



- (51) **International Patent Classification:**
A61K 31/11 (2006.01) *A61P 35/00* (2006.01)
A61K 31/404 (2006.01)
- (21) **International Application Number:**
PCT/US2013/072055
- (22) **International Filing Date:**
26 November 2013 (26.11.2013)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
61/730,832 28 November 2012 (28.11.2012) US
- (71) **Applicant:** THE REGENTS OF THE UNIVERSITY OF MICHIGAN [US/US]; 1600 Huron Parkway, 2nd Floor, Ann Arbor, Michigan 48109 (US).
- (72) **Inventors:** BUCKANOVICH, Ronald; 518 W. Summit, Ann Arbor, Michigan 48103 (US). LARSEN, Scott D.; 13638 Cantaberry Ct., South Lyon, Michigan 48178 (US). YANG, Kun; 4420 Cancer Center, 1500 E. Medical Center Drive, Ann Arbor, Michigan 48109 (US).
- (74) **Agent:** ARENSON, Tanya A.; Casimir Jones, S.C., 2275 Deming Way, Suite 310, Middleton, Wisconsin 53562 (US).

- (81) **Designated States** (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) **Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) **Title:** METHODS AND COMPOSITIONS FOR TARGETING CANCER STEM CELLS

(57) **Abstract:** The present invention relates to chemical compounds, methods for their discovery, and their therapeutic and research use. In particular, the present invention provides compounds as therapeutic agents against cancer stem cells.



WO 2014/085485 A1

METHODS AND COMPOSITIONS FOR TARGETING CANCER STEM CELLS

This application claims priority to U.S. Provisional Patent Application Serial No. 61/730,832 filed November 28, 2012, which is herein incorporated by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under W81XWH-12-1-0325 awarded by the U. S. Army Medical Research and Materiel Command. The government has certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates to chemical compounds, methods for their discovery, and their therapeutic and research use. In particular, the present invention provides compounds as therapeutic agents against cancer stem cells.

BACKGROUND OF THE INVENTION

Approximately 22,000 women will be diagnosed with ovarian cancer in 2011 and approximately 15,000 will die of this disease (Edwards et al., J Natl Cancer Inst. 2005; 97:1407-27).

Ovarian cancer symptoms are often vague. Women and their doctors often blame the symptoms on other, more common conditions. By the time the cancer is diagnosed, the tumor has often spread beyond the ovaries. Treatment of ovarian cancer usually involves a combination of surgery and chemotherapy.

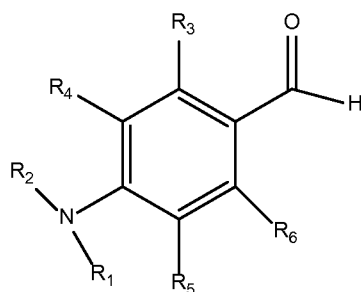
While most ovarian cancer patients will have a complete response with surgery and chemotherapy, the majority will relapse and die of their disease. The high relapse rate in ovarian cancer after complete clinical response may be due to a cancer stem cell model in which rare, inherently chemoresistant cancer stem cells capable of proliferating and differentiating to regenerate the various cell types within a tumor, thereby causing relapse of the disease.

Additional treatments for ovarian cancer are needed. Treatment that target cancer stem cells are particularly needed.

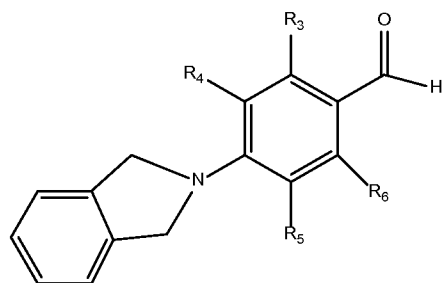
SUMMARY

5 The present invention relates to chemical compounds, methods for their discovery, and their therapeutic and research use. In particular, the present invention provides compounds as therapeutic agents against cancer stem cells.

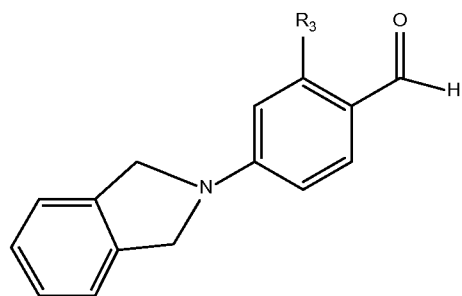
For example, in some embodiments, the present invention provides a composition comprising one or more compounds that inhibit aldehyde dehydrogenase (ALDH). The
10 present invention is not limited to a particular ALDH inhibitor. In some embodiments, the



inhibitors have the structure , wherein R1 and R2 are independently or together, H, an alkyl, a cycloalkyl, together form a cycloalkyl or heterocyclic alkyl or aryl, an aryl, an alkenyl, a cycloalkyl, alkