a heavy chain variable region CDR3 comprising SEQ ID NO:3;
- (d) a kappa light chain variable region CDR1 comprising SEQ ID NO:4;
- (e) a kappa light chain variable region CDR2 comprising SEQ ID NO:5; and
- 10 (f) a kappa light chain variable region CDR3 comprising SEQ ID NO:6.

[0009] As reflected by the subscript m, each antibody Ab can conjugate with more than one drug moiety, depending on the number of sites antibody Ab has available for conjugation and the experimental conditions employed. Those skilled in the art will appreciate that, while each individual antibody Ab is conjugated to an integer number of drug moieties, a conjugate preparation of the conjugate may analyze for a non-integer ratio of drug moieties to antibody Ab, reflecting a statistical average. This ratio is referred to as the substitution ratio (SR) or, synonymously, the drug-antibody ratio (DAR). Preferably, each antibody Ab is conjugated to 3 or 4 drug moieties (*i.e.*, m is 3 or 4). The average m for a conjugate preparation preferably is between 3 and 3.5 (*i.e.*, the DAR is 3 to 3.5).

20 [0010] In a preferred embodiment, the antibody Ab has a heavy chain variable region amino acid sequence according to SEQ ID NO:7 and a kappa light chain variable region amino acid sequence according to SEQ ID NO:8.

[0011] In another preferred embodiment, antibody Ab has a heavy chain constant region comprising comprising SEQ ID NO:9 and a kappa light chain constant region comprising SEQ ID NO:10. The heavy chain constant region may further have a lysine at its C-terminus..

[0012] In yet another preferred embodiment, antibody Ab has a heavy chain comprising SEQ ID NO:11 and a kappa light chain comprising SEQ ID NO:12. Such antibody is referred to herein as GPC3.1. Correspondingly, the ADC of formula I where the antibody is GPC3.1 is referred to herein as ADC3.1.

5 [0013] The heavy chain of antibody GPC3.1 optionally may further have a lysine at its C-terminus.

[0014] This disclosure also provides a method of treating a cancer in a human subject suffering from such cancer, comprising administering to the human subject a therapeutically effective amount of an antibody-drug conjugate of this disclosure, where the cancer is
10 hepatocellular carcinoma, ovarian, or lung cancer, especially liver or lung cancer. The antibody-drug conjugate preferably is administered intravenously, at a dose of between 0.1 and 20 mg/kg, preferably between 0.5 and 15 mg/kg, and more preferably between 1.0 and 5 mg/kg.

[0015] This disclosure also provides a pharmaceutical formulation comprising an antibody-drug conjugate of this disclosure and a pharmaceutically acceptable excipient.

15 [0016] This disclosure also provides an isolated nucleic acid molecule encoding an antibody heavy chain comprising SEQ ID NO:11, which nucleic acid molecule preferably comprises SEQ ID NO:13.

[0017] This disclosure also provides an expression vector comprising the nucleic acid molecule of SEQ ID NO:13, and a host cell comprising such expression vector.

20 [0018] This disclosure also provides an isolated nucleic acid molecule encoding an antibody kappa chain comprising SEQ ID NO:12, which nucleic acid molecule comprises SEQ ID NO:15.

[0019] This disclosure also provides an expression vector comprising the nucleic acid molecule of SEQ ID NO:15, and a host cell comprising such expression vector.

BRIEF DESCRIPTION OF THE DRAWING(S)

25 [0020] **FIGs. 1A-1C** show in combination the alignment between a nucleotide sequence encoding a signal peptide and the heavy chain of an anti-glypican-3 antibody of this disclosure (SEQ ID NO:13) and the amino acid sequence so encoded (SEQ ID NO:14).

[0021] **FIGs. 2A-2B** show in combination the alignment between a nucleotide sequence encoding a signal peptide and the kappa light chain of an anti-glypican-3 antibody of this
30 disclosure (SEQ ID NO:15) and the amino acid sequence so encoded (SEQ ID NO:16).

[0022] FIGs. 3A-3C show, in combination, that antibody GPC3.1 binds to Hep3B hepatocellular carcinoma and H446 small-cell lung cancer cells, and that, when conjugated to a drug, it effectively delivers the drug to inhibit proliferation of such cells.

[0023] FIGs. 4A-4B show the dose dependent efficacy of ADC3.1 in a Hep3B xenograft model, as measured by tumor volume shrinkage and per cent body weight change, respectively.

[0024] FIGs. 5A-5B and 6A-6B compare the efficacy of ADC3.1 in single dosing and split dosing administration regimens, against HuH7D12 hepatocellular carcinoma tumors.

[0025] FIGs. 7A-7B and 8A-8B compare the efficacy of ADC3.1 in single dosing and split dosing administration regimens, against H446 small-cell lung cancer tumors.

[0026] FIGs. 9 and 10 are xenograft studies showing the efficacy of ADC3.1 against ovarian and squamous lung patient derived tumor cells, respectively.

[0027] FIGs. 11A-11C compare the properties of antibody GPC3.1 and three anti-glypican-3 antibodies that are variants of antibody GPC3.1, and of their respective ADCs.

[0028] FIG. 12A compares the pharmacokinetic and *in vitro* properties of GPC3.1 against those of other anti-glypican-3 antibodies prepared by independent immunizations of transgenic mice. FIG. 12B compares the *in vitro* potencies of ADC3.1 and an ADC prepared from one of those other antibodies.

[0029] FIGs. 13A-13B compare the *in vivo* efficacy of ADC3.1 against those of ADCs made with other anti-glypican-3 antibodies.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS

[0030] “Antibody” means whole antibodies and any antigen binding fragment (*i.e.*, “antigen-binding portion”) or single chain variants thereof. A whole antibody is a protein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain comprises a heavy chain variable region (V_H) and a heavy chain constant region comprising three domains, C_{H1}, C_{H2} and C_{H3}. Each light chain comprises a light chain variable region (V_L or V_k) and a light chain constant region comprising one single domain, C_L. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with more conserved framework regions (FRs). Each V_H and V_L comprises three CDRs and four FRs, arranged from amino- to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. The variable regions contain a binding domain that interacts with an antigen. The constant regions

may mediate the binding of the antibody to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (C1q) of the classical complement system. An antibody is said to “specifically bind” to an antigen X if the antibody binds to antigen X with a K_D of 5×10^{-8} M or less, more preferably 1×10^{-8} M or less, more preferably 6×10^{-9} M or less, more preferably 3×10^{-9} M or less, even more preferably 2×10^{-9} M or less. The antibody can be chimeric, humanized, or, preferably, human. The heavy chain constant region can be engineered to affect glycosylation type or extent, to extend antibody half-life, to enhance or reduce interactions with effector cells or the complement system, or to modulate some other property. The engineering can be accomplished by replacement, addition, or deletion of one or more amino acids or by replacement of a domain with a domain from another immunoglobulin type, or a combination of the foregoing.

[0031] “Antigen binding fragment” and “antigen binding portion” of an antibody (or simply “antibody portion” or “antibody fragment”) mean one or more fragments of an antibody that retain the ability to specifically bind to an antigen. It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody, such as (i) a Fab fragment, a monovalent fragment consisting of the V_L , V_H , C_L and C_{H1} domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fab' fragment, which is essentially an Fab with part of the hinge region (see, for example, Abbas *et al.*, *Cellular and Molecular Immunology*, 6th Ed., Saunders Elsevier 2007); (iv) a Fd fragment consisting of the V_H and C_{H1} domains; (v) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (vi) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a V_H domain; (vii) an isolated complementarity determining region (CDR); and (viii) a nanobody, a heavy chain variable region containing a single variable domain and two constant domains. Preferred antigen binding fragments are Fab, $F(ab')_2$, Fab' , Fv, and Fd fragments. Furthermore, although the two domains of the Fv fragment, V_L and V_H , are encoded by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv, or scFv); see, *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also encompassed within the term “antigen-binding portion” of an antibody.

[0032] An “isolated antibody” means an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds antigen

X is substantially free of antibodies that specifically bind antigens other than antigen X). An isolated antibody that specifically binds antigen X may, however, have cross-reactivity to other antigens, such as antigen X molecules from other species. In certain embodiments, an isolated antibody specifically binds to human antigen X and does not cross-react with other (non-human) antigen X antigens. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

[0033] “Monoclonal antibody” or “monoclonal antibody composition” means a preparation of antibody molecules of single molecular composition, which displays a single binding specificity and affinity for a particular epitope.

[0034] “Human antibody” means an antibody having variable regions in which both the framework and CDR regions (and the constant region, if present) are derived from human germline immunoglobulin sequences. Human antibodies may include later modifications, including natural or synthetic modifications. Human antibodies may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, “human antibody” does not include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[0035] “Human monoclonal antibody” means an antibody displaying a single binding specificity, which has variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. In one embodiment, human monoclonal antibodies are produced by a hybridoma that includes a B cell obtained from a transgenic nonhuman animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell.

ANTIBODY GPC3.1

[0036] At a first glance, the role to be performed by an antibody in an ADC appears to be a simple one: lead the conjugated drug to the target cell, and once there, release its drug payload, either inside the target cell or in the environs thereof. However, the selection of a suitable antibody for a successful ADC entails many variables beyond merely binding to the antigen of interest. Multiple factors may affect the overall efficacy of an ADC, including stability in circulation prior to reaching the target cell, binding affinity the antigen, safety vis-a-vis non-target cells that also express the antigen, and pharmacokinetics. The interplay among these

factors is difficult to predict. As the data presented hereinbelow demonstrates, not all antibodies binding to glypican-3 produce an ADC as efficacious as antibody GPC3.1.

[0037] CDR1, CDR2, and CDR3 of the heavy chain of antibody GPC3.1 comprise the amino acids of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3, respectively. CDR1, CDR2, and
5 CDR3 of the light (kappa) chain of antibody GPC3.1 comprise the amino acids of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, respectively. The amino acid sequences of the heavy and kappa chain variable regions are given by SEQ ID NO:7 and SEQ ID NO:8, respectively.

[0038] The heavy chain constant region of antibody GPC3.1 is of the IgG1 isotype, comprising the R214, E356, and M358 allotypes (numbering per EU index as set forth in Kabat
10 et al., "Sequences of proteins of immunological interest, 5th ed., Pub. No. 91-3242, U.S. Dept. Health & Human Services, NIH, Bethesda, Md., 1991; hereinafter "Kabat"). Its amino acid sequence is set forth in SEQ ID NO:9. This allotype combination has a high prevalence in the Caucasian population.

[0039] The kappa light chain constant region of antibody GPC3.1 has an amino acid
15 sequence as set forth in SEQ ID NO:10.

[0040] The complete heavy and kappa light chain amino acid sequences of antibody GPC3.1 are set forth in SEQ ID NO:11 and NO:12, respectively.

[0041] Terrett *et al.* 2014 disclose an anti-glypican-3 antibody 4A6 that has the same heavy and light chain variable regions as antibody GPC3.1, of the IgG1 or IgG4 isotype. It further
20 generically discloses that antibody 4A6 can be used in ADCs, but does not provide any working examples.

[0042] Antibody GPC3.1 can be produced by recombinant expression of its heavy and kappa chains in a suitable host cell. SEQ ID NO:13 shows a DNA sequence, inclusive of a signal peptide, that can be used for recombinant production of the heavy chain, while SEQ ID NO:14
25 shows the amino acid sequence encoded thereby. The alignment between the DNA and amino acid sequences is shown in **FIGs. 1A-1C**. SEQ ID NO:15 shows a DNA sequence, inclusive of a signal peptide, that can be used for recombinant production of the kappa chain, while SEQ ID NO:16 shows the amino acid sequence encoded thereby. The alignment between the DNA and amino acid sequences is shown in **FIGs. 2A-2B**.

[0043] Those skilled in the art will know that, when an antibody is produced recombinantly
30 with a heavy chain C-terminal lysine group, such lysine is often removed by endogenous carboxypeptidases during cell culture production (Luo *et al.* 2012). Therefore antibody GPC3.1 can also be produced employing a DNA sequence corresponding to SEQ ID NO:13 but with an

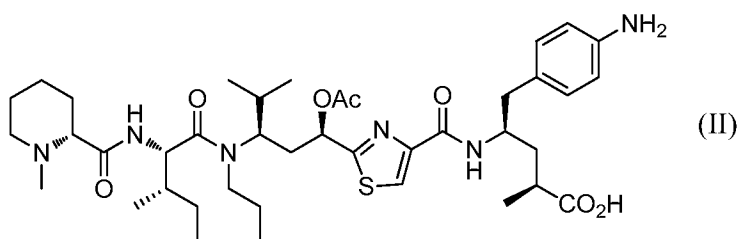
added codon for lysine at the C-terminal position and then allowing post-translational enzymatic removal of the lysine.

[0044] This disclosure also provides nucleic acids encoding antibody GPC3.1, in particular a nucleic acid (SEQ ID NO:13) encoding its heavy chain (SEQ ID NO:11), and conservative
 5 modifications of such nucleic acids. A “conservative modification” means, in respect of a nucleic acid sequence, a modification that replaces a nucleic acid therein with another but the modification results in the modified nucleic acid sequence encoding the same or a conservatively
 10 modified amino acid sequence compared to the one encoded by the original nucleic acid sequence or, where the original nucleic acid does not encode an amino acid sequence, the resultant modified nucleic acid sequence is essentially the same as the original nucleic acid sequence. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acid sequences may encode any given protein. A nucleic acid sequence may have plural conservative modifications.

[0045] Where a polypeptide or nucleic acid molecule is associated with a particular SEQ ID
 15 NO:., preferably such polypeptide or nucleic acid molecule consists of the amino acid or nucleic acid sequence of the associated SEQ ID NO:.

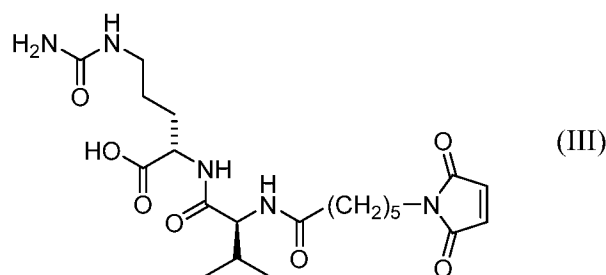
THERAPEUTIC AGENT AND LINKER

[0046] The therapeutic agent in the conjugate of this disclosure is a synthetic tubulysin analog and has a structure represented by formula (II) (Cheng *et al.* 2013):



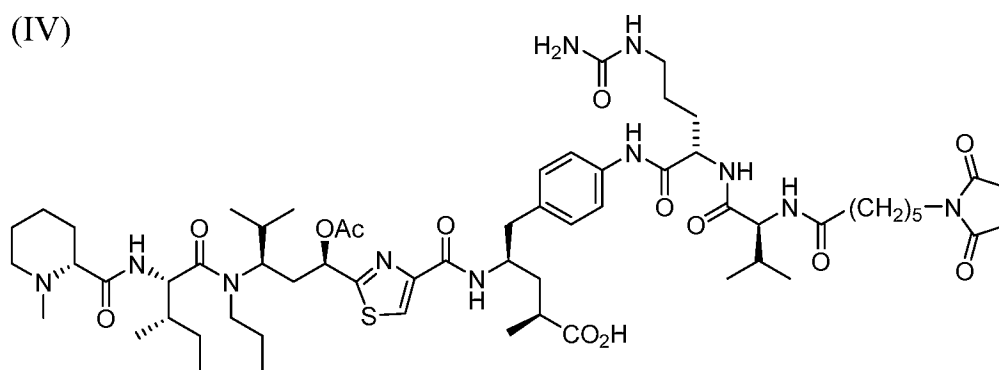
[0047] The tubulysins are potent naturally occurring cytotoxins, which act as anti-mitotic agents that interfere with mitosis by preventing the assembly of the tubulins into microtubules. The affected cells to accumulate in the G₂/M phase and undergo apoptosis.

[0048] To conjugate a therapeutic agent to an antibody, a linker moiety is needed. In the
 25 instance of the present invention, the linker moiety has a structure represented by formula (III):

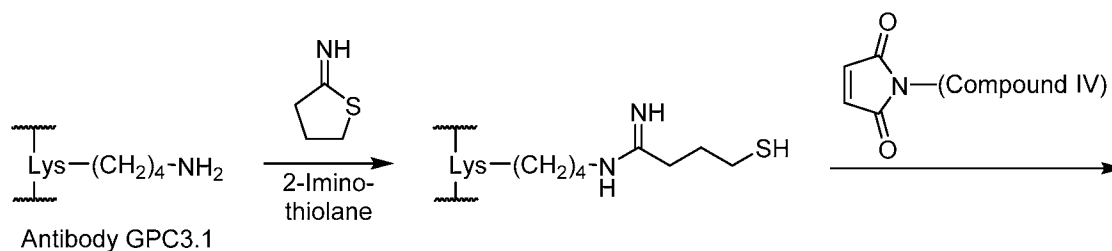


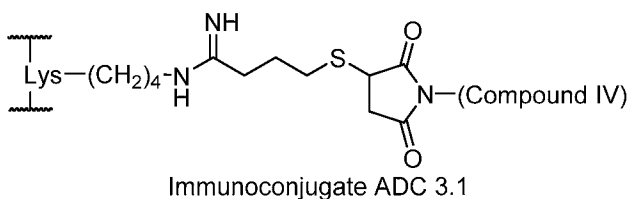
It comprises a valine-citrulline dipeptide (Val-Cit, recited in the conventional N-to-C direction), which is designed to be cleaved by the intracellular enzyme cathepsin B after the ADC has reached a target cancer cell and has been internalized by it, thus releasing the therapeutic agent to exert its cytotoxic effect. See Dubowchik *et al.* 1998a, 1998b, and 2002.

[0049] In the preparation of the conjugate of this disclosure, drug (II) and linker (III) are coupled to produce a therapeutic agent-linker compound having a structure represented by formula (IV), by forming an amide bond between the $-CO_2H$ group of the citrulline in linker (II) and the aromatic $-NH_2$ of compound (II).



[0050] Compound (IV) is then conjugated to the antibody to prepare an ADC of formula (I). An ϵ -amino group in the side chain of a lysine residue of antibody GPC3.1 is reacted with 2-iminothiolane to introduce a free thiol ($-SH$) group. The thiol group can react with the maleimide group in compound (IV) to effect conjugation:

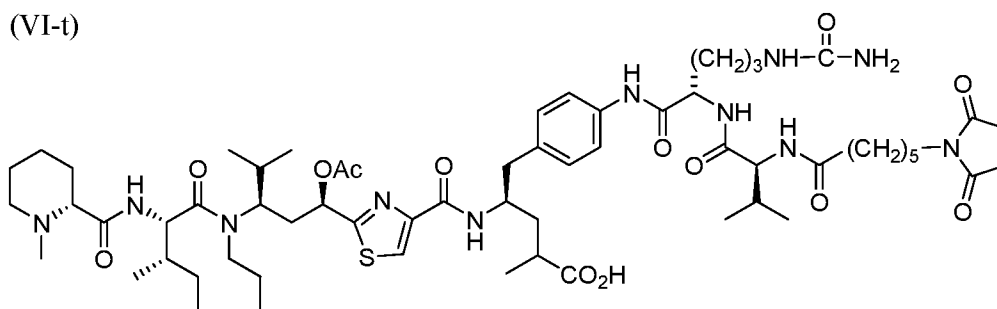




[0051] Typically, a thiolation level of two to four thiols per antibody is achieved. For a representative procedure, see Cong *et al.* 2015, the disclosure of which is incorporated herein by reference.

5 **[0052]** In addition to the naturally occurring tubulysins, synthetic tubulysin analogs with potent cytotoxic activity are known, for example as disclosed in Cheng *et al.* 2013 and Cong 2015. These references further disclose that such tubulysin analogs can be used in ADCs.

[0053] In particular, Cheng *et al.* 2013 discloses the preparation of a tubulysin analog-linker compound referred to there as formula (VI-t) (i.e., identical to formula (IV) above excepting the racemic methyl group alpha to the carboxylic acid) and, at Table 4 therein, its conjugates with an anti-CD70 antibody or anti-mesothelin antibody.



EXAMPLES

[0054] The practice of this invention can be further understood by reference to the following
15 examples, which are provided by way of illustration and not of limitation.

Example 1

[0055] The antibody GPC3.1 V_H and V_K sequences were cloned into expression vectors containing the osteonectin signal sequence and the human IgG1 and kappa constant regions. The resulting heavy and light chain expression vectors were co-transfected into CHO cells and stable clones were selected and screened for IgG expression. One clone was chosen and expanded for antibody production.

Example 2

[0056] This general procedure can be used to make ADC3.1 and other antibody-drug conjugates disclosed herein. Initially the antibody is buffer exchanged into 0.1 M phosphate buffer (pH 8.0) containing 50 mM NaCl and 2 mM diethylene triamine pentaacetic acid (DTPA) and concentrated to 5-10 mg/mL. Thiolation is achieved through addition of 2-iminothiolane to the antibody. The amount of 2-iminothiolane to be added can be determined by a preliminary experiment and varies from antibody to antibody. In the preliminary experiment, a titration of increasing amounts of 2-iminothiolane is added to the antibody, and following incubation with the antibody for 1 h at RT (room temperature, circa 25 °C), the antibody is desalted into 50 mM HEPES, 5 mM Glycine, 2 mM DTPA, pH 5.5 using a SEPHADEX™ G-25 column and the number of thiol groups introduced determined rapidly by reaction with dithiodipyridine (DTDP). Reaction of thiol groups with DTDP results in liberation of thiopyridine, which can be monitored spectroscopically at 324 nm. Samples at a protein concentration of 0.5-1.0 mg/mL are typically used. The absorbance at 280 nm can be used to accurately determine the concentration of protein in the samples, and then an aliquot of each sample (0.9 mL) is incubated with 0.1 mL DTDP (5 mM stock solution in ethanol) for 10 min at RT. Blank samples of buffer alone plus DTDP are also incubated alongside. After 10 min, absorbance at 324 nm is measured and the number of thiol groups is quantitated using an extinction coefficient for thiopyridine of 19,800 M⁻¹.

[0057] Typically a thiolation level of about two to three thiol groups per antibody is desirable. For example, with some antibodies this can be achieved by adding a 15-fold molar excess of 2-iminothiolane followed by incubation at RT for 1 h. The antibody is then incubated with 2-iminothiolane at the desired molar ratio and then desalted into conjugation buffer (50 mM HEPES, 5 mM glycine, 2 mM DTPA, pH 5.5)). The thiolated material is maintained on ice while the number of thiols introduced is quantitated as described above.

[0058] After verification of the number of thiols introduced, the dimer-linker of formula (IV) is added at a 2.5-fold molar excess per thiol. The conjugation reaction is allowed to proceed in conjugation buffer containing a final concentration of 25% propylene glycol and 5% trehalose. Commonly, the drug-linker stock solution is dissolved in 100% DMSO. The stock solution is added directly to the thiolated antibody.

[0059] The conjugation reaction mixture is incubated at RT for 2 h with gentle stirring. A 10-fold molar excess of N-ethyl maleimide (100 mM stock in DMSO) is then added to the conjugation mixture and stirred for an additional hour to block any unreacted thiols.

[0060] The sample is then filtered via a 0.2 μ filter. The material is buffer exchanged via TFF VivaFlow 50 Sartorius 30 MWCO PES membrane into 10 mg/mL glycine, 20 mg/mL sorbitol, 15% acetonitrile (MeCN) pH 5.0 (5X TFF buffer exchange volume), to remove any unreacted drug. The final formulation is carried out by TFF into 20 mg/mL sorbitol, 10 mg/mL glycine, pH 5.0.

Example 3

[0061] **FIG. 3A** compares the binding of ADC3.1 to Hep3B hepatocellular carcinoma (liver cancer) and H446 small-cell lung cancer (SCLC) cells. The higher binding to Hep3B cells indicates that they express a higher level of glypican-3 than H446 cells. The isotype control was an anti-CD70 antibody.

[0062] A ^3H thymidine assay, where the inhibition of incorporation of ^3H thymidine indicates inhibition of proliferation of the tested cell line, was used to assess the dose-dependent inhibitory effect of ADC3.1 on the proliferation of Hep3B and H446 cells. The human tumor cell lines were obtained from the American Type Culture Collection (ATCC), P.O. Box 1549, Manassas, VA 20108, USA, and cultured according to instructions from ATCC. Cells were seeded at 1.0×10^4 cells/well in 96-well plates. 1:3 serial dilutions of ADC3.1 were added to the wells. Plates were allowed to incubate for 72 h. The plates were pulsed with 1.0 μCi of ^3H -thymidine per well for the last 24 hours of the total incubation period, harvested, and read on a Top Count Scintillation Counter (Packard Instruments, Meriden, CT). The EC_{50} values – the ADC concentration at which cell proliferation was reduced by 50% – were determined using PRISM™ software, version 4.0 (GraphPad Software, La Jolla, CA, USA).

[0063] **FIGS. 3B** and **3C** show that ADC3.1 inhibits proliferation of the tested cell lines in a dose-dependent manner, while the isotype ADC (control) had essentially no inhibitory effect. In **FIG. 3B** the control was a conjugate of an anti-mesothelin antibody and the drug-linker compound of formula (IV). In **FIG. 3C** the control was a conjugate of an anti-CD70 antibody and the drug-linker compound of formula (IV).

[0064] The EC_{50} values, along with those against HepG2 and HuH7D12 hepatocellular carcinoma cell lines, are shown in Table 1, plus data for the drug alone (formula (II)). These results show that, generally, ADC3.1 is effective in delivering the drug to the target cell, at a level comparable to that of unconjugated drug. That is, release of the drug from the conjugate is efficient.

Table 1 – Efficacy of ADC Compared to Unconjugated Drug				
Agent	EC ₅₀ (nM)			
	Hep3B	HepG2	HuH7D12	H446
ADC3.1	0.2	0.3	0.2	1.0
Unconjugated Drug (II)	0.2	0.3	0.2	0.2

Example 4

[0065] FIGs. 4A and 4B present the data for a xenograft study on the dose dependent efficacy of ADC3.1 against Hep3B tumors. ADC3.1 was administered twice, seven days apart (Q7Dx2), at doses of 0.01, 0.03, and 0.1 $\mu\text{mol/kg}$. (A dose of 0.1 $\mu\text{mol/kg}$ corresponds approximately to 5 mg/kg. Thus, the dosages convert to 0.5, 1.5, and 5 mg/kg, respectively.) The isotype ADC was the same as in FIG. 3C. In this and other xenograft studies described herein, CB17.SCID mice were used.

[0066] The data show that, at 0.1 $\mu\text{mol/kg}$, ADC3.1 was highly effective in causing tumor regression (FIG. 4A), was well tolerated, and relieved tumor growth related cachexia. The vehicle (formulation buffer) and ADC isotype controls were ineffective, with the selectivity between ADC3.1 and the isotype control being greater than 3:1. Lower doses of ADC3.1 (0.01 and 0.03 $\mu\text{mol/kg}$) were significantly less effective. This steep dose dependent response is believed to be partially due to the nonlinear pharmacokinetics of ADC3.1.

Example 5

[0067] The efficacy of ADC3.1 in a xenograft study of the single dose efficacy of ADC3.1 against HuH7D12 cells is shown in FIG. 5A (tumor volume regression) and FIG. 5B (per cent body weight change). The dosages were 0.1 and 0.3 $\mu\text{mol/kg}$ (5 and 15 mg/kg, respectively).

[0068] The corresponding split dosing study is shown in FIGs. 6A and 6B, with three doses administered in one-week intervals (Q7Dx3). The doses were 0.033 and 0.1 $\mu\text{mol/kg}$.

[0069] The results indicate that the single dosing regimen was more efficacious, with tumor regression noted with a single dose of 0.3 $\mu\text{mol/kg}$ (seven of eight mice becoming tumor-free).

Example 6

[0070] A similar xenograft study was performed, comparing single dose (FIGs. 7A-7B) and split dose (Q7Dx3, FIGs. 8A-8B) administration schedules on the efficacies of ADC3.1 against H446 cells. The doses amounts noted parenthetically in the figures are in $\mu\text{mol/kg}$,

corresponding to 5 and 15 mg/kg, respectively. This study included a control (isotype ADC), which was an ADC of an anti-mesothelin antibody and the dimer-linker of formula (IV).

[0071] Again, the single dose regimen was somewhat more efficacious, although exhibiting higher transient body weight loss. In the single dose study, tumor regression was observed in
5 eight of eight mice at 0.3 $\mu\text{mol/kg}$ and in four of eight mice at 0.1 $\mu\text{mol/kg}$. In the split dose study, tumor regression was observed in four of eight mice at 0.1 $\mu\text{mol/kg}$.

Example 7

[0072] This example provides results of patient derived xenograft (PDX) studies.

[0073] **FIG. 9** shows the reduction in a patient-derived ovarian tumor volume upon treatment
10 with ADC3.1. The dosing schedule was Q7Dx6 (six weekly doses) and the dosage was 3 mg/kg. The control was a CD70 ADC carrying the same linker and drug moiety (formula (IV)), at a dosage of also 3 mg/kg but with a dosing schedule of three weekly doses (Q7Dx3). First dosing was 30 days post-implantation.

[0074] **FIG. 10** shows the reduction in a patient derived squamous lung tumor volume upon
15 treatment with ADC3.1. The dosing schedule was X7Dx6 (six weekly doses) and the dosage was 3 mg/kg. The control was a mesothelin ADC carrying the same linker and drug moiety (formula (IV)), at a dosage of also 3 mg/kg but a dosing schedule was three weekly doses (Q7Dx3). The first dosing was 30 days post-implantation.

Example 8

20 [0075] This example describes a study seeking to identify variants of antibody GPC3.1, which might be better targeting agents for an ADC.

[0076] Anti-glypican-3 antibodies internalize with similar efficiencies into target cells such as cancerous cells that express high levels of glypican-3, regardless of whether they are low affinity binders (fast k_{off} , $K_D \approx 10 \text{ nM}$) or high affinity binders (slow k_{off} , $K_D \leq 1 \text{ nM}$).
25 Hypothetically, it is possible that an ADC of anti-glypican-3 antibody with relatively low affinity may exhibit reduced toxicity against normal cells, which have a lower expression level of glypican-3. Further, an ADC of an anti-glypican-3 antibody with relatively low affinity may distribute more facilely into distant tumor tissues. To evaluate this hypothesis, 83 variants of Antibody GPC3.1 were prepared, containing modifications in the variable region. Of these, the
30 three most promising (designated antibodies A, B, and C) were selected for head-to-head comparisons against antibody GPC3.1.

[0077] Compared to Antibody GPC3.1, Antibody A has the same heavy chain CDR1 and light (kappa) chain CDR1 and CDR2 (SEQ ID NO:1, NO:4, and NO:5, respectively) but different heavy chain CDR2 and CDR3 (SEQ ID NO:17 and NO:18, respectively) and kappa chain CDR3 (SEQ ID NO:19). Also, Antibody A differs from Antibody GPC3.1 in certain heavy chain framework amino acids, as noted in its heavy chain variable region sequence (SEQ ID NO:23). Its kappa chain variable region sequence is provided in SEQ ID NO:24. Its heavy and kappa chain constant regions have the same sequence as those in Antibody GPC3.1 (SEQ ID NO:9 and NO:10, respectively).

[0078] Compared to Antibody GPC3.1, Antibody B has the same heavy chain CDR3 and light (kappa) chain CDR1 and CDR2 (SEQ ID NO:3, NO:4 and NO:5, respectively) but different heavy chain CDR1 and CDR2 (SEQ ID NO:20 and NO:21, respectively) and kappa chain CDR3 (SEQ ID NO:22). Also, Antibody B differs from Antibody GPC3.1 in certain heavy chain framework amino acids, as noted in its heavy chain variable region sequence (SEQ ID NO:25). Its kappa chain variable region sequence is provided in SEQ ID NO:26. Its heavy and kappa chain constant regions have the same sequence as those in Antibody GPC3.1 (SEQ ID NO:9 and NO:10, respectively).

[0079] Compared to Antibody GPC3.1, Antibody C has the same heavy chain CDR3 and light (kappa) chain CDR1 and CDR2 (SEQ ID NO:3, NO:4 and NO:5, respectively) but different heavy chain CDR1 and CDR2 (SEQ ID NO:20 and NO:17, respectively) and kappa chain CDR3 (SEQ ID NO:19). Also, Antibody C differs from Antibody GPC3.1 in certain heavy chain framework amino acids, as noted in its heavy chain variable region sequence (SEQ ID NO:27). Its kappa chain variable region sequence is provided in SEQ ID NO:28. Its heavy and kappa chain constant regions have the same sequence as those in Antibody GPC3.1 (SEQ ID NO:9 and NO:10, respectively).

[0080] The respective k_{off} and K_D values for antibodies A, B, C and GPC3.1 are shown in Table 2.

Table 2 – Binding Affinity of Variant Antibodies to Glypican-3			
Antibody	$K_D \times 10^{-9}$ (M)	$k_{on} \times 10^4$ (1/Ms)	$k_{off} \times 10^{-4}$ (1/s)
A	2.7	110	29
B	2.2	170	36
C	0.9	170	16
GPC3.1	28	150	415

[0081] Compared to antibody GPC3.1, variant antibodies A, B, and C exhibited between 10- and 30-fold improvement in K_D and k_{off} according to Biacore™ assays. They also exhibited faster clearance in mice.

5 [0082] FIG. 11A is a comparison of the pharmacokinetic (PK) profiles in SCID mice at an intravenous dose of 0.5 mg/kg. The profiles were similar, although that of antibody GPC3.1 was slightly better.

[0083] FIG. 11B presents the results of a 3H thymidine incorporation *in vitro* assay using Hep3B hepatocellular carcinoma cells. According to these results, ADCs of antibodies A, B, and
10 C with the drug-linker of formula (IV) (ADCs A, B, and C, respectively) were slightly more active than ADC3.1

[0084] FIG. 11C shows the results of a FACS (fluorescence activated cell sorting) study of the binding of the four ADCs of FIG. 11B. ADC A was slightly more active than the other three, which were similarly active.

15 [0085] Thus, in view of the foregoing *in vitro* results, ADCs A, B, and C were viewed as promising candidates for *in vivo* comparative studies against ADC3.1. The results of such studies are presented and discussed in Example 10 hereinbelow.

Example 9

[0086] This example describes a different study with a similar objective, that is, to identify
20 other anti-glypican-3 antibodies, which might be more efficacious as a targeting agent in an ADC than antibody GPC3.1.

[0087] Rather than modifying antibody GPC3.1, anti-glypican-3 antibodies were made *de novo* by immunizing HuMab® transgenic mice. Methods for raising of human antibodies by immunizing HuMab® transgenic mice are disclosed in Terrett *et al.*, US 8,680,247 B2 (2014),
25 the disclosure of which is incorporated herein by reference. The binding properties of four antibodies so raised and antibody GPC3.1 are shown in Table 3 below.

Table 3 – Binding Affinity of HuMab® Antibodies to Glypican-3			
Antibody	$K_D \times 10^{-9}$ (M)	$k_{on} \times 10^4$ (1/Ms)	$k_{off} \times 10^{-4}$ (1/s)
D	3.9	19	74
E	0.5	15	0.7
F	13	27	36
G	133	2.1	28
GPC3.1	28	147	415

[0088] Binning studies showed that antibody E bound to a different epitope than antibody GPC3.1 and was non-blocking *vis-a-vis* it. The epitope grouping of antibody D was not determined.

