WEE1 INHIBITOR COMBINATION THERAPY

INCORPORATION BY REFERENCE TO ANY PRIORITY APPLICATIONS

[0001] Any and all applications for which a foreign or domestic priority claim is identified, for example, in the Application Data Sheet or Request as filed with the present application, are hereby incorporated by reference under 37 CFR 1.57, and Rules 4.18 and 20.6, including U.S. Provisional Application Nos. 63/565,372, filed March 14, 2024 and 63/641,592, filed May 2, 2024, each of which is incorporated by reference in their entireties.

SEQUENCE STATEMENT

[0002] This application contains a Sequence Listing, which has been submitted electronically and is hereby incorporated by reference in its entirety. The sequence listing, was created on March 6, 2025, is named ZENO177WO.xml and is 13 kb in size.

Field

[0003] The present application relates to the fields of chemistry, biochemistry and medicine. More particularly, disclosed herein are combination therapies, and methods of treating diseases and/or conditions with a combination therapy described herein.

Description

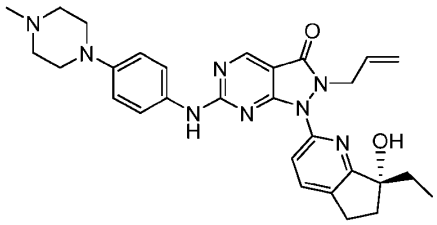
[0004] Maytansine and its derivatives maytansinoids are highly cytotoxic drugs. Maytansine was first isolated from east African shrub *Maytenus serrata* and shown to be 100- to 1000-fold more cytotoxic than conventional cancer chemotherapeutic agents like methotrexate, daunorubicin, doxorubicin, and vincristine. However, due to the narrow therapeutic window and high systemic toxicity of maytansine, it is not an oncological chemotherapeutic agent when used alone and has been clinically banned from direct use in human therapy.

[0005] Antibody-drug conjugates (ADCs) as targeted therapy has emerged as an approach to address the limitations of single-agent therapy. In November 2022, the U.S. Food and Drug Administration (FDA) granted approval to mirvetuximab soravtansine-gynx (brand name Elahere) for adult patients selected with folate receptor alpha-positive, platinum-resistant epithelial ovarian, fallopian tube or primary peritoneal cancer, who have

received one to three prior systemic treatment regimens. Mirvetuximab soravtansine-gynx is a folate receptor alpha-directed antibody and DM4 maytansinoid conjugate. While the FDA approval represents a milestone in the ongoing development of therapeutic ADCs, there remains a need for improved ADCs to help address the long-felt need for additional options to treat different types of cancer and to deliver therapeutic payloads to selected cells and tissues.

SUMMARY

[0006] Some embodiments described herein relate to the use of a combination of Compound (A), or a pharmaceutically acceptable salt thereof, and (i) a maytansinoid, or a pharmaceutically acceptable salt thereof; or (ii) an antibody-drug conjugate comprising a maytansinoid, or a pharmaceutically acceptable salt thereof, for treating a cancer in a subject. Compound (A) is



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BRIEF DESCRIPTION OF THE DRAWINGS

[0007] **FIG. 1A** shows 2D heatmaps of *in vitro* synergy between Compound (A) and DM4 in OVCAR3 and OV-90 cancer cells. **FIG. 1B** illustrates protein biomarker changes in response to Compound (A) monotherapy, DM4 monotherapy and a combination of Compound (A) and DM4 in OVCAR3 cells in cultures *in vitro*. **FIG. 1C** shows 2D heatmaps of *in vitro* synergy between Compound (A) and mirvetuximab soravtansine in OVCAR3 and OV-90 cancer cells. **FIG. 1D** illustrates protein biomarker changes in response to Compound (A) monotherapy, mirvetuximab soravtansine monotherapy and a combination of Compound (A) and mirvetuximab soravtansine in OVCAR3 cells in cultures *in vitro*.

[0008] **FIG. 2A** is a panel of graphs showing the percentage of cells with pHH3-positive staining in cell cycle, which are indicative of mitotic arrest, when OVCAR3 cancer cells were treated with DMSO (vehicle), Compound (A) monotherapy, DM4 or mirvetuximab soravtansine monotherapy, or the combination of Compound (A) + DM4 or Compound (A) + mirvetuximab soravtansine. **FIGS. 2B** and **2C** are bar graphs showing

cell cycle analyses by flow cytometry with indicated markers as shown in **FIG. 2A**, when OVCAR3 cancer cells were treated with Compound (A) monotherapy, DM4 (**FIG. 2B**) or mirvetuximab soravtansine (**FIG. 2C**) monotherapy, or the combination of Compound (A) + DM4 (**FIG. 2B**) or Compound (A) + mirvetuximab soravtansine (**FIG. 2C**).

[0009] **FIG. 3A** is a panel of images showing OVCAR3 cells with pHH3 (white), α-tubulin (light grey), and DAPI (dark grey) immunostaining, when OVCAR3 cancer cells were treated with DMSO (vehicle), Compound (A) monotherapy, mirvetuximab soravtansine monotherapy, or the combination of Compound (A) + mirvetuximab soravtansine. **FIG. 3B** is a corresponding bar graph to **FIG. 3A**, depicting the percentage of cells with pHH3-positive staining. **FIG. 3C** is a panel of representative images showing mitotic phenotypes of OVCAR3 cells. **FIG. 3D** is a corresponding bar graph to **FIG. 3C** showing the percentage of cells with mitotic defects vs. the percentage of cells with normal mitosis, when OVCAR3 cancer cells were treated as described above for **FIG. 3A**.

[0010] **FIG. 4A** shows tumor volume measurements over a course of 25 days when mice inoculated with OV-90 high-grade serous ovarian cancer/carcinoma (HGSOC) tumor cells were treated with vehicle (negative control), Compound (A) monotherapy, mirvetuximab soravtansine monotherapy, and the combination of Compound (A) + mirvetuximab soravtansine. **FIG. 4B** shows individual tumor volume changes on day 25 when mice inoculated with OV-90 cells were treated as described above for **FIG. 4A**. **FIG. 4C** shows the body weight changes of the mice subject to the treatment regimens of **FIG. 4A**.

DETAILED DESCRIPTION

Definitions

[0011] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art. All patents, applications, published applications and other publications referenced herein are incorporated by reference in their entirety unless stated otherwise. In the event that there is a plurality of definitions for a term herein, those in this section prevail unless stated otherwise.

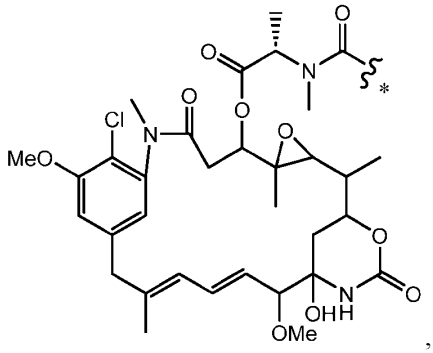
[0012] As used herein, an "immunoconjugate" is a conjugate comprising an immunological substance and a drug moiety that are joined together by a linker. Typically,

an immunoconjugate is prepared by reacting the immunological substance with a conjugate as defined herein. An "antibody-drug conjugate" (ADC) is one class of immunoconjugate whereby the immunological substance is an antibody or an antigen-binding fragment thereof.

[0013] As used herein, an "antibody" (Ab) is a protein made by the immune system, or a synthetic or engineered variant thereof, that binds to specific sites on cells or tissues. The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multi-specific antibodies (*e.g.*, bispecific antibodies), chimeric antibodies, nanobodies, and antigen-binding fragments so long as they exhibit the desired antigen-binding activity. Monoclonal antibodies are a type of synthetic antibody. In cancer treatment, monoclonal antibodies may kill cancer cells directly, they may block development of tumor blood vessels, and/or they may help the immune system kill cancer cells. Humanized antibodies are antibodies from non-human species whose protein sequences have been modified to increase their similarity to antibody variants produced naturally in humans. For example, a murine monoclonal antibody, 4D5, directed against the extracellular domain of HER2 and a potent inhibitor of growth of human breast cancer cells overexpressing HER2 *in vitro* and in xenograft models, was humanized by inserting the complementary determining regions of 4D5 into the framework of a consensus human IgG1. The resulting recombinant humanized anti-HER2 mAb was trastuzumab. An antibody typically is composed of two heavy chains and two light chains; and the heavy chain and the light chain typically each contain three complementarity-determining regions (CDRs) immunoglobulin (Ig) hypervariable domains that determine specific antibody binding.

[0014] An "antigen-binding fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')2, diabodies, linear antibodies, single-chain antibody molecules (*e.g.*, scFv), and single-domain antibodies.

[0015] "Maytansinoid" refers to an analog or a derivative (*e.g.*, synthetic analog or derivative) of maytansine having a general structure of:



, which is the structure of maytansine having modifications, such as adding substituted or unsubstituted alkyls or alkenyls, alcohols (R-OH), thiol (R-SH), ether (R-O-R'), thioether (R-S-R'), and/or disulfide groups at * in the foregoing structural formula. In some embodiments, these functional groups are introduced to facilitate covalent attachment to an antibody or an antigen-fragment thereof. Non-limiting examples of maytansinoids include DM1, S-methyl DM1 or DM1-SMe, DM4, S-methyl DM4 or DM4-SMe, DM3, DM3-SMe (*see, e.g.*, U.S. Patent No. 7,276,497).

[0016] The term "pharmaceutically acceptable salt" refers to a salt of a compound that does not cause significant irritation to an organism to which it is administered and does not abrogate the biological activity and properties of the compound. In some embodiments, the salt is an acid addition salt of the compound. Pharmaceutical salts can be obtained by reacting a compound with inorganic acids such as hydrohalic acid (e.g., hydrochloric acid or hydrobromic acid), a sulfuric acid, a nitric acid and a phosphoric acid (such as 2,3-dihydroxypropyl dihydrogen phosphate). Pharmaceutical salts can also be obtained by reacting a compound with an organic acid such as aliphatic or aromatic carboxylic or sulfonic acids, for example formic, acetic, succinic, lactic, malic, tartaric, citric, ascorbic, nicotinic, methanesulfonic, ethanesulfonic, p-toluensulfonic, trifluoroacetic, benzoic, salicylic, 2-oxopentanedioic or naphthalenesulfonic acid. Pharmaceutical salts can also be obtained by reacting a compound with a base to form a salt such as an ammonium salt, an alkali metal salt, such as a sodium, a potassium or a lithium salt, an alkaline earth metal salt, such as a calcium or a magnesium salt, a salt of a carbonate, a salt of a bicarbonate, a salt of organic bases such as dicyclohexylamine, N-methyl-D-glucamine, tris(hydroxymethyl)methylamine, C_1-C_7 alkylamine, cyclohexylamine, triethanolamine, ethylenediamine and salts with amino acids such as arginine and lysine. Those skilled in the art understand that when a salt is formed by protonation of a nitrogen-based group (for example, NH_2), the nitrogen-based group can be

associated with a positive charge (for example, NH_2 can become NH_3^+) and the positive charge can be balanced by a negatively charged counterion (such as Cl^-).

[0017] It is understood that, in any compound described herein having one or more chiral centers, if an absolute stereochemistry is not expressly indicated, then each center may independently be of *R*-configuration or *S*-configuration or a mixture thereof. Thus, the compounds provided herein may be enantiomerically pure, enantiomerically enriched, racemic mixture, diastereomerically pure, diastereomerically enriched or a stereoisomeric mixture. In addition, it is understood that, in any compound described herein having one or more double bond(s) generating geometrical isomers that can be defined as E or Z, each double bond may independently be E or Z a mixture thereof. Likewise, it is understood that, in any compound described, all tautomeric forms are also intended to be included.

[0018] It is to be understood that where compounds disclosed herein have unfilled valencies, then the valencies are to be filled with hydrogens or isotopes thereof, e.g., hydrogen-1 (protium) and hydrogen-2 (deuterium).

[0019] It is understood that the compounds described herein can be labeled isotopically. Substitution with isotopes such as deuterium may afford certain therapeutic advantages resulting from greater metabolic stability, such as, for example, increased *in vivo* half-life or reduced dosage requirements. Each chemical element as represented in a compound structure may include any isotope of said element. For example, in a compound structure a hydrogen atom may be explicitly disclosed or understood to be present in the compound. At any position of the compound that a hydrogen atom may be present, the hydrogen atom can be any isotope of hydrogen, including but not limited to hydrogen-1 (protium) and hydrogen-2 (deuterium). Thus, reference herein to a compound encompasses all potential isotopic forms unless the context clearly dictates otherwise.

[0020] It is understood that the methods and combinations described herein include crystalline forms (also known as polymorphs, which include the different crystal packing arrangements of the same elemental composition of a compound), amorphous phases, salts, solvates and hydrates. In some embodiments, the compounds described herein exist in solvated forms with pharmaceutically acceptable solvents such as water, ethanol or the like. In other embodiments, the compounds described herein exist in unsolvated form. Solvates contain either stoichiometric or non-stoichiometric amounts of a solvent and may be formed during the process of crystallization with pharmaceutically acceptable solvents such as water, ethanol or the like. Hydrates are formed when the solvent is water or

alcoholates are formed when the solvent is alcohol. In addition, the compounds provided herein can exist in unsolvated as well as solvated forms. In general, the solvated forms are considered equivalent to the unsolvated forms for the purposes of the compounds and methods provided herein.

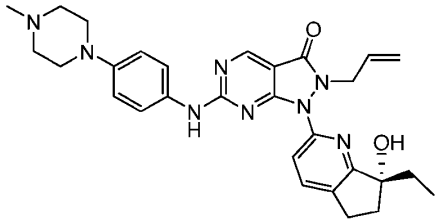
[0021] Where a range of values is provided, it is understood that the upper and lower limit, and each intervening value between the upper and lower limit of the range is encompassed within the embodiments.

[0022] Terms and phrases used in this application, and variations thereof, especially in the appended claims, unless otherwise expressly stated, should be construed as open ended as opposed to limiting. As examples of the foregoing, the term 'including' should be read to mean "including, without limitation," "including but not limited to," or the like; the term "comprising" as used herein is synonymous with "including," "containing," or "characterized by," and is inclusive or open-ended and does not exclude additional, unrecited elements or method steps; the term "having" should be interpreted as "having at least;" the term "includes" should be interpreted as "includes but is not limited to;" the term "example" is used to provide exemplary instances of the item in discussion, not an exhaustive or limiting list thereof. In addition, the term "comprising" is to be interpreted synonymously with the phrases "having at least" or "including at least." When used in the context of a compound, composition or device, the term "comprising" means that the compound, composition or device includes at least the recited features or components but may also include additional features or components.

[0023] With respect to the use of substantially any plural and/or singular terms herein, those having skill in the art can translate from the plural to the singular and/or from the singular to the plural as is appropriate to the context and/or application. The various singular/plural permutations may be expressly set forth herein for sake of clarity. The indefinite article "a" or "an" does not exclude a plurality. The mere fact that certain measures are recited in mutually different dependent claims does not indicate that a combination of these measures cannot be used to advantage. Any reference signs in the claims should not be construed as limiting the scope.

Compounds and Antibody-Drug Conjugates

[0024] Some embodiments described herein relate to the use of a combination of Compound (A), or a pharmaceutically acceptable salt thereof, and (i) a maytansinoid, or a pharmaceutically acceptable salt thereof; or (ii) an antibody-drug conjugate comprising a

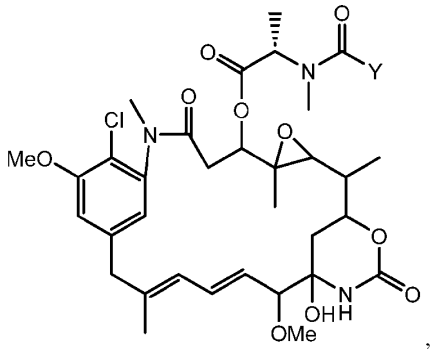
maytansinoid, or a pharmaceutically acceptable salt thereof, for treating a cancer in a subject. Compound (A) is  (also known as azenosertib or ZN-c3), or a pharmaceutically acceptable salt thereof. One embodiment relates to the use of a combination of Compound (A) as defined above, or a pharmaceutically acceptable salt thereof, and an antibody-drug conjugate comprising a maytansinoid, or a pharmaceutically acceptable salt thereof, for treating a cancer in a subject. One embodiment relates to the use of a combination of Compound (A) as defined above, or a pharmaceutically acceptable salt thereof, and an antibody-drug conjugate comprising DM4 or DM4-SMe maytansinoid (*see* below for structure), or a pharmaceutically acceptable salt of any of the foregoing, for treating a cancer in a subject.

[0025] Another aspect of this disclosure relates to a method of treating a cancer in a subject comprising administering an effective amount of Compound (A) as defined above, or a pharmaceutically acceptable salt thereof, and an effective amount of: (i) a maytansinoid, or a pharmaceutically acceptable salt thereof; or (ii) an antibody-drug conjugate comprising a maytansinoid, or a pharmaceutically acceptable salt thereof, to the subject. One embodiment relates to a method of treating a cancer in a subject comprising administering an effective amount of Compound (A) as defined above, or a pharmaceutically acceptable salt thereof, and an effective amount of an antibody-drug conjugate comprising a maytansinoid, or a pharmaceutically acceptable salt thereof, to the subject. . One embodiment relates to a method of treating a cancer in a subject comprising administering an effective amount of Compound (A) as defined above, or a pharmaceutically acceptable salt thereof, and an effective amount of an antibody-drug conjugate comprising DM4 or DM4-SMe maytansinoid (*see* below for structure), or a pharmaceutically acceptable salt of any of the foregoing, to the subject.

[0026] Yet another aspect of this disclosure relates to a combination of Compound (A), or a pharmaceutically acceptable salt thereof, and (i) a maytansinoid, or a pharmaceutically acceptable salt thereof; or (ii) an antibody-drug conjugate comprising a maytansinoid, or a pharmaceutically acceptable salt thereof, for use in treating a cancer. One embodiment relates to a combination of Compound (A), or a pharmaceutically

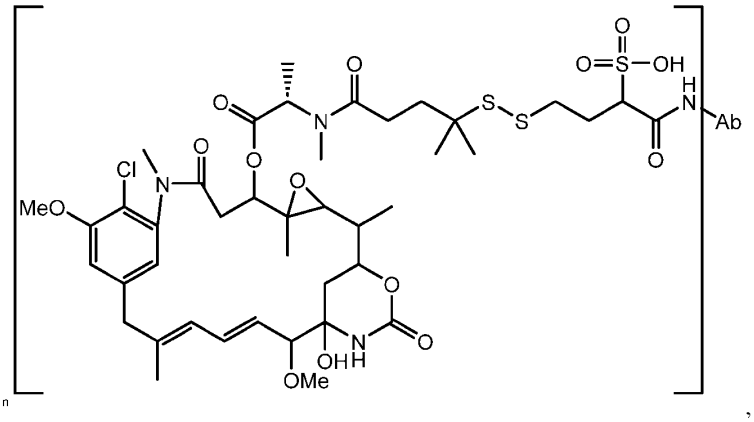
acceptable salt thereof, and an antibody-drug conjugate comprising a maytansinoid, or a pharmaceutically acceptable salt thereof, for use in treating a cancer. One embodiment relates to a combination of Compound (A), or a pharmaceutically acceptable salt thereof, and an antibody-drug conjugate comprising DM4 or DM4-SMe maytansinoid (*see* below for structure) or a pharmaceutically acceptable salt of any of the foregoing, for use in treating a cancer.

[0027] In some embodiments, the maytansinoid in the uses and methods of the present disclosure is represented by the following structural formula:



or a pharmaceutically acceptable salt thereof, wherein **Y** is $-(CH_2)_2SH$ (DM1), $-(CH_2)_2SHMe$ (DM1-SMe), $-(CH_2)_2CHCH_3SH$ (DM3), $-(CH_2)_2CHCH_3SMe$ (DM3-SMe), $-(CH_2)_2C(CH_3)_2SH$ (DM4) or $-(CH_2)_2C(CH_3)_2SMe$ (DM4-SMe). In one embodiment, **Y** is $-(CH_2)_2C(CH_3)_2SH$ (DM4). In one embodiment, **Y** is $-(CH_2)_2C(CH_3)_2SMe$ (DM4-SMe).

[0028] In some embodiments, the antibody-drug conjugate comprising a maytansinoid is represented by the following structural formula:

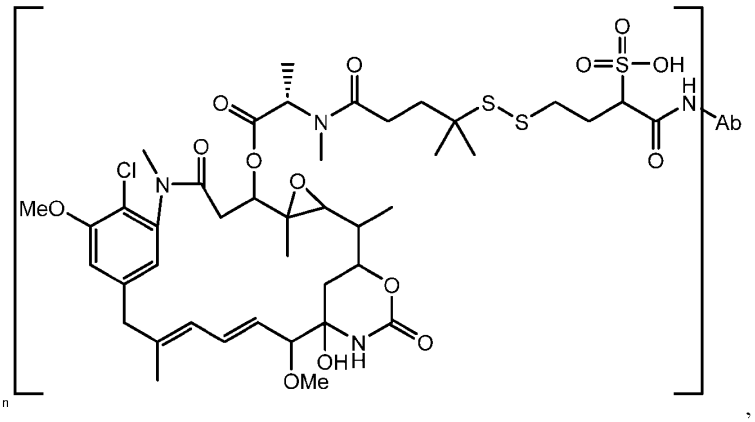


or a pharmaceutically acceptable salt thereof, wherein:

Ab is an antibody or an antigen-binding fragment thereof; and

n represents an average number of maytansinoid compounds conjugated to a single Ab and is in a range of about 1 to about 10. In one embodiment, **n** is in a range of about 3 to about 4.

[0029] One further aspect of this disclosure relates to the use of a combination of Compound (A) as defined above, or a pharmaceutically acceptable salt thereof, and an antibody-drug conjugate for treating a cancer in a subject, wherein the antibody-drug conjugate comprising a maytansinoid is represented by the following structural formula:



or a pharmaceutically acceptable salt thereof, wherein:

Ab is an antibody or an antigen-binding fragment thereof; and

n represents an average number of maytansinoid compounds conjugated to a single Ab and is in a range of about 1 to about 10. In one embodiment, **n** is in a range of about 3 to about 4.

[0030] In some embodiments, the antibody-drug conjugate or **Ab** specifically binds to a target selected from the extracellular domain of human receptor tyrosine kinase-like orphan receptor 1 (ROR1), human epidermal growth factor receptor 2 (HER2), folate receptor alpha (FRα), cadherin 6 (CDH6), sodium-dependent phosphate transporter NaPi2b, tissue factor (TF or also known as platelet tissue factor, coagulation factor III (F3)) and CD142), mesothelin (MSLN), mucin 16 (MUC16 or also known as ovarian cancer-related tumor marker CA125), protein tyrosine kinase 7 (PTK7), receptor tyrosine kinase AXL (AXL), antigen CD166 (CD166), ectonucleotide pyrophosphatase/phosphodiesterase family member 3 (ENPP3), T-cell immunoglobulin and mucin domain 1 (TIM-1), cluster of differentiation 70 (CD70, also known as CD27L), B7 homolog 3 (B7-H3, also known as

CD276), B7 homolog 4 (B7-H4), trophoblast cell surface antigen 2 (TROP-2), Nectin-4, CD22, CD30, CD79b, CD19, and B-cell maturation antigen (BCMA).

[0031] In some embodiments, the antibody-drug conjugate comprises an antibody or an antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof or **Ab** comprises:

(a) a heavy chain CDR1 comprising GYFMN (SEQ ID NO:1), a heavy chain CDR2 comprising RIHPYDGDTFYNQKFQG (SEQ ID NO:2) and a heavy chain CDR3 comprising YDGSRAMDY (SEQ ID NO:3); and

(b) a light chain CDR1 comprising KASQSVSFAGTSLMH (SEQ ID NO:4), a light chain CDR2 comprising RASNLEA (SEQ ID NO:5) and a light chain CDR3 comprising QQSREYPYT (SEQ ID NO:6).

[0032] In one embodiment, the antibody-drug conjugate comprises an antibody or an antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof or **Ab** comprises:

(a) a heavy chain consisting of CDR1 of GYFMN (SEQ ID NO:1), a heavy chain CDR2 of RIHPYDGDTFYNQKFQG (SEQ ID NO:2) and a heavy chain CDR3 of YDGSRAMDY (SEQ ID NO:3); and

(b) a light chain consisting of CDR1 of KASQSVSFAGTSLMH (SEQ ID NO:4), a light chain CDR2 of RASNLEA (SEQ ID NO:5) and a light chain CDR3 of QQSREYPYT (SEQ ID NO:6).

[0033] In some embodiments, the antibody-drug conjugate comprises an antibody or an antigen-binding fragment thereof and wherein the antibody or antigen-binding fragment thereof or **Ab** comprises a heavy chain variable domain comprising SEQ ID NO:7, and a light chain variable domain comprising SEQ ID NO:8 or SEQ ID NO:9. In one embodiment, the antibody-drug conjugate comprises an antibody or an antigen-binding fragment thereof and wherein the antibody or antigen-binding fragment thereof or **Ab** comprises a heavy chain variable domain consisting of SEQ ID NO:7, and a light chain variable domain consisting of SEQ ID NO:8 or SEQ ID NO:9.

[0034] In some embodiments, the antibody-drug conjugate comprises an antibody or an antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof or **Ab** comprises a heavy chain comprising SEQ ID NO:10, and a light chain comprising SEQ ID NO:11. In one embodiment, the antibody-drug conjugate comprises an antibody or an antigen-binding fragment thereof, wherein the antibody or

antigen-binding fragment thereof or **Ab** comprises a heavy chain consisting of SEQ ID NO:10, and a light chain consisting of SEQ ID NO:11.

[0035] In some embodiments, an antibody or antigen-binding fragment thereof (Ab) has at least 90% sequence identity to an amino acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11. In some embodiments, an antibody or antigen-binding fragment thereof (Ab) has at least 95% sequence identity to an amino acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11. In some embodiments, an antibody or antigen-binding fragment thereof (Ab) has at least 98% sequence identity to an amino acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11. In some embodiments, an antibody or antigen-binding fragment thereof (Ab) has an amino acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, wherein the amino acid sequence may contain one, two, three, four, five or more additional, deleted or substituted amino acids. For example, the addition, deletion or substituted amino acid(s) can be at either end, at both ends or in the middle of the sequence. In some embodiments, an antibody or antigen-binding fragment thereof (Ab) can have an amino acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, wherein amino acid sequence may be chemically modified with one or more functional groups.

[0036] The uses and methods of the present disclosure include uses and methods of treating a cancer selected from a brain cancer, a cervicocerebral cancer, an esophageal cancer, a thyroid cancer, a small cell cancer, a non-small cell cancer, a breast cancer, a lung cancer (*e.g.*, non-small cell lung cancer and small cell lung cancer), a stomach cancer, a urothelial cancer, a gallbladder/bile duct cancer, a liver cancer, a pancreatic cancer, a colon cancer, a rectal cancer, a colorectal cancer, an ovarian cancer (*e.g.*, epithelial ovarian cancer), a fallopian tube cancer, a primary peritoneal cancer, an uterine cancer, a choriocarcinoma, an uterus body cancer, an uterocervical cancer, a renal pelvis/ureter cancer, a bladder cancer, a prostate cancer, a penis cancer, a testicular cancer,

a fetal cancer, Wilms' cancer, a skin cancer, malignant melanoma, a neuroblastoma, an osteosarcoma, an Ewing's tumor, a soft part sarcoma, an acute leukemia, a chronic lymphatic leukemia, a chronic myelocytic leukemia, polycythemia vera, a malignant lymphoma, multiple myeloma, a Hodgkin's lymphoma and a non-Hodgkin's lymphoma. In one embodiment, the cancer is an ovarian cancer. In one embodiment, the ovarian cancer is high-grade serous ovarian cancer or high-grade serous ovarian carcinoma (HGSOC).

[0037] In some embodiments, the cancer treated in the uses and methods of provided herein is a "platinum-sensitive cancer," which as used herein and per Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 refers to a cancer or tumor that is sensitive to platinum-based chemotherapy (*e.g.*, cisplatin, carboplatin, oxaliplatin and pharmaceutically acceptable salts thereof) that is defined as (1) $\geq$ 30% decrease from baseline, confirmed at 4 weeks, (2) no $\geq$ 20% increase over smallest sum observed or (3) no new lesions.

[0038] In some embodiments, the cancer treated in the uses and methods of provided herein is a "platinum-resistant cancer," which as used herein and per Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 refers to a cancer or tumor that may have previously responded to, but no longer responds, to platinum-based chemotherapy that is defined as (1) $\geq$ 30% decrease from baseline or (2) no $\geq$ 20% increase over smallest sum observed and no new lesions at 4 weeks. In one embodiment, the cancer acquires resistance, for example, via prior exposure to platinum-based therapy, either as monotherapy or in combination with other compounds (such as other chemotherapy, immune checkpoint inhibitors, etc.).

[0039] In some embodiments, the cancer treated in the uses and methods of provided herein is a "platinum-refractory cancer," which as used herein and per Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 refers to a cancer or tumor which never responded to platinum-based chemotherapy that is defined as (1) $\geq$ 30% decrease from baseline or (2) no $\geq$ 20% increase over smallest sum observed and no new lesions at 4 weeks. In one embodiment, the cancer is inherently resistant.

[0040] In some embodiments, the subject with the cancer has received at least one prior systemic treatment regimens. In one embodiment, the subject has received one to three prior systemic treatment regimens. Such first-line standard therapies include immunotherapy, such as an immune checkpoint inhibitor, alone or in combination with chemotherapy, such as platinum-based chemotherapy. Non-limiting examples of platinum-

based chemotherapy include cisplatin, carboplatin and oxaliplatin (including pharmaceutically acceptable salts of any of the foregoing).

[0041] Non-limiting examples of immune checkpoint inhibitors include PD-1 inhibitors, PD-L1 inhibitors, CTLA-4 inhibitors and LAG-3 inhibitors (including pharmaceutically acceptable salts of any of the foregoing). In some embodiments, an immune checkpoint inhibitor may be bi- or multi-specific and may target at least two proteins, including, but not limited to, two proteins selected from PD-1, PD-L1, CTLA-4 and LAG-3. Non-limiting examples of PD-1 inhibitors include retifanlimab, pucotenlimab, cadonilimab, serplulimab, nivolumab (relatimab), zimberelimab, penpulimab, dostarlimab (dostarlimab-gxly), prolgolimab, tislelizumab, camrelizumab, sintilimab, toripalimab, cemiplimab (cemiplimab-RWLC), pembrolizumab, nivolumab, balstilimab, finotonlimab, iparomlimab, ivonescimab, tuvonralimab/iparomlimab, cetrelimab, favezelimab/pembrolizumab, genolimzumab, nofazinlimab, pembrolizumab/hyaluronidase, pembrolizumab/quavonlimab, pembrolizumab/vibostolimab, pembrolizumab/quavonlimab, rilvegostomig, sasanlimab, spartalizumab, tebotelimab and volrustomig (including pharmaceutically acceptable salts of any of the foregoing).

[0042] Non-limiting examples of PD-L1 inhibitors include socazolimab, adebrelimab, sugemalimab, envafolimab, durvalumab, avelumab, atezolizumab, benmelstobart, tagitanlimab, bintrafusp alfa, erfonrilimab and retlirafusp alfa (including pharmaceutically acceptable salts of any of the foregoing).

[0043] Non-limiting examples of CTLA-4 inhibitors include tremelimumab, cadonilimab, ipilimumab, tuvonralimab/paromlimab, erfonrilimab, gotistobart, pembrolizumab/quavonlimab, pembrolizumab/quavonlimab, quavonlimab and volrustomig (including pharmaceutically acceptable salts of any of the foregoing).

[0044] Non-limiting examples of LAG-3 inhibitors include nivolumab/relatlimab, favezelimab/pembrolizumab, fianlimab, relatlimab, tebotelimab, eftilagimod alpha and favezelimab (including pharmaceutically acceptable salts of any of the foregoing).

[0045] When the treatment is a combination of compounds, the order of administration or use of the combination of Compound (A) and maytansinoid or an antibody-drug conjugate comprising maytansinoid (including pharmaceutically acceptable salts of any of the foregoing) described herein can vary. In some embodiments, Compound (A), or a pharmaceutically acceptable salt thereof, and a maytansinoid, or a

pharmaceutically acceptable salt thereof, or an antibody-drug conjugate comprising a maytansinoid, or a pharmaceutically acceptable salt thereof, are administered or used sequentially. In some embodiments, Compound (A), or a pharmaceutically acceptable salt thereof, can be administered prior to a maytansinoid or an antibody-drug conjugate comprising a maytansinoid, or a pharmaceutically acceptable salt of any of the foregoing. In other embodiments, Compound (A), or a pharmaceutically acceptable salt thereof, can be administered subsequent to or after a maytansinoid or an antibody-drug conjugate comprising a maytansinoid, or a pharmaceutically acceptable salt of any of the foregoing. In still other embodiments, Compound (A), or a pharmaceutically acceptable salt thereof, can be administered concomitantly or concurrently with maytansinoid or an antibody-drug conjugate comprising maytansinoid, or a pharmaceutically acceptable salt of any of the foregoing.

[0046] In some embodiments, Compound (A), or a pharmaceutically acceptable salt thereof, is in use on an intermittent dosing schedule. In some embodiments, Compound (A), or a pharmaceutically acceptable salt thereof, is in use on an intermittent 5 days on, 2 days off dosing schedule.

[0047] In some embodiments, the cancer treated in the uses and methods of provided herein may be further characterized by one or more biomarkers. In one embodiment, the cancer treated in the uses and methods of provided herein may be further characterized by the folate receptor alpha (FRα) status. In one embodiment, the cancer has a folate receptor alpha (FRα)-positive status. In one embodiment, the cancer has a folate receptor alpha (FRα)-high status. In one embodiment, the cancer has a folate receptor alpha (FRα)-medium status. In one embodiment, the cancer has a folate receptor alpha (FRα)-low status. In one embodiment, the cancer has a folate receptor alpha (FRα)-negative status.

[0048] In one embodiment, the cancer treated in the uses and methods of provided herein may be further characterized by the Cyclin E1 status. In one embodiment, the cancer has a Cyclin E1-positive status. In one embodiment, the cancer has a Cyclin E1-high status. In one embodiment, the cancer has a Cyclin E1-low status. In one embodiment, the cancer has a Cyclin E1-negative status.

[0049] The folate receptor alpha (FRα) status and the Cyclin E1 status the respective receptor or protein levels, or mRNA or transcript levels thereof, in a tumor sample obtained from a subject; and a H-score, an immunochemistry (IHC) staining intensity score (*e.g.*, 1+, 2+, 3+) may be derived or calculated. The expression of each

protein is assessed using a 0 to 3+ intensity score by a trained and qualified observer with categorization of ambiguous cases confirmed by a pathologist and the percentage of tumor cells having an IHC 1+, 2+, or 3+ intensity staining score (*i.e.*, score 1 and above, score 2 and above, score 3 and above) is obtained and used to determine protein expression level.

[0050] In some embodiments, the cancer treated in the uses and methods of provided herein may be characterized by the presence of a *TP53* mutation.

[0051] In some embodiments, the use of a combination of Compound (A), or a pharmaceutically acceptable salt thereof, and a maytansinoid or an antibody-drug conjugate comprising maytansinoid, or a pharmaceutically acceptable salt of any of the foregoing, for treating a cancer or methods of treatment with a combination of Compound (A), or a pharmaceutically acceptable salt thereof, and a maytansinoid or an antibody-drug conjugate comprising a maytansinoid, or a pharmaceutically acceptable salt of any of the foregoing, provided herein can decrease the number and/or severity of side effects that can be attributed to monotherapy of maytansinoid or an antibody-drug conjugate comprising maytansinoid, or a pharmaceutically acceptable salt of any of the foregoing.

[0052] Using a combination of Compound (A), or a pharmaceutically acceptable salt thereof, and a maytansinoid or an antibody-drug conjugate comprising a maytansinoid, or a pharmaceutically acceptable salt of any of the foregoing, as described herein can result in additive, synergistic or strongly synergistic effect. A combination of compounds described herein can result in an effect that is not antagonistic.

[0053] As used herein, the term "antagonistic" means that the activity of the combination of compounds is less compared to the sum of the activities of the compounds in combination when the activity of each compound is determined individually (i.e., as a single compound). As used herein, the term "synergistic effect" means that the activity of the combination of compounds is greater than the sum of the individual activities of the compounds in the combination when the activity of each compound is determined individually. As used herein, the term "additive effect" means that the activity of the combination of compounds is about equal to the sum of the individual activities of the compounds in the combination when the activity of each compound is determined individually.

[0054] A potential advantage of utilizing a combination as described herein may be a reduction in the required amount(s) of the compound(s) that is effective in treating a disease condition disclosed herein compared to when a maytansinoid or an antibody-drug conjugate comprising a maytansinoid, or a pharmaceutically acceptable salt of any of the

foregoing, is administered as a monotherapy. For example, the amount of a maytansinoid or an antibody-drug conjugate comprising maytansinoid, or a pharmaceutically acceptable salt of any of the foregoing, used in a combination described herein can be less compared to the amount of a maytansinoid or an antibody-drug conjugate comprising maytansinoid, or a pharmaceutically acceptable salt of any of the foregoing, needed to achieve the same reduction in a disease marker (for example, tumor size) when administered as a monotherapy. Another potential advantage of utilizing a combination as described herein is that the use of two or more compounds having different mechanisms of action can create a higher barrier to the development of resistance compared to when a maytansinoid or an antibody-drug conjugate comprising maytansinoid, or a pharmaceutically acceptable salt thereof, is administered as monotherapy. Additional advantages of utilizing a combination as described herein may include little to no cross resistance between the compounds of a combination described herein; different routes for elimination of the compounds of a combination described herein; and/or little to no overlapping toxicities between the compounds of a combination described herein.

Pharmaceutical Compositions

[0055] Compound (A), or a pharmaceutically acceptable salt thereof, can be provided in a pharmaceutical composition. Likewise, a maytansinoid or an antibody-drug conjugate comprising maytansinoid, including pharmaceutically acceptable salts of any of the foregoing, can be provided in a pharmaceutical composition.

[0056] The term "pharmaceutical composition" refers to a mixture of one or more compounds and/or salts disclosed herein with other chemical components, such as diluents, carriers and/or excipients. The pharmaceutical composition facilitates administration of the compound to an organism. Pharmaceutical compositions can also be obtained by reacting compounds with inorganic or organic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, and salicylic acid. Pharmaceutical compositions will generally be tailored to the specific intended route of administration.

[0057] As used herein, a "carrier" refers to a compound that facilitates the incorporation of a compound into cells or tissues. For example, without limitation, dimethyl sulfoxide (DMSO) is a commonly utilized carrier that facilitates the uptake of many organic compounds into cells or tissues of a subject.

[0058] As used herein, a "diluent" refers to an ingredient in a pharmaceutical composition that lacks appreciable pharmacological activity but may be pharmaceutically necessary or desirable. For example, a diluent may be used to increase the bulk of a potent drug whose mass is too small for manufacture and/or administration. It may also be a liquid for the dissolution of a drug to be administered by injection, ingestion or inhalation. A common form of diluent in the art is a buffered aqueous solution such as, without limitation, phosphate buffered saline that mimics the pH and isotonicity of human blood.

[0059] As used herein, an "excipient" refers to an essentially inert substance that is added to a pharmaceutical composition to provide, without limitation, bulk, consistency, stability, binding ability, lubrication, disintegrating ability etc., to the composition. For example, stabilizers such as anti-oxidants and metal-chelating agents are excipients. In an embodiment, the pharmaceutical composition comprises an anti-oxidant and/or a metal-chelating agent. A "diluent" is a type of excipient.

[0060] In some embodiments, a maytansinoid or an antibody-drug conjugate comprising maytansinoid, along with pharmaceutically acceptable salts of any of the foregoing, can be provided in a pharmaceutical composition that includes Compound (A), or a pharmaceutically acceptable salt thereof. In other embodiments, a maytansinoid or an antibody-drug conjugate comprising maytansinoid, along with pharmaceutically acceptable salts of any of the foregoing, can be administered in a pharmaceutical composition that is separate from a pharmaceutical composition that includes Compound (A), or a pharmaceutically acceptable salt thereof.

[0061] The pharmaceutical compositions described herein can be administered to a human patient *per se*, or in pharmaceutical compositions where they are mixed with other active ingredients, as in combination therapy, or carriers, diluents, excipients or combinations thereof. Proper formulation is dependent upon the route of administration chosen. Techniques for formulation and administration of the compounds described herein are known to those skilled in the art.

[0062] The pharmaceutical compositions disclosed herein may be manufactured in a manner that is itself known, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or tableting processes. Additionally, the active ingredients are contained in an amount effective to achieve its intended purpose. Many of the compounds used in the pharmaceutical combinations disclosed herein may be provided as salts with pharmaceutically compatible counterions.

[0063] Multiple techniques of administering a compound, salt and/or composition exist in the art including, but not limited to, oral, rectal, pulmonary, topical, aerosol, injection, infusion and parenteral delivery, including intramuscular, subcutaneous, intravenous, intramedullary injections, intrathecal, direct intraventricular, intraperitoneal, intranasal and intraocular injections. In some embodiments, Compound (A), or a pharmaceutically acceptable salt thereof, can be administered orally. In some embodiments, Compound (A), or a pharmaceutically acceptable salt thereof can be provided to a subject by the same route of administration as a maytansinoid or an antibody-drug conjugate comprising maytansinoid, along with pharmaceutically acceptable salts thereof. In other embodiments, Compound (A), or a pharmaceutically acceptable salt thereof, can be provided to a subject by a different route of administration as a maytansinoid or an antibody-drug conjugate comprising maytansinoid, along with pharmaceutically acceptable salts thereof.

[0064] One may also administer the compound, salt and/or composition in a local rather than systemic manner, for example, via injection or implantation of the compound directly into the affected area, often in a depot or sustained release formulation. Furthermore, one may administer the compound in a targeted drug delivery system, for example, in a liposome coated with a tissue-specific antibody. The liposomes will be targeted to and taken up selectively by the organ. For example, intranasal or pulmonary delivery to target a respiratory disease or condition may be desirable.

[0065] The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accompanied with a notice associated with the container in form prescribed by a governmental agency regulating the manufacture, use, or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the drug for human or veterinary administration. Such notice, for example, may be the labeling approved by the U.S. Food and Drug Administration for prescription drugs, or the approved product insert. Compositions that can include a compound and/or salt described herein formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

Uses and Methods of Treatment

[0066] As provided herein, in some embodiments, a combination of compounds that includes an effective amount of Compound (A), or a pharmaceutically acceptable salt thereof, and an effective amount of a maytansinoid or an antibody-drug conjugate comprising maytansinoid, or a pharmaceutically acceptable salt of any of the foregoing, can be used to treat a cancer.

[0067] In some cases, following cancer treatment, a subject can relapse or have reoccurrence of the cancer. As used herein, the terms "relapse" and "reoccurrence" are used in their normal sense as understood by those skilled in the art. Thus, the cancer can be a recurrent cancer.

[0068] As used herein, a "subject" refers to an animal that is the object of treatment, observation or experiment. "Animal" includes cold- and warm-blooded vertebrates and invertebrates such as fish, shellfish, reptiles and, in particular, mammals. "Mammal" includes, without limitation, mice, rats, rabbits, guinea pigs, dogs, cats, sheep, goats, cows, horses, primates, such as monkeys, chimpanzees, and apes, and, in particular, humans. In some embodiments, the subject can be human. In some embodiments, the subject can be a child and/or an infant. In other embodiments, the subject can be an adult.

[0069] As used herein, the terms "treat," "treating," "treatment," "therapeutic," and "therapy" do not necessarily mean total cure or abolition of the disease or condition. Any alleviation of any undesired signs or symptoms of the disease or condition, to any extent can be considered treatment and/or therapy. Furthermore, treatment may include acts that may worsen the subject's overall feeling of well-being or appearance.

[0070] The term "effective amount" is used to indicate an amount of an active compound, or pharmaceutical agent, that elicits the biological or medicinal response indicated. For example, an effective amount of compound, salt or composition can be the amount needed to prevent, alleviate or ameliorate symptoms of the disease or condition, or prolong the survival of the subject being treated. This response may occur in a tissue, system, animal or human and includes alleviation of the signs or symptoms of the disease or condition being treated. Determination of an effective amount is well within the capability of those skilled in the art, in view of the disclosure provided herein. The effective amount of the compounds disclosed herein required as a dose will depend on the route of administration, the type of animal, including human, being treated and the physical characteristics of the specific animal under consideration. The dose can be tailored to

achieve a desired effect, but will depend on such factors as weight, diet, concurrent medication and other factors which those skilled in the medical arts will recognize.

[0071] For example, an effective amount of a compound, or radiation, is the amount that results in: (a) the reduction, alleviation or disappearance of one or more symptoms caused by the cancer, (b) the reduction of tumor size, (c) the elimination of the tumor, and/or (d) long-term disease stabilization (growth arrest) of the tumor.

[0072] The amount of compound, salt and/or composition required for use in treatment will vary not only with the particular compound or salt selected but also with the route of administration, the nature and/or symptoms of the disease or condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician. In cases of administration of a pharmaceutically acceptable salt, dosages may be calculated as the free base. As will be understood by those of skill in the art, in certain situations it may be necessary to administer the compounds disclosed herein in amounts that exceed, or even far exceed, the dosage ranges described herein in order to effectively and/or aggressively treat particularly aggressive diseases or conditions.

[0073] As will be readily apparent to one skilled in the art, the useful *in vivo* dosage to be administered and the particular mode of administration will vary depending upon the age, weight, the severity of the affliction, the mammalian species treated, the particular compounds employed and the specific use for which these compounds are employed. The determination of effective dosage levels, that is the dosage levels necessary to achieve the desired result, can be accomplished by one skilled in the art using routine methods, for example, human clinical trials, *in vivo* studies and *in vitro* studies. For example, useful dosages of Compounds (A) and a maytansinoid or an antibody-drug conjugate comprising maytansinoid, or pharmaceutically acceptable salts of the foregoing, can be determined by comparing their *in vitro* activity, and *in vivo* activity in animal models. Such comparison can be done by comparison against an established drug, such as cisplatin and/or gemcitabine.

[0074] Dosage amount and interval may be adjusted individually to provide plasma levels of the active moiety which are sufficient to maintain the modulating effects, or minimal effective concentration (MEC). The MEC will vary for each compound but can be estimated from *in vivo* and/or *in vitro* data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. However, HPLC assays or bioassays can be used to determine plasma concentrations. Dosage intervals can also be

determined using MEC value. Compositions should be administered using a regimen which maintains plasma levels above the MEC for 10-90% of the time, such as between 30-90% and between 50-90%. In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration. A dosing regimen may comprise an "intermittent" dosing, during which one or more dosing parameters such as dosage amount and/or dosage interval are varied or changed. For example, an intermittent dosing phase may comprise a period of continuous administration (*e.g.*, 6 days, 5 days, 4 days in a week) followed by a "rest" phase (*e.g.*, 1 day, 2 days, 3 days in the same week) during which Compound (A) is not administered or is administered at a reduced dosage amount and/or less frequently. A dosing regimen may further comprise one or more repeated cycles of intermittent dosing regimens.

[0075] It should be noted that the attending physician would know how to and when to terminate, interrupt or adjust administration due to toxicity or organ dysfunctions. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response was not adequate (precluding toxicity). The magnitude of an administrated dose in the management of the disorder of interest will vary with the severity of the disease or condition to be treated and to the route of administration. The severity of the disease or condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency, will also vary according to the age, body weight and response of the individual patient. A program comparable to that discussed above may be used in veterinary medicine.

[0076] Compounds, conjugates, salts and compositions disclosed herein can be evaluated for efficacy and toxicity using known methods. For example, the toxicology of a particular compound, or of a subset of the compounds, sharing certain chemical moieties, may be established by determining *in vitro* toxicity towards a cell line, such as a mammalian, such as human, cell line. The results of such studies are often predictive of toxicity in animals, such as mammals, or more specifically, humans. Alternatively, the toxicity of particular compounds in an animal model, such as mice, rats, rabbits, dogs or monkeys, may be determined using known methods. The efficacy of a particular compound may be established using several recognized methods, such as *in vitro* methods, animal models, or human clinical trials. When selecting a model to determine efficacy, the skilled artisan can be guided by the state of the art to choose an appropriate model, dose, route of administration and/or regime.

EXAMPLES

[0077] Additional embodiments are disclosed in further detail in the following examples, which are not in any way intended to limit the scope of the claims.

Example 1: Compound (A) and DM4 or Mirvetuximab Soravtansine *In Vitro* Synergy and Increased *In Vitro* DNA Damage/Mitotic Arrest/Apoptosis

[0078] OVCAR3 ovarian cancer cells (HGSOC, folate receptor alpha-positive, *TP53*-mutated, *CCNE1*-amplified) were cultured in RPMI 1640 medium (ATCC modification) (Gibco) supplemented with 20% FBS (Gibco), 10 µg/mL insulin (Sigma), and 1% penicillin-streptomycin (Gibco). OV-90 high-grade serous ovarian cancer cells (HGSOC, folate receptor alpha-positive, *TP53*-mutated, Cyclin E1-low) were cultured in 1:1 mixture of MCDB 105 (1.5 g/L sodium bicarbonate) (Cell Applications) and Medium 199 (2.2 g/L sodium bicarbonate) (Gibco) supplemented with 15% FBS (Gibco) and 1% penicillin-streptomycin (Gibco). Cells were maintained at 37 °C in an atmosphere containing 5% CO_2.

[0079] OVCAR3 or OV-90 cells were seeded at optimal density in 96-well plates for evaluation of combination treatments on cell viability. Treatments were performed the following day with Compound (A) and DM4 (0-0.5 nM for OVCAR3, 0-1 nM for OV90) or mirvetuximab soravtansine (0-10 nM) as monotherapies or in a matrix combination format. Cell viability was then measured 7 days following treatment by CellTiter-Glo 2.0 assay. Synergies between Compound (A) and DM4 were then calculated using ZIP synergy score from SynergyFinder tool (http://www.synergyfinderplus.org/).

[0080] OVCAR3 cells were seeded at optimal density in 6 cm dishes for the evaluation of combination treatments on biomarker changes. Treatment doses were determined from combination CellTiter-Glo assays and treatments were performed the following day with Compound (A) (250 nM) and DM4 (0.25 nM) or mirvetuximab soravtansine (5 nM) as monotherapies or as combination therapy. After indicated time of drug exposure, cells were harvested, rinsed with PBS, lysed and sonicated in cold RIPA buffer (Sigma) containing protease and phosphatase inhibitors for 15 minutes, and centrifuged at 4 °C, 15,000 rpm for 15 minutes. Protein concentrations were measured using the Pierce BCA Protein Assay kit (Thermo Fisher). JESS plates were loaded and run according to the manufacturer's instructions. Primary antibodies anti-Cyclin E1 was purchased from Abcam, anti-GAPDH was purchased from Invitrogen, anti-phospho-H3,

anti-γH2AX, anti-phospho-CDK1, anti-phsopho-Chk1, anti-cleaved caspase 3, anti-Wee1, and anti-CDK1 were purchased from Cell Signaling Technology.

[0081] As shown in **FIGS. 1A** and **1C**, Compound (A) in combination with DM4 (**FIG. 1A**) or mirvetuximab soravtansine (**FIG. 1C**) consistently showed significant synergy in OVCAR3 and OV-90 cells, *i.e.*, demonstrated a clear synergy (ZIP synergy score ≥ 10) at depicted dose ranges, with increased DNA damage, mitosis arrest, and apoptosis as indicated by γH2AX, phosphor-Histone H3, and cleaved caspase 3, respectively (*see* **FIGS. 1B** and **1D**).

Example 2: Compound (A) and DM4 or Mirvetuximab Soravtansine Combinations Increased *In Vitro* Mitotic Arrest/Mitotic Defects

[0082] OVCAR3 high-grade serous ovarian cancer cells (HGSOC, folate receptor alpha-positive, *TP53*-mutated, *CCNE1*-amplified) were cultured in RPMI 1640 medium (ATCC modification) (Gibco) supplemented with 20% FBS (Gibco), 10 μg/mL insulin (Sigma), and 1% penicillin-streptomycin (Gibco). Cells were maintained at 37 °C in an atmosphere containing 5% CO_2.

[0083] OVCAR3 cells were seeded at optimal density in 12-well plates for evaluation of combination treatments on cell cycle progression. The cells were treated the following day with Compound (A) (300 nM) and DM4 (0.25 nM) for 24 hours or Compound (A) (300 nM) and mirvetuximab soravtansine (5 nM) for 48 hours as monotherapies or as combination therapy. Cells were then harvested, fixed, and permeabilized before Alexa Fluor 488 anti-Histone H3 Phospho (Ser10) (BioLegend) staining, Click-iT Plus EdU Alexa Fluor 647 (Thermo Fisher) detection, and DNA content staining with FxCycle Violet (Thermo Fisher). Cell cycle progression was measured using Attune Nxt flow cytometer, analyzed using FlowJo, and plotted using GraphPad Prism.

[0084] As shown in **FIGS. 2A** (top panel) and **2B**, while Compound (A) and DM4 alone induced limited mitotic arrest as indicated by the percentage of cells with pHH3-positive staining of 7.4% and 7.77%, respectively, the combination treatment significantly increased mitotic arrest to 39.3% with decreased cell population in G1 phase. Similarly, as shown in **FIGS. 2A** (bottom panel) and **2C**, Compound (A) in combination with mirvetuximab soravtansine increased mitotic arrest to 18.79%, compared to 7.86% and 0.96% of cells arrested in mitosis with Compound (A) and mirvetuximab soravtansine monotherapies, respectively.

[0085] OVCAR3 cells were cultured as described above. OVCAR3 cells were seeded at optimal density in 96-well plates for evaluation of combination treatments on mitotic cells. Treatment was performed the following day with mirvetuximab soravtansine (5 nM) for 48 hours and Compound (A) (300 nM) for 24 hours as monotherapies or as combination therapy. Cells were then fixed and permeabilized before primary antibodies staining (anti-α-tubulin and anti-pHH3 antibodies) followed by secondary antibodies and DAPI staining. Images were captured using Operetta CLS high-content analysis system. Mitotic cells were determined by pHH3 positive staining and plotted using GraphPad Prism. Mitotic phenotypes were determined by α-tubulin and DAPI staining and plotted using GraphPad Prism.

[0086] As shown in **FIGS. 3A** and **3B**, Compound (A) in combination with mirvetuximab soravtansine increased mitotic arrest as indicated by the percentage of cells with pHH3-positive staining. While Compound (A) and mirvetuximab soravtansine alone induced mitotic defects, the combination further increased the number of cells with abnormal mitosis (*see* **FIGS. 3C** and **3D**). In **FIGS. 3A** and **3C**, α-tubulin (light grey) indicates spindles, pHH3 (white) serves as a mitosis marker, and DAPI (dark grey) indicates chromosome.

Example 3: *In Vivo* Tumor-Bearing Mice Assay

[0087] The OV-90 high-grade serous ovarian cancer/carcinoma (HGSOC, *TP53*-mutated, Cyclin E1-low, folate receptor alpha-low) tumor cell line was maintained *in vitro* as monolayer culture in 1:1 mixture of MCDB 105 (1.5 g/L sodium bicarbonate) and Medium 199 (2.2 g/L sodium bicarbonate) supplemented with 15% heat inactivated fetal bovine serum at 37°C in an atmosphere of 5% CO_2 in air. The tumor cells were routinely sub-cultured before confluence by trypsin-EDTA treatment, not to exceed 4-5 passages. The cells growing in an exponential growth phase were harvested and counted for tumor inoculation.

[0088] Each mouse was inoculated subcutaneously on the right flank with single cell suspension of tumor cells (1×10^7) in 100 µL 1:1 mixture of MCDB 105 (1.5 g/L sodium bicarbonate) and Medium 199 (2.2 g/L sodium bicarbonate) and Matrigel mixture (1:1 ratio) without serum for the tumor development. The treatments were administered to the tumor-bearing mice ($n = 8$ in each group) according to the study design shown in Table 1.

Table 1. Study design

Group	Treatment	Dose (mg/kg)	Volume (μL/g)	Route
1	Vehicle, qd × 26	-	10	p.o.
2	Compound (A), [(qd 5:2) × 3 cycles] + qd × 5	60	10	p.o.
3	Mirvetuximab soravtansine, single dose	2.5	10	i.v.
4	Compound (A), [(qd 5:2) × 3 cycles] + qd × 5 + Mirvetuximab soravtansine, single dose	60 + 2.5	10 + 10	p.o. + i.v.

[0089] For routine monitoring, all study animals were monitored not only for tumor growth, but also for behavior, such as mobility, food and water consumption (by cage side checking only), body weight, eye/hair matting and any other abnormal effect. Any mortality and/or abnormal clinical signs were recorded.

[0090] Body weights of animals were measured twice weekly throughout the study. Body weight change, expressed in %, was calculated using the following formula: BW change (%) = $((BW_{Day\ X} - BW_{Day\ 0})/BW_{Day\ 0}) \times 100$, where $BW_{Day\ x}$ is BW on a given day, and BW_{Day0} is BW on Day 0 (initiation of treatment).

[0091] The measurement of tumor size was conducted twice a week with a caliper and the tumor volume (mm^3) was estimated using the formula: $TV = a \times b^2/2$ throughout the study, where "a" and "b" was long and short diameters of a tumor, respectively. The TVs were used for calculation of the tumor growth inhibition (TGI, an indicator of antitumor effectiveness) value using the formula: $(1 - (T_d - T_0) / (C_d - C_0)) \times 100\%$. T_d and C_d were the mean tumor volumes of the treated and control animals, and T_0 and C_0 were the mean tumor volumes of the treated and control animals at the start of the experiment.

[0092] As shown in **FIGS. 4A** and **4B**, the combination therapy of Compound (A) and mirvetuximab soravtansine induced tumor growth inhibition or tumor regressions that were superior to their respective monotherapies, when the tumor volumes were measured at day 25. In **FIG. 4A**, the top line is vehicle, the bottom line with open circles is the combination therapy, and the middle two lines are the monotherapies. In **FIG. 4B**, the set of bars closest to the y-axis are vehicle, the middle sets of bars are the monotherapies and the right most set of bars is the combination therapy. The body weight measurements in **FIG. 4C** indicated minimal body weight changes throughout the study in this exemplary

combination therapy. Typically, a body weight loss of greater than 15% indicates that the treatment regimen is not well-tolerated.

[0093] Furthermore, although the foregoing has been described in some detail by way of illustrations and examples for purposes of clarity and understanding, it will be understood by those of skill in the art that numerous and various modifications can be made without departing from the spirit of the present disclosure. Therefore, it should be clearly understood that the forms disclosed herein are illustrative only and are not intended to limit the scope of the present disclosure, but rather to also cover all modification and alternatives coming with the true scope and spirit of the present disclosure.

SEQUENCE LISTINGS

SEQ ID NO:1
GYFMN

SEQ ID NO:2
RIHPYDGDTFYNQKFQG

SEQ ID NO:3
YDGSRAMDY

SEQ ID NO:4
KASQSVSFAGTSLMH

SEQ ID NO:5
RASNLEA

SEQ ID NO:6
QQSREYPYT

SEQ ID NO:7
QVQLVQSGAEVVKPGASVKISCKASGYTFTGYFMNWVKQSPGQSLEWIGRIHPY
DGDTFYNQKFQGKATLTVDKSSNTAHMELLSLTSEDFAVYYCTRYDGSRAMDY
WGQGTTVTVSS

SEQ ID NO:8
DIVLTQSPLSLAVSLGQPAIISCKASQSVSFAGTSLMHWYHQKPGQQPRLLIYRAS
NLEAGVPDRFSGSGSKTDFTLTISPVEAEDAATYYCQQSREYPYTFGGGTKLEIKR

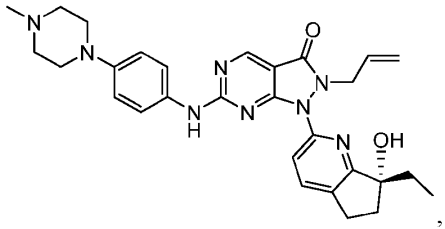
SEQ ID NO:9
DIVLTQSPLSLAVSLGQPAIISCKASQSVSFAGTSLMHWYHQKPGQQPRLLIYRAS
NLEAGVPDRFSGSGSKTDFTLNISPVEAEDAATYYCQQSREYPYTFGGGTKLEIK
R

SEQ ID NO:10
QVQLVQSGAEVVKPGASVKISCKASGYTFTGYFMNWVKQSPGQSLEWIGRIHPY
DGDTFYNQKFQGKATLTVDKSSNTAHMELLSLTSEDFAVYYCTRYDGSRAMDY
WGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE
PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS
NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH
NHYTQKSLSLSPG

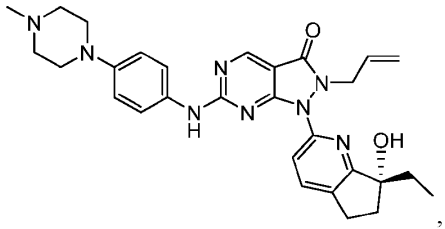
SEQ ID NO:11
DIVLTQSPLSLAVSLGQPAIISCKASQSVSFAGTSLMHWYHQKPGQQPRLLIYRAS
NLEAGVPDRFSGSGSKTDFTLTISPVEAEDAATYYCQQSREYPYTFGGGTKLEIKR
TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQEST
EQ DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

WHAT IS CLAIMED IS:

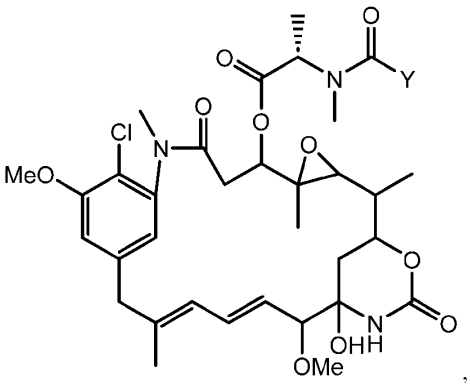
1. Use of a combination of Compound (A), or a pharmaceutically acceptable salt thereof, and (i) a maytansinoid, or a pharmaceutically acceptable salt thereof; or (ii) an antibody-drug conjugate comprising a maytansinoid, or a pharmaceutically acceptable salt thereof for treating a cancer in a subject, wherein:

Compound (A) is  , or a pharmaceutically acceptable salt thereof.

2. Use of a combination of Compound (A), or a pharmaceutically acceptable salt thereof, and an antibody-drug conjugate comprising a maytansinoid, or a pharmaceutically acceptable salt thereof, for treating a cancer in a subject, wherein:

Compound (A) is  , or a pharmaceutically acceptable salt thereof.

3. The use of claim 1, wherein the maytansinoid is represented by the following structural formula:

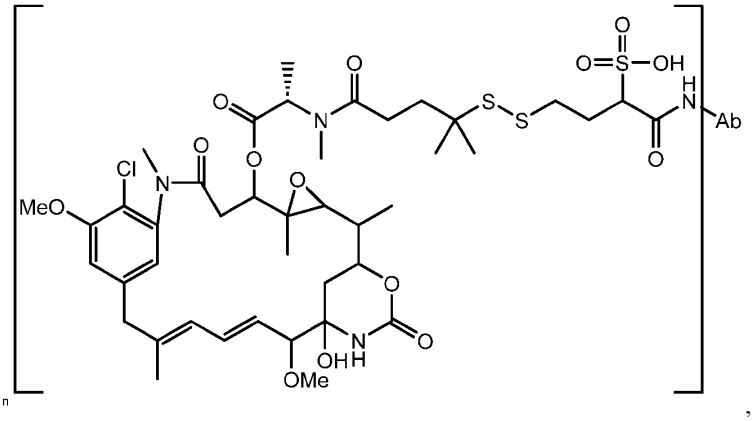


or a pharmaceutically acceptable salt thereof, wherein **Y** is $-(CH_2)_2SH$ (DM1), $-(CH_2)_2SHMe$ (DM1-SMe), $-(CH_2)_2CHCH_3SH$ (DM3), $-(CH_2)_2CHCH_3SMe$ (DM3-SMe), $-(CH_2)_2C(CH_3)_2SH$ (DM4) or $-(CH_2)_2C(CH_3)_2SMe$ (DM4-SMe).

4. The use of claim 3, wherein **Y** is $-(CH_2)_2C(CH_3)_2SH$ (DM4).

5. The use of claim 3, wherein **Y** is $-(CH_2)_2C(CH_3)_2SMe$ (DM4-SMe).

6. The use of any one of claims 1 to 5, wherein the antibody-drug conjugate comprising a maytansinoid is represented by the following structural formula:



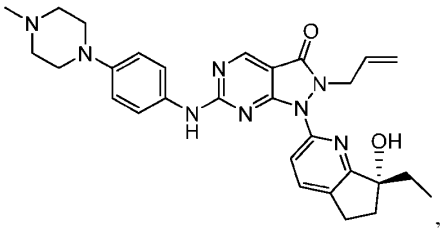
,

or a pharmaceutically acceptable salt thereof, wherein:

Ab is an antibody or an antigen-binding fragment thereof; and

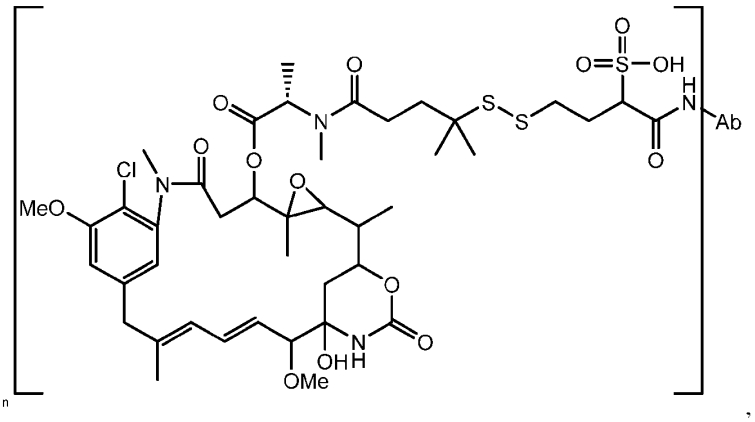
n represents an average number of maytansinoid compounds conjugated to a single Ab and is in a range of about 1 to about 10.

7. Use of a combination of Compound (A), or a pharmaceutically acceptable salt thereof, and an antibody-drug conjugate for treating a cancer in a subject, wherein:

Compound (A) is 

, or a pharmaceutically acceptable salt thereof; and

the antibody-drug conjugate is represented by the following structural formula:



or a pharmaceutically acceptable salt thereof, wherein:

Ab is an antibody or an antigen-binding fragment thereof; and

n represents an average number of maytansinoid compounds conjugated to a single Ab and is in a range of about 1 to about 10.

8. The use of any one of claims 1 to 7, wherein the antibody-drug conjugate or **Ab** specifically binds to a target selected from the extracellular domain of human receptor tyrosine kinase-like orphan receptor 1 (ROR1), human epidermal growth factor receptor 2 (HER2), folate receptor alpha (FRα), cadherin 6 (CDH6), sodium-dependent phosphate transporter NaPi2b, tissue factor (TF or also known as platelet tissue factor, coagulation factor III (F3)) and CD142), mesothelin (MSLN), mucin 16 (MUC16 or also known as ovarian cancer-related tumor marker CA125), protein tyrosine kinase 7 (PTK7), receptor tyrosine kinase AXL (AXL), antigen CD166 (CD166), ectonucleotide pyrophosphatase/phosphodiesterase family member 3 (ENPP3), T-cell immunoglobulin and mucin domain 1 (TIM-1), cluster of differentiation 70 (CD70, also known as CD27L), B7 homolog 3 (B7-H3, also known as CD276), B7 homolog 4 (B7-H4), trophoblast cell surface antigen 2 (TROP-2), Nectin-4, CD22, CD30, CD79b, CD19, and B-cell maturation antigen (BCMA).

9. The use of any one of claims 1 to 8, wherein the antibody-drug conjugate comprises an antibody or an antigen-binding fragment thereof and wherein the antibody or antigen-binding fragment thereof or **Ab** comprises:

(a) a heavy chain CDR1 comprising GYFMN (SEQ ID NO:1), a heavy chain CDR2 comprising RIHPYDGDTFYNQKFQG (SEQ ID NO:2) and a heavy chain CDR3 comprising YDGSRAMDY (SEQ ID NO:3); and

(b) a light chain CDR1 comprising KASQSVSFAGTSLMH (SEQ ID NO:4), a light chain CDR2 comprising RASNLEA (SEQ ID NO:5) and a light chain CDR3 comprising QQSREYPYT (SEQ ID NO:6).

10. The use of any one of claims 1 to 9, wherein the antibody-drug conjugate comprises an antibody or an antigen-binding fragment thereof and wherein the antibody or antigen-binding fragment thereof or **Ab** comprises a heavy chain variable domain comprising SEQ ID NO:7, and a light chain variable domain comprising SEQ ID NO:8 or SEQ ID NO:9.

11. The use of any one of claims 1 to 9, wherein the antibody-drug conjugate comprises an antibody or an antigen-binding fragment thereof and wherein the antibody or antigen-binding fragment thereof or **Ab** comprises a heavy chain comprising SEQ ID NO:10, and a light chain comprising SEQ ID NO:11.

12. The use of any one of claims 1 to 11, wherein the cancer is selected from a brain cancer, a cervicocerebral cancer, an esophageal cancer, a thyroid cancer, a small cell cancer, a non-small cell cancer, a breast cancer, a lung cancer (*e.g.*, non-small cell lung cancer and small cell lung cancer), a stomach cancer, a urothelial cancer, a gallbladder/bile duct cancer, a liver cancer, a pancreatic cancer, a colon cancer, a rectal cancer, a colorectal cancer, an ovarian cancer (*e.g.*, epithelial ovarian cancer), a fallopian tube cancer, a primary peritoneal cancer, an uterine cancer, a choriocarcinoma, an uterus body cancer, an uterocervical cancer, a renal pelvis/ureter cancer, a bladder cancer, a prostate cancer, a penis cancer, a testicular cancer, a fetal cancer, Wilms' cancer, a skin cancer, malignant melanoma, a neuroblastoma, an osteosarcoma, an Ewing's tumor, a soft part sarcoma, an acute leukemia, a chronic lymphatic leukemia, a chronic myelocytic leukemia, polycythemia vera, a malignant lymphoma, multiple myeloma, a Hodgkin's lymphoma and a non-Hodgkin`s lymphoma.

13. The use of any one of claims 1 to 12, wherein the cancer is an ovarian cancer.

14. The use of claim 13, wherein the ovarian cancer is high-grade serous ovarian cancer or carcinoma (HGSOC).

15. The use of any one of claims 1 to 14, wherein the cancer is a platinum-sensitive cancer, a platinum-resistant cancer or a platinum-refractory cancer.

16. The use of any one of claims 1 to 14, wherein the cancer is a platinum-resistant cancer.

17. The use of any one of claims 1 to 15, wherein the subject has received at least one prior systemic treatment regimens (*e.g.*, one to three prior systemic treatment regimens).

18. The use of claim 17, wherein the at least one prior systemic treatment regimen comprises immunotherapy, platinum-based chemotherapy or a combination thereof.

19. The use of any one of claims 1 to 18, wherein the cancer has a folate receptor alpha (FRα)-positive status.

20. The use of any one of claims 1 to 18, wherein the cancer has a folate receptor alpha (FRα)-high status.

21. The use of any one of claims 1 to 18, wherein the cancer has a folate receptor alpha (FRα)-medium status.

22. The use of any one of claims 1 to 18, wherein the cancer has a folate receptor alpha (FRα)-low status.

23. The use of any one of claims 1 to 22, wherein the cancer has a Cyclin E1-low status.

24. The use of any one of claims 1 to 23, wherein the cancer has a *TP53* mutation.

25. The use of any one of claims 1 to 24, wherein Compound (A), or a pharmaceutically acceptable salt thereof, is in use on an intermittent dosing schedule.

26. The use of any one of claims 1 to 25, wherein Compound (A), or a pharmaceutically acceptable salt thereof, is in use on an intermittent 5 days on, 2 days off dosing schedule.