5 [0089] **FIG. 12A** shows that the PK profiles of antibodies GPC3.1, E, and E are similar. SCID mice were used, with a dose of 0.5 mg/kg administered intravenously. **FIG. 12B** shows that, in the *in vitro* Hep3B ^3H thymidine incorporation assay, the activities of ADC3.1 and an ADC of antibody E with drug-linker (IV) (ADC E) are very close, with EC_{50} 's of 0.17 and 0.13 nM, respectively.

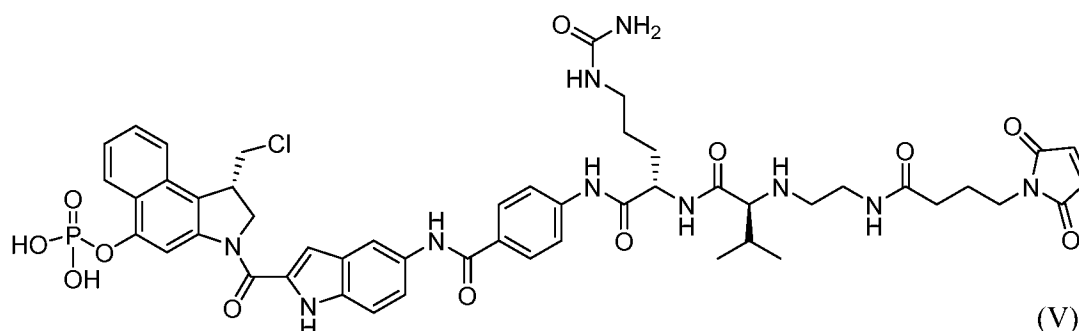
10 [0090] In view of the above results antibody E was selected for ADC comparative studies against ADC 3.1.

Example 10

[0091] ADCs were prepared combining drug linker compound (IV) and antibodies A, B, C, and E, to produce ADCs respectively designated ADC A, ADC B, ADC C, and ADC E. *In vivo* 15 xenograft studies were performed for these four ADCs alongside ADC 3.1, a vehicle (formulation buffer) control, and an ADC control (an ADC of an anti-CD70 antibody with drug-linker (IV)), using Hep3B cells. Dosing was 0.1 $\mu\text{mol/kg}$ (5 mg/kg) in one case (**FIG. 13A**) and 0.03 $\mu\text{mol/kg}$ (1.5 mg/kg) in the other case (**FIG. 13B**). The dosing frequency was Q7Dx2. As can be seen from these figures, none of ADCs A, B, C or E was as efficacious as antibody 20 GPC3.1 in reducing the tumor volume. These results evidence the unpredictable nature of developing an efficacious ADC by extrapolating from *in vitro* results. The superior *in vivo* efficacy of ADC 3.1 in comparison with the other four ADCs was unexpected in view of the similarity in *in vitro* properties.

Example 11

[0092] For comparative purposes, an ADC of anti-GPC3 antibody GPC3.1 and drug-linker compound (V) was prepared.



5 [0093] The drug moiety in compound (V) belongs to the class of cytotoxins known as cyclopropabenzindoles (CBIs) and have been used in ADCs (Zhang *et al.* 2015).

[0094] When tested against Hep3B cancer cells by the ³H thymidine assay, the ADC of compound (V) had a potency (EC₅₀ 0.079 nM) that compared favorably against that of ADC3.1 (EC₅₀ 0.15 nM). However, the former's pharmacokinetic (PK) properties were not as desirable
10 as that of ADC3.1.

[0095] The foregoing detailed description of the invention includes passages that are chiefly or exclusively concerned with particular parts or aspects of the invention. It is to be understood that this is for clarity and convenience, that a particular feature may be relevant in more than just the passage in which it is disclosed, and that the disclosure herein includes all the appropriate
15 combinations of information found in the different passages. Similarly, although the various figures and descriptions herein relate to specific embodiments of the invention, it is to be understood that where a specific feature is disclosed in the context of a particular figure or embodiment, such feature can also be used, to the extent appropriate, in the context of another figure or embodiment, in combination with another feature, or in the invention in general.

20 [0096] Further, while the present invention has been particularly described in terms of certain preferred embodiments, the invention is not limited to such preferred embodiments. Rather, the scope of the invention is defined by the appended claims.

REFERENCES

[0097] Full citations for the following references cited in abbreviated fashion by first author
25 (or inventor) and date earlier in this specification are provided below. Each of these references is incorporated herein by reference for all purposes.

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- [00112] Terrett *et al.*, US 8,680,247 B2 (2014).
- [00113] Zhang *et al.*, US 8,852,599 B2 (2014).
- [00114] Zhang *et al.*, US 9,186,416 B2 (2015).

SEQUENCES

[00115] Incorporated herein by reference in its entirety is a Sequence Listing named “20170322_SEQT_12526WOPCT_YC.txt,” comprising SEQ ID NO:1 through SEQ ID NO:28, which include nucleic acid and/or amino acid sequences disclosed herein. The Sequence Listing has been submitted herewith in ASCII text format via EFS-Web, and thus constitutes both the paper and computer readable form thereof. The Sequence Listing was first created using PatentIn 3.5 on March 22, 2017, and is approximately 32 KB in size.

[00116] Table 4 below provides a short summary of the sequence listings filed together with this specification.

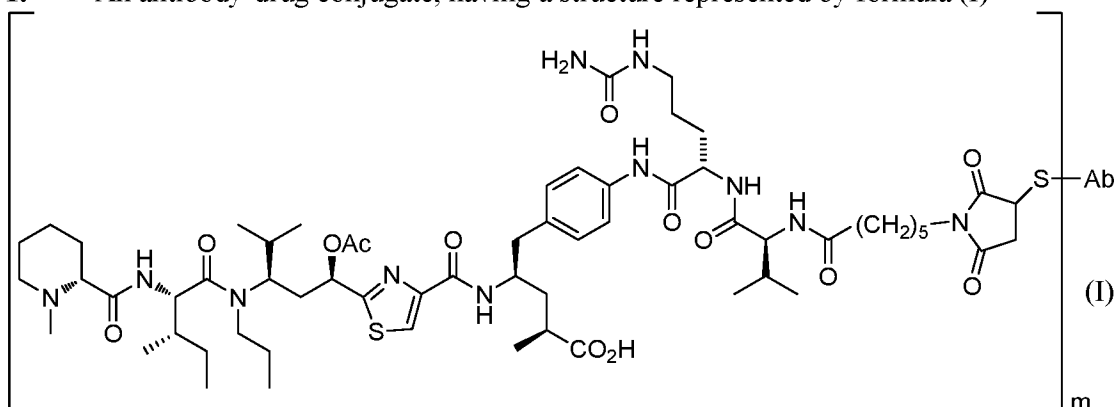
Table 4 – Sequence Listing Summary	
SEQ ID NO:	SEQUENCE DESCRIPTION
1	GPC3.1 heavy chain CDR1 a.a.
2	GPC3.1 heavy chain CDR2 a.a.
3	GPC3.1 heavy chain CDR3 a.a.
4	GPC3.1 kappa chain CDR1 a.a.
5	GPC3.1 kappa CDR2 a.a.
6	GPC3.1 kappa CDR3 a.a.
7	GPC3.1 heavy chain variable region a.a.
8	GPC3.1 kappa chain variable region a.a.
9	GPC3.1 heavy chain constant region a.a.
10	GPC3.1 kappa chain constant region a.a.
11	GPC3.1 heavy chain a.a.
12	GPC3.1 kappa chain a.a.
13	GPC3.1 heavy chain with signal peptide n.t.
14	GPC3.1 heavy chain with signal peptide a.a.
15	GPC3.1 kappa chain with signal peptide n.t.
16	GPC3.1 kappa chain with signal peptide a.a.
17	Antibodies A and C heavy chain CDR2 a.a.
18	Antibody A heavy chain CDR3 a.a.
19	Antibodies A and C kappa chain CDR3 a.a.
20	Antibodies B and C heavy chain CDR1 a.a.
21	Antibody B heavy chain CDR2 a.a.
22	Antibody B kappa chain CDR3 a.a.

Table 4 – Sequence Listing Summary	
SEQ ID NO:	SEQUENCE DESCRIPTION
23	Antibody A heavy chain variable region a.a.
24	Antibody A kappa chain variable region a.a.
25	Antibody B heavy chain variable region a.a.
26	Antibody B kappa chain variable region a.a.
27	Antibody C heavy chain variable region a.a.
28	Antibody C kappa chain variable region a.a.

CLAIMS

What is claimed is:

1. An antibody-drug conjugate, having a structure represented by formula (I)



5 wherein

m is 1, 2, 3, or 4 and

Ab is an anti-glypican-3 antibody having

- 10 (a) a heavy chain variable region CDR1 comprising SEQ ID NO:1;
 (b) a heavy chain variable region CDR2 comprising SEQ ID NO:2;
 (c) a heavy chain variable region CDR3 comprising SEQ ID NO:3;
 (d) a kappa light chain variable region CDR1 comprising SEQ ID NO:4;
 (e) a kappa light chain variable region CDR2 comprising SEQ ID NO:5; and
 (f) a kappa light chain variable region CDR3 comprising SEQ ID NO:6.

- 15 2. An antibody-drug conjugate according to claim 1, wherein the antibody Ab has a heavy chain variable region comprising SEQ ID NO:7 and a kappa light chain variable region comprising SEQ ID NO:8.

3. An antibody-drug conjugate according to claim 1, wherein the antibody Ab has a heavy chain constant region comprising SEQ ID NO:9 and a kappa light chain constant region comprising SEQ ID NO:10.

- 20 4. An antibody-drug conjugate according to claim 3, wherein the heavy chain constant region further comprises a C-terminal lysine.

5. An antibody-drug conjugate according to claim 1, wherein the antibody Ab has a heavy chain comprising SEQ ID NO:11 and a kappa light chain comprising SEQ ID NO:12.

6. An antibody-drug conjugate according to claim 5, wherein the heavy chain further comprises a C-terminal lysine.
7. A method of treating a cancer in a human subject suffering from such cancer, comprising administering to the human subject a therapeutically effective amount of an antibody-drug
5 conjugate according to claim 1, 2, 3, 4, 5, or 6, where the cancer is hepatocellular carcinoma, ovarian, or lung cancer.
8. A method according to claim 7, wherein the antibody-drug conjugate is administered intravenously, at a dose of between 0.1 and 20 mg/kg.
9. A pharmaceutical formulation comprising an antibody-drug conjugate according to claim
10 1, 2, 3, 4, 5, or 6, and a pharmaceutically acceptable excipient.

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FIG. 1A**Antibody GPC3.1 Heavy Chain with Signal Peptide**

Signal Peptide																	
atg	agg	gct	tgg	atc	ttc	ttt	ctg	ctc	tgc	ctg	gcc	ggg	aga	gcg	ctc		48
Met	Arg	Ala	Trp	Ile	Phe	Phe	Leu	Leu	Cys	Leu	Ala	Gly	Arg	Ala	Leu		
1				5					10					15			
~~~~~																	
gca	gag	gtg	cag	ctg	gtg	cag	tct	gga	gca	gag	gtg	aaa	aag	ccc	ggg		96
Ala	Glu	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly		
			20					25					30				
~~~~~																	
gag	tct	ctg	aag	atc	tcc	tgt	aag	ggg	tct	gga	tac	agc	ttt	acc	agc		144
Glu	Ser	Leu	Lys	Ile	Ser	Cys	Lys	Gly	Ser	Gly	Tyr	Ser	Phe	Thr	Ser		
		35					40					45					
~~~~~																	
CDR1																	
tac	tgg	atc	gcc	tgg	gtg	cgc	cag	atg	ccc	ggg	aaa	ggc	ctg	gag	tgg		192
Tyr	Trp	Ile	Ala	Trp	Val	Arg	Gln	Met	Pro	Gly	Lys	Gly	Leu	Glu	Trp		
	50					55					60						
~~~~~																	
CDR2																	
atg	ggg	atc	atc	ttt	cct	ggg	gac	tct	gat	acc	aga	tac	agc	ccg	tcc		240
Met	Gly	Ile	Ile	Phe	Pro	Gly	Asp	Ser	Asp	Thr	Arg	Tyr	Ser	Pro	Ser		
65					70					75					80		
~~~~~																	
ttc	caa	ggc	cag	gtc	acc	atc	tca	gcc	gac	agg	tcc	atc	aga	acc	gcc		288
Phe	Gln	Gly	Gln	Val	Thr	Ile	Ser	Ala	Asp	Arg	Ser	Ile	Arg	Thr	Ala		
				85					90					95			
~~~~~																	
tac	ctg	cag	tgg	agc	agc	ctg	aag	gcc	tgc	gac	acc	gcc	ttg	tat	tac		336
Tyr	Leu	Gln	Trp	Ser	Ser	Leu	Lys	Ala	Ser	Asp	Thr	Ala	Leu	Tyr	Tyr		
			100					105					110				
~~~~~																	
CDR3																	
tgt	gcg	aga	acc	cgg	gag	ggg	tac	ttt	gac	tac	tgg	ggc	cag	gga	acc		384
Cys	Ala	Arg	Thr	Arg	Glu	Gly	Tyr	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr		
		115				120						125					
~~~~~																	
ctg	gtc	acc	gtc	tcc	tca	gct	agc	acc	aag	ggc	cca	tcg	gtc	ttc	ccc		432
Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro		
	130					135					140						
~~~~~																	
ctg	gca	ccc	tcc	tcc	aag	agc	acc	tct	ggg	ggc	aca	gcg	gcc	ctg	ggc		480
Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly		
145					150					155					160		
~~~~~																	
tgc	ctg	gtc	aag	gac	tac	ttc	ccc	gaa	ccg	gtg	acg	gtg	tcg	tgg	aac		528
Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn		
				165					170					175			
~~~~~																	
tca	ggc	gcc	ctg	acc	agc	ggc	gtg	cac	acc	ttc	ccg	gct	gtc	cta	cag		576
Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln		
			180					185					190				

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**FIG. 1B****Antibody GPC3.1 Heavy Chain with Signal Peptide (continued)**

tcc tca gga ctc tac tcc ctc agc agc gtg gtg acc gtg ccc tcc agc Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser	624
195 200 205	
agc ttg ggc acc cag acc tac atc tgc aac gtg aat cac aag ccc agc Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser	672
210 215 220	
aac acc aag gtg gac aag aga gtt gag ccc aaa tct tgt gac aaa act Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr	720
225 230 235 240	
cac aca tgc cca ccg tgc cca gca cct gaa ctc ctg ggg gga ccg tca His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser	768
245 250 255	
gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cg Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg	816
260 265 270	
acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro	864
275 280 285	
gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala	912
290 295 300	
aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgt gtg gtc Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val	960
305 310 315 320	
agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr	1008
325 330 335	
aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr	1056
340 345 350	
atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu	1104
355 360 365	
ccc cca tcc ccg gag gag atg acc aag aac cag gtc agc ctg acc tgc Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys	1152
370 375 380	
ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser	1200
385 390 395 400	
aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp	1248
405 410 415	

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**FIG. 1C****Antibody GPC3.1 Heavy Chain with Signal Peptide (continued)**

tcc gac ggc tcc ttc ttc ctc tat agc aag ctc acc gtg gac aag agc	1296
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser	
420 425 430	
agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct	1344
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala	
435 440 445	
ctg cac aac cac tac acg cag aag agc ctc tcc ctg tcc ccg ggt tga	1392
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly	
450 455 460	

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**FIG. 2A****GPC3.1 Kappa Chain with Signal Peptide**

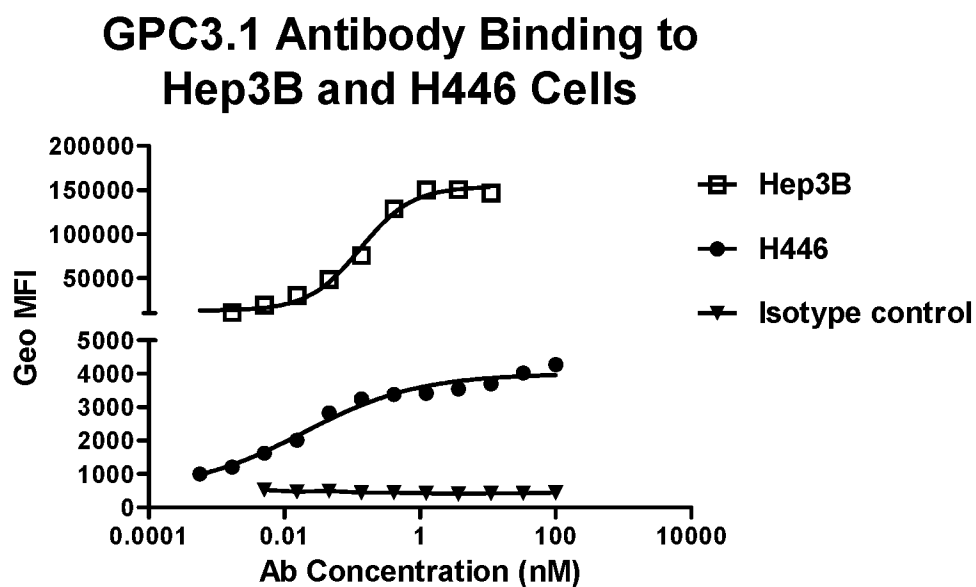
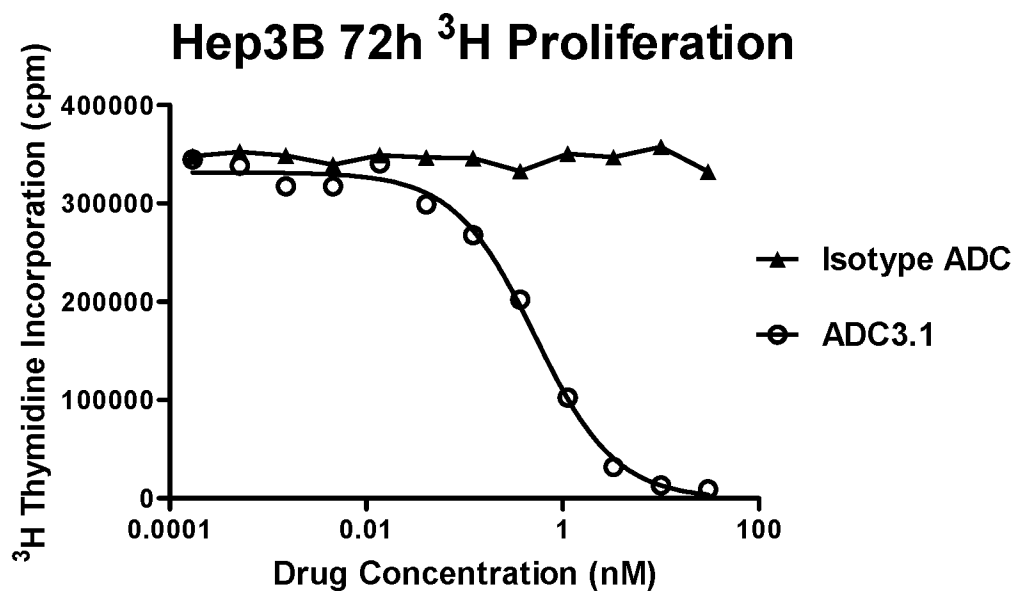
Signal Peptide																
~~~~~																
atg	agg	gct	tgg	atc	ttc	ttt	ctg	ctc	tgc	ctg	gcc	ggg	cgc	gcc	ttg	48
Met	Arg	Ala	Trp	Ile	Phe	Phe	Leu	Leu	Cys	Leu	Ala	Gly	Arg	Ala	Leu	
1				5					10					15		
~~~~~																
gcc	gaa	att	gtg	ttg	acg	cag	tct	cca	ggc	acc	ctg	tct	ttg	tct	cca	96
Ala	Glu	Ile	Val	Leu	Thr	Gln	Ser	Pro	Gly	Thr	Leu	Ser	Leu	Ser	Pro	
			20					25					30			
~~~~~																
CDR1																
~~~~~																
ggg	gaa	aga	gcc	acc	ctc	tcc	tgc	agg	gcc	gtt	cag	agt	gtt	agc	agc	144
Gly	Glu	Arg	Ala	Thr	Leu	Ser	Cys	Arg	Ala	Val	Gln	Ser	Val	Ser	Ser	
		35					40					45				
~~~~~																
agc	tat	tta	gcc	tgg	tac	cag	cag	aaa	cct	ggc	cag	gct	ccc	agg	ctc	192
Ser	Tyr	Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Ala	Pro	Arg	Leu	
	50					55					60					
~~~~~																
CDR2																
~~~~~																
ctc	atc	tat	ggt	gca	tcc	agc	agg	gcc	act	ggc	atc	cca	gac	agg	ttc	240
Leu	Ile	Tyr	Gly	Ala	Ser	Ser	Arg	Ala	Thr	Gly	Ile	Pro	Asp	Arg	Phe	
65				70						75					80	
~~~~~																
agt	ggc	agt	ggg	tct	ggg	aca	gac	ttc	act	ctc	acc	atc	agc	aga	ctg	288
Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Arg	Leu	
				85					90					95		
~~~~~																
CDR3																
~~~~~																
gag	cct	gaa	gat	ttt	gca	gtg	tat	tac	tgt	cag	cag	tat	ggt	agc	tca	336
Glu	Pro	Glu	Asp	Phe	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	Tyr	Gly	Ser	Ser	
			100					105					110			
~~~~~																
ccc	act	ttc	ggc	gga	ggg	acc	aag	gtg	gag	atc	aaa	cgt	acg	gtg	gct	384
Pro	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Val	Glu	Ile	Lys	Arg	Thr	Val	Ala	
		115					120					125				
~~~~~																
gca	cca	tct	gtc	ttc	atc	ttc	ccg	cca	tct	gat	gag	cag	ttg	aaa	tct	432
Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	
	130					135					140					
~~~~~																
gga	act	gcc	tct	gtt	gtg	tgc	ctg	ctg	aat	aac	ttc	tat	ccc	aga	gag	480
Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	
145					150					155					160	
~~~~~																
gcc	aaa	gta	cag	tgg	aag	gtg	gat	aac	gcc	ctc	caa	tcg	ggt	aac	tcc	528
Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	
				165					170					175		

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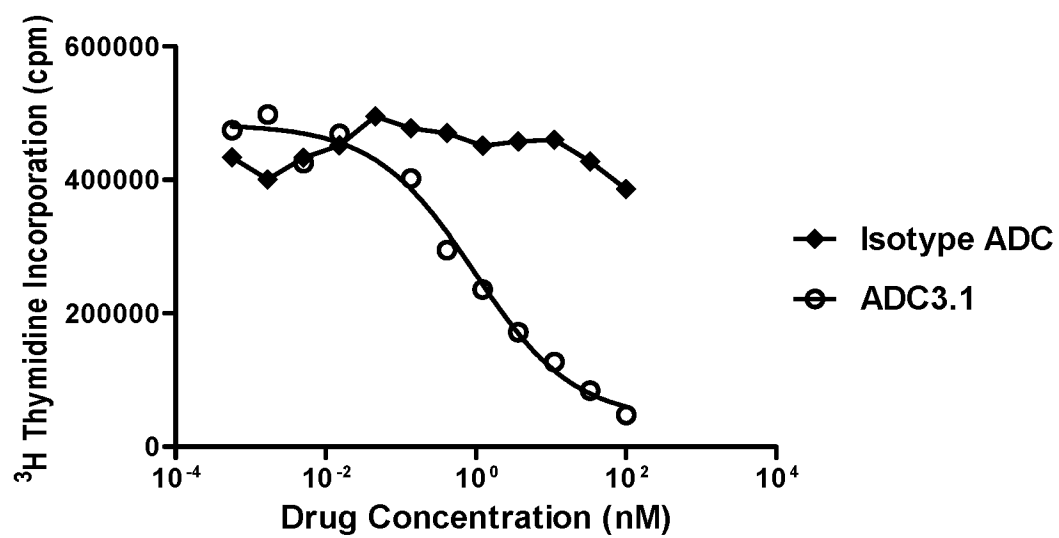
**FIG. 2B****GPC3.1 Kappa Chain with Signal Peptide (continued)**

cag gag agt gtc aca gag cag gac agc aag gac agc acc tac agc ctc	576
Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu	
180 185 190	
agc agc acc ctg acg ctg agc aaa gca gac tac gag aaa cac aaa gtc	624
Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val	
195 200 205	
tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg ccc gtc aca aag	672
Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys	
210 215 220	
agc ttc aac agg gga gag tgt tag	696
Ser Phe Asn Arg Gly Glu Cys	
225 230	

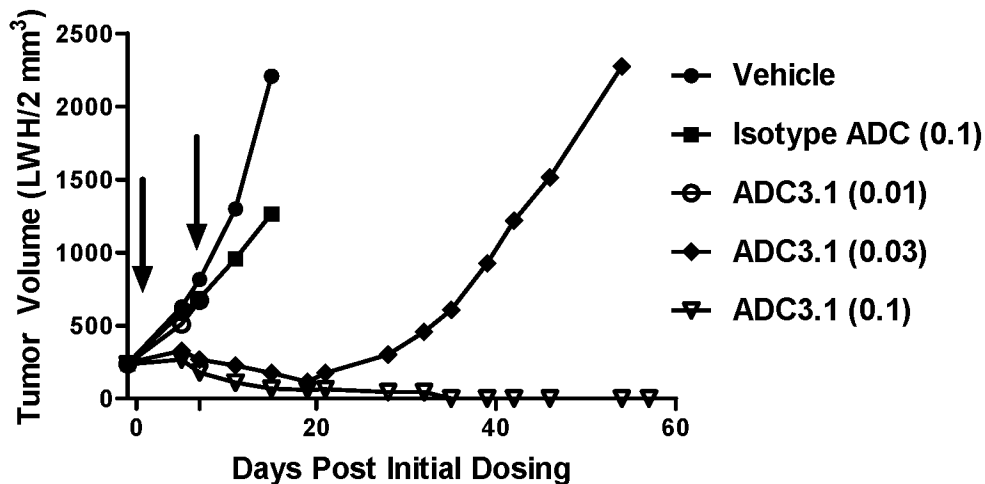
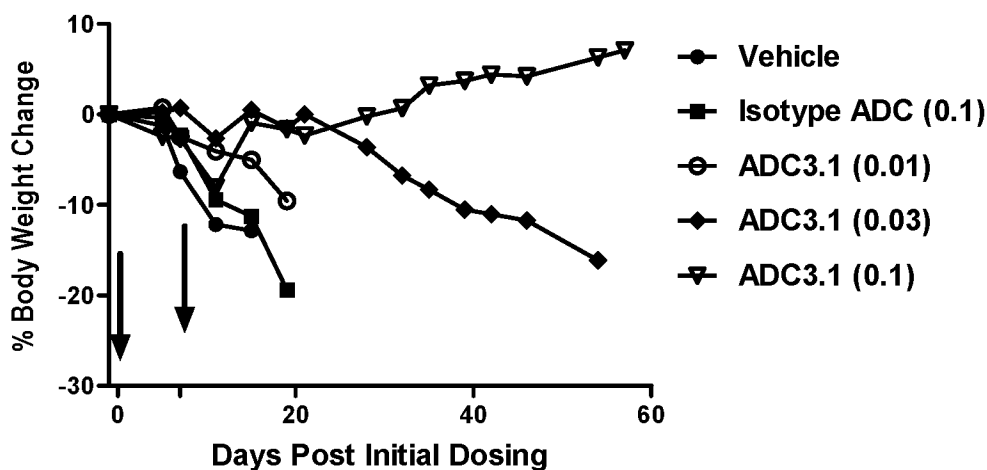
6/18

**FIG. 3A****FIG. 3B**

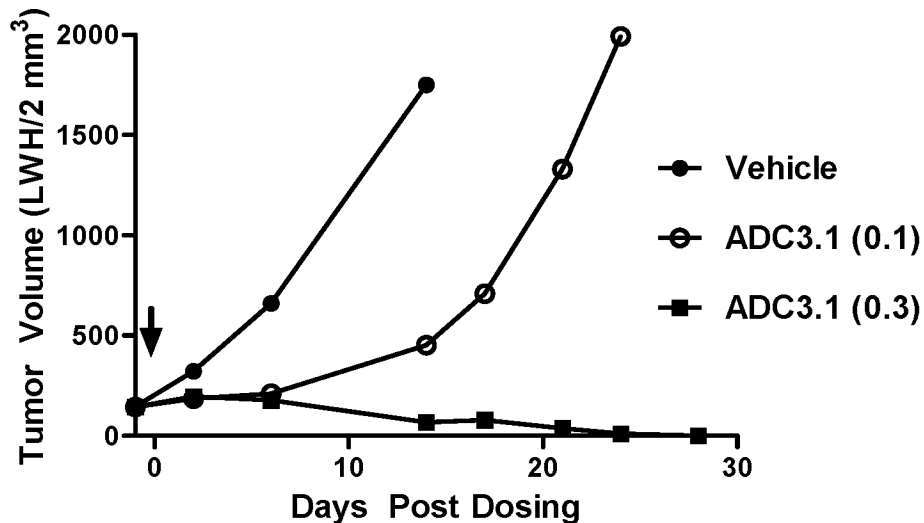
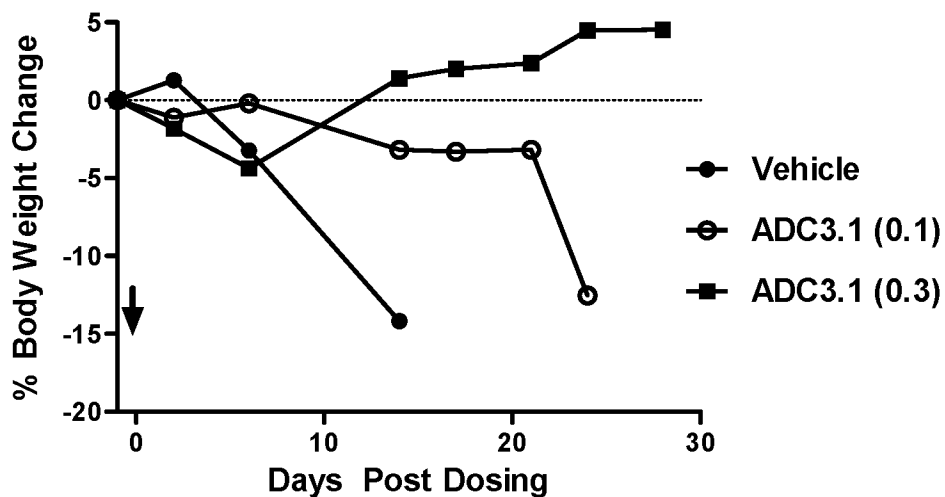
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**FIG. 3C****H446 72h  $^3\text{H}$  Proliferation**

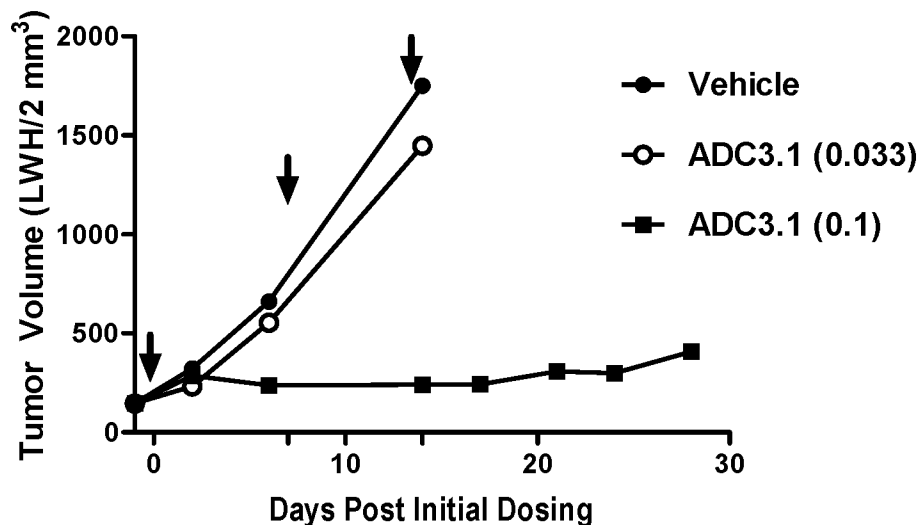
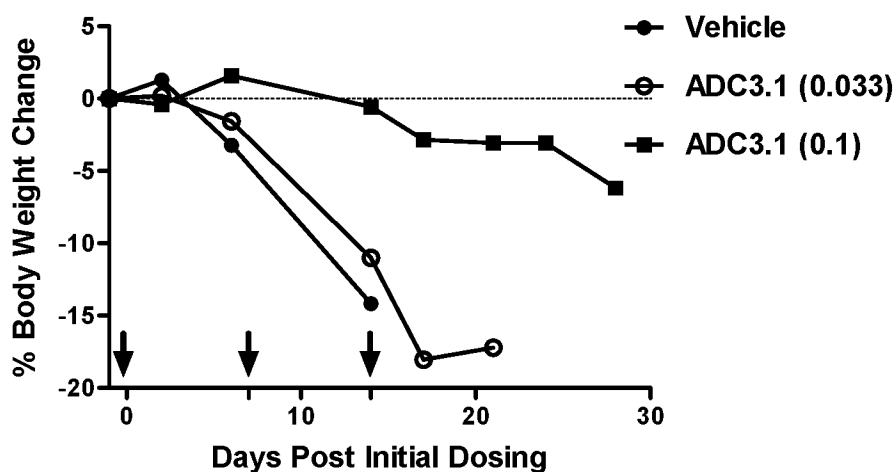
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**FIG. 4A****Dose Dependent Efficacy of ADC in Hep3B Xenograft Model (Tumor volume)****FIG. 4B****Dose Dependent Efficacy of ADC in Hep3B Xenograft Model (Body Weight)**

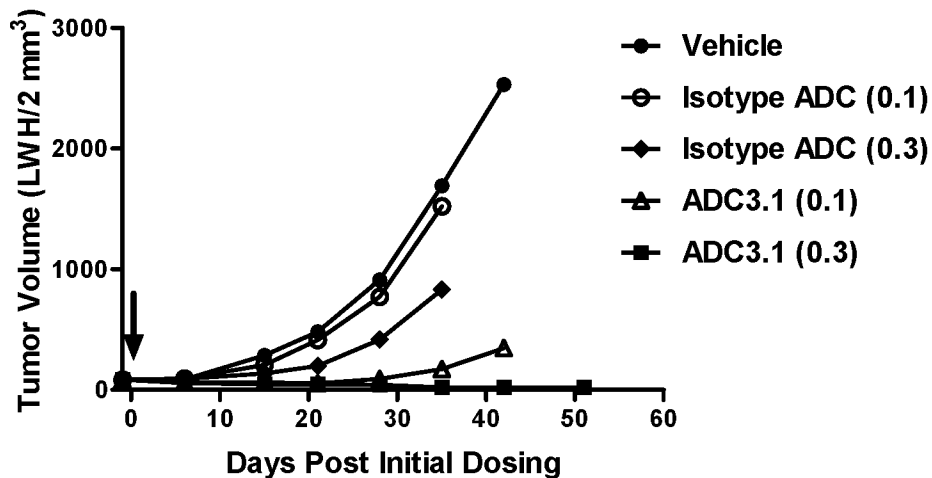
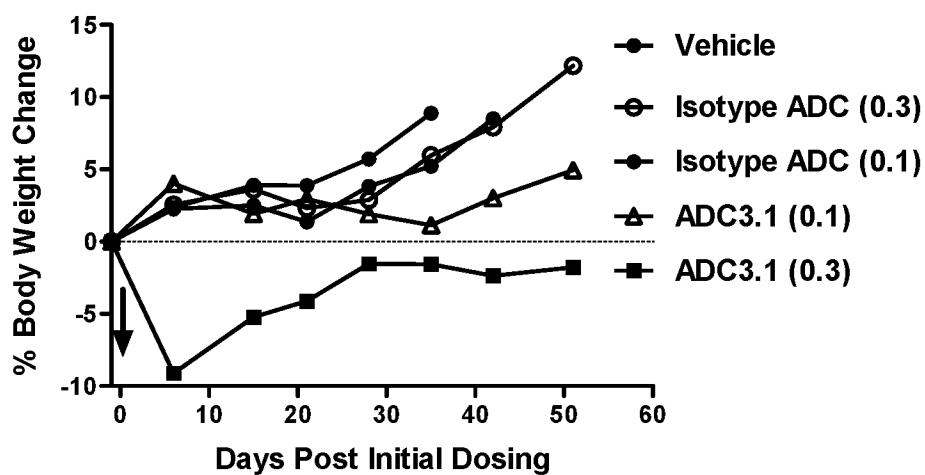
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**FIG. 5A****Single Dose Efficacy of ADC in HuH7D12 Xenograft Model (Tumor Volume)****FIG. 5B****Single Dose Efficacy of ADC in HuH7D12 Xenograft Model (Body weight)**

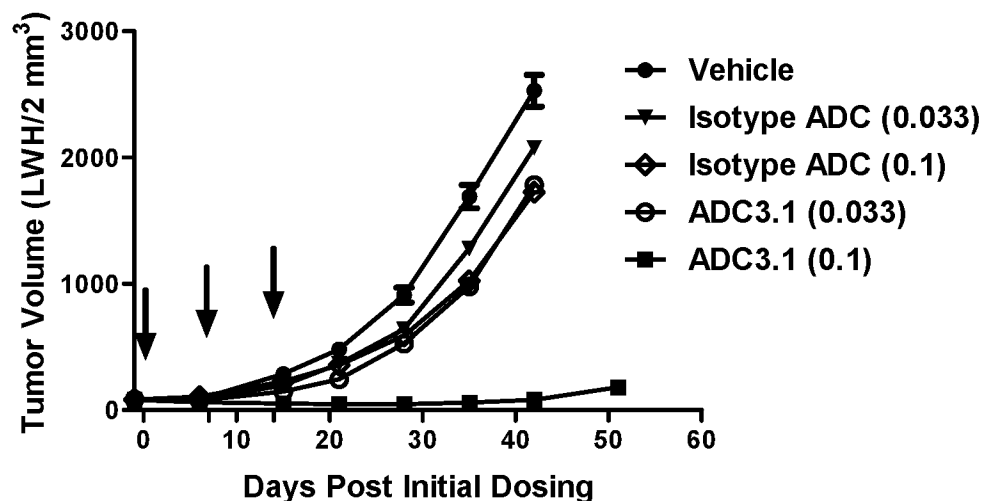
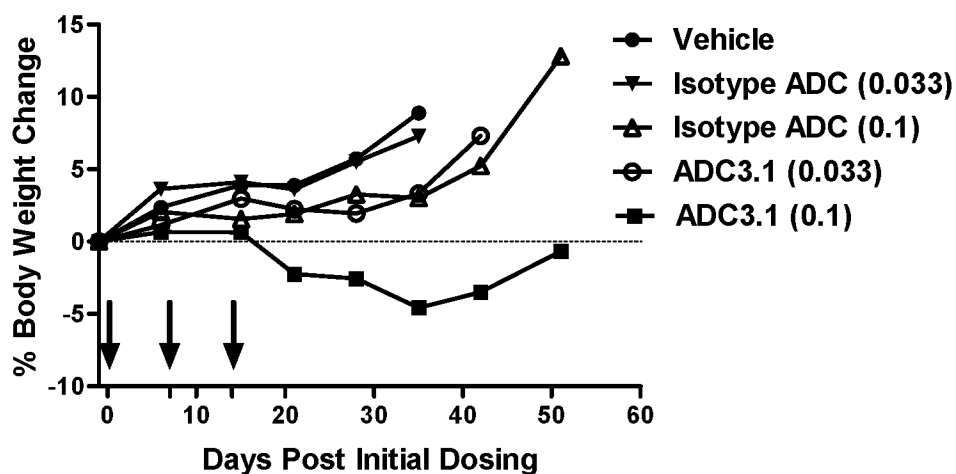
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**FIG. 6A****Split Dosing Efficacy of ADC in HuH7D12  
Xenograft Model (Tumor Volume)****FIG. 6B****Split Dosing Efficacy of ADC in HuH7D12  
Xenograft Model (Body Weight)**

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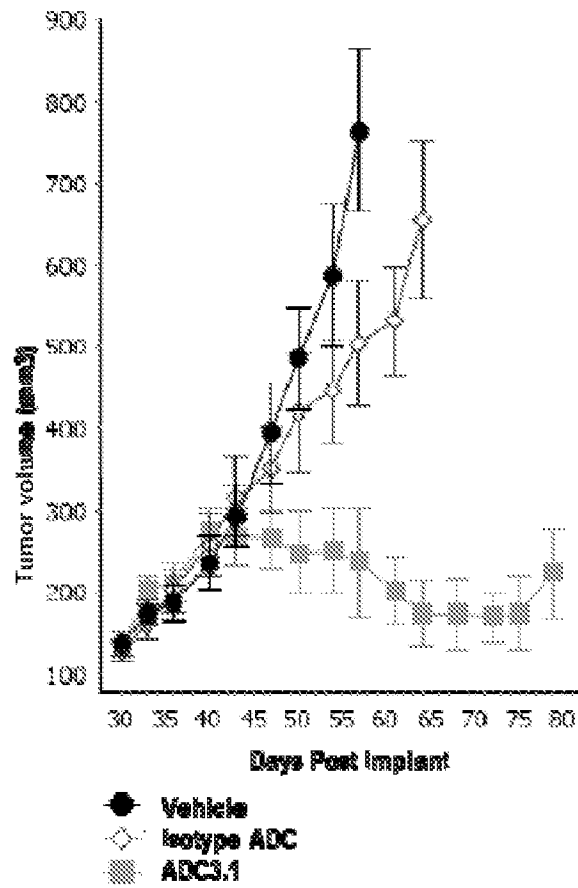
**FIG. 7A****Single Dose Efficacy of ADC in H446  
Xenograft Model (Tumor Volume)****FIG. 7B****Single Dose Efficacy of ADC in H446  
Xenograft Model (Body Weight)**

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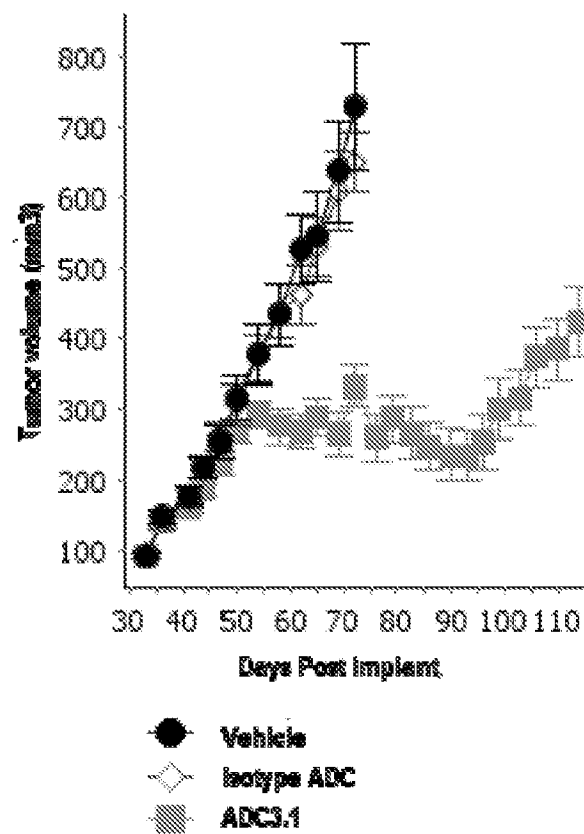
**FIG. 8A****Split Dosing Efficacy of ADC in H446  
Xenograft Model (Tumor Volume)****FIG. 8B****Split Dosing Efficacy of ADC in H446  
Xenograft Model (Body Weight)**

**FIG. 9**

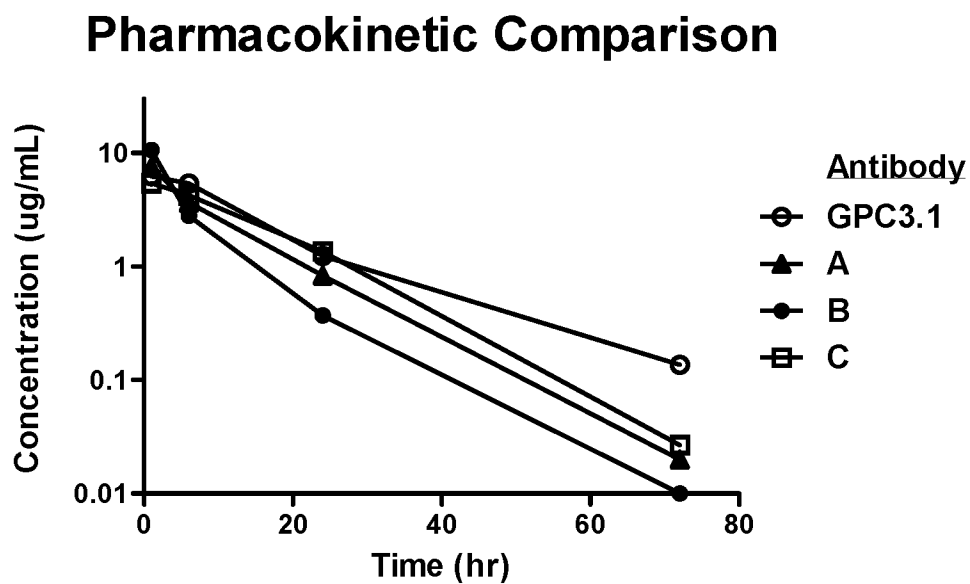
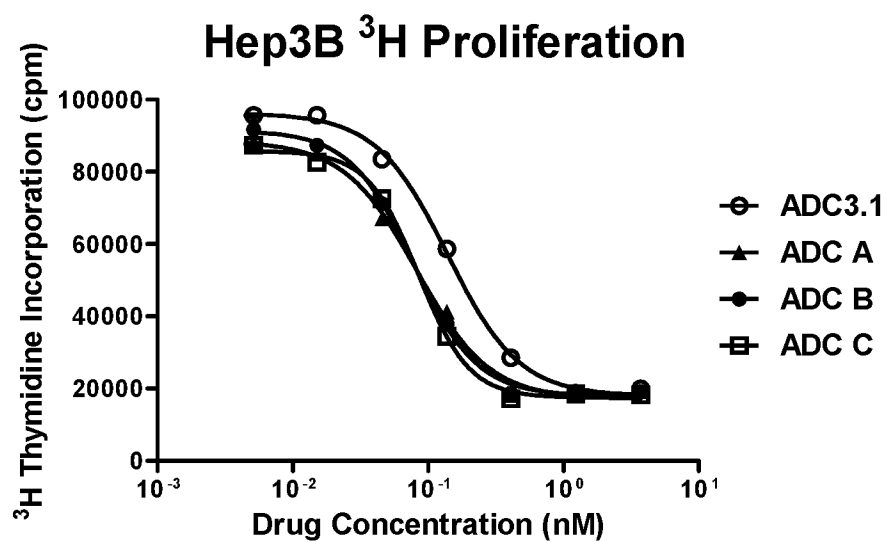
**Ovarian Cancer PDX Study**



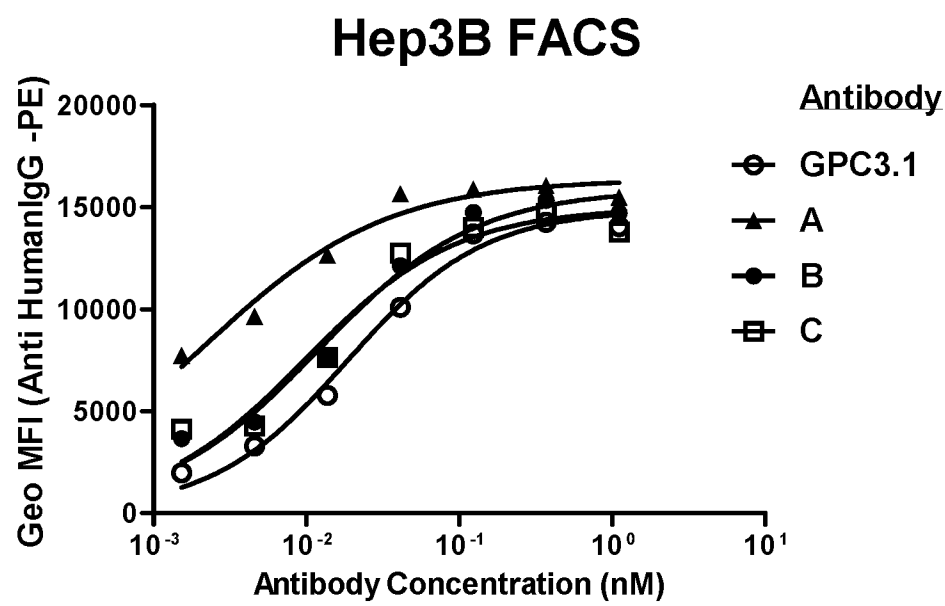
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**FIG. 10****Squamous Lung Cancer PDX Study**

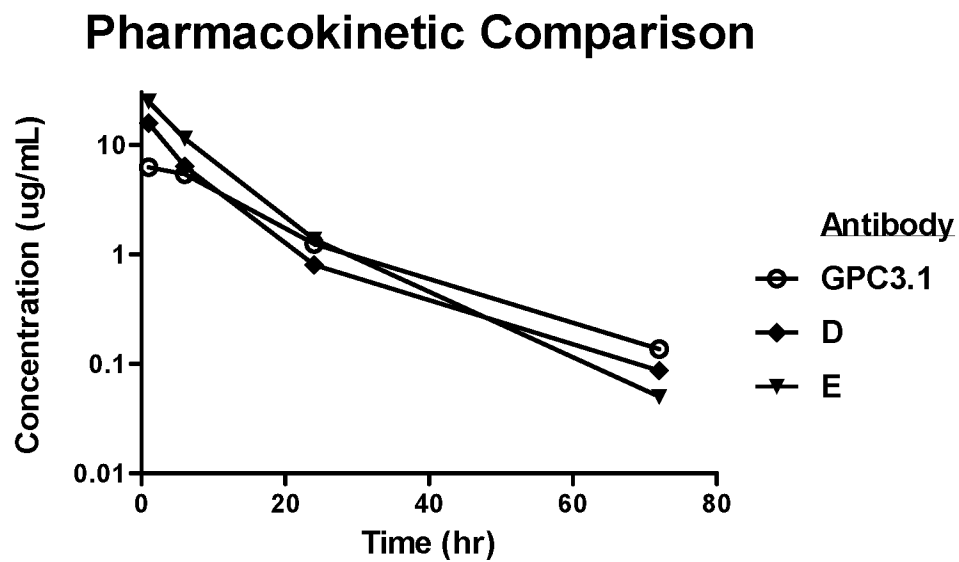
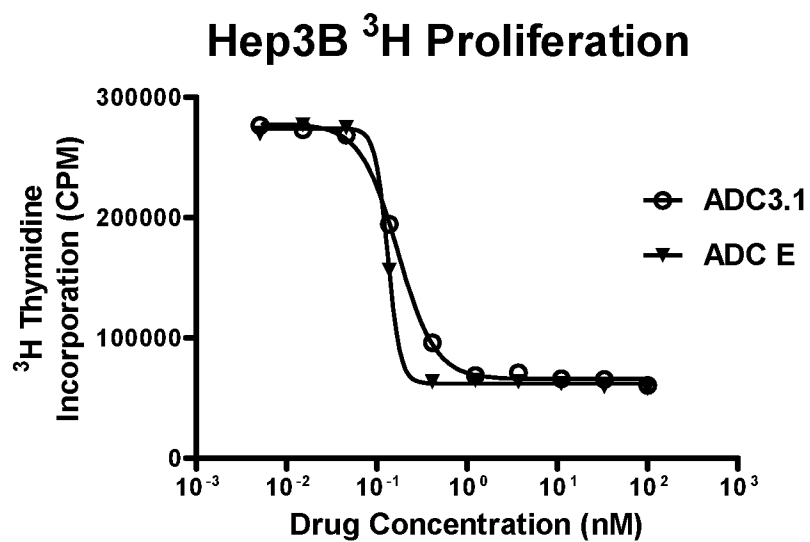
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**FIG. 11A****FIG. 11B**

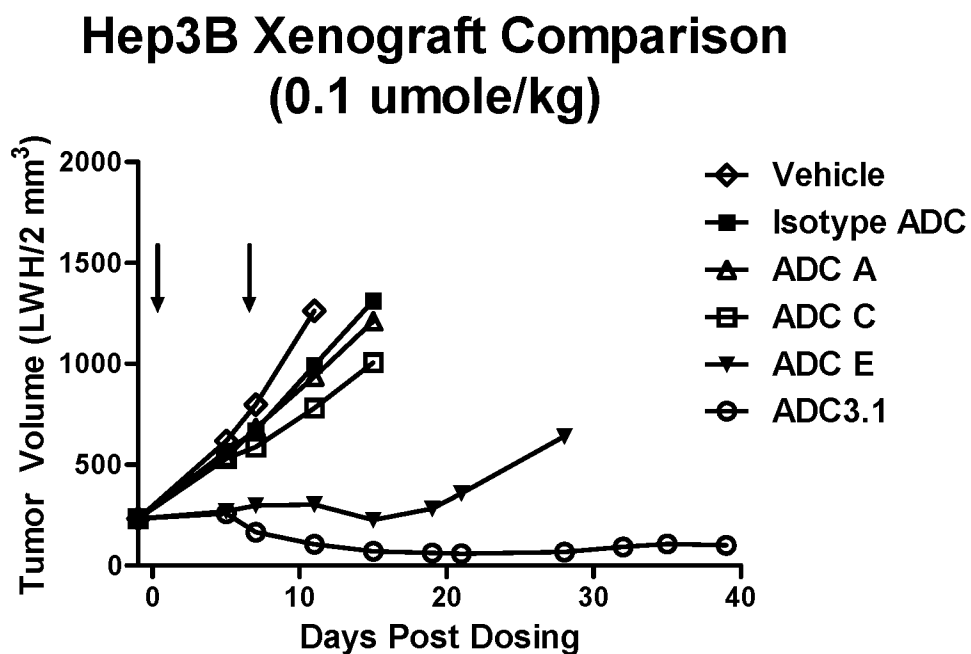
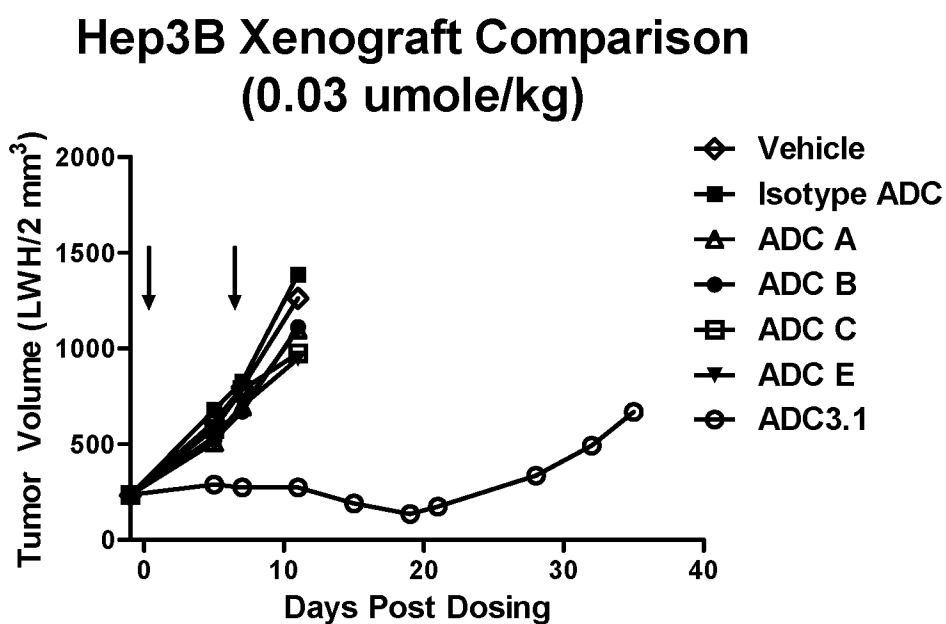
**FIG. 11C**



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**FIG. 12A****FIG. 12B**

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**FIG. 13A****FIG. 13B**

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2017/031634

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> INV. A61K47/68 A61P35/00 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
Minimum documentation searched (classification system followed by classification symbols) A61K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, WPI Data		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2014/227295 A1 (CONG QIANG [US] ET AL) 14 August 2014 (2014-08-14) claims 7, 8 figure 2; example 19 paragraph [0170]	1,2,4, 6-9
Y	US 2011/027274 A1 (CHENG HENG [US] ET AL) 3 February 2011 (2011-02-03) page 36; example 5; compound 39 ----- -/--	1,2,4, 6-9
<span><input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C.</span> <span><input checked="" type="checkbox"/> See patent family annex.</span>		
* Special categories of cited documents :   "A" document defining the general state of the art which is not considered to be of particular relevance   "E" earlier application or patent but published on or after the international filing date   "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)   "O" document referring to an oral disclosure, use, exhibition or other means   "P" document published prior to the international filing date but later than the priority date claimed       "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention   "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone   "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art   "&amp;" document member of the same patent family		
Date of the actual completion of the international search   13 July 2017		Date of mailing of the international search report   28/07/2017
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer   Langer, Miren

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International application No  
PCT/US2017/031634

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JAKUBOVIC ET AL: "Glypican-3: From the mutations of Simpson-Golabi-Behmel genetic syndrome to a tumor marker for hepatocellular carcinoma", EXPERIMENTAL AND MOLECULAR PATHOLOGY, ACADEMIC PRESS, US, vol. 82, no. 2, 30 March 2007 (2007-03-30), pages 184-189, XP022005680, ISSN: 0014-4800, DOI: 10.1016/J.YEXMP.2006.10.010 -----	1-9
A	US 2015/132324 A1 (CONG QIANG [US] ET AL) 14 May 2015 (2015-05-14) -----	1-9
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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/031634

### Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
  - a. ☒ forming part of the international application as filed:
    - ☒ in the form of an Annex C/ST.25 text file.
    - ☐ on paper or in the form of an image file.
  - b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
  - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
    - ☐ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
    - ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2017/031634

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		EA 201591317 A1 30-12-2015	
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Information on patent family members

International application No

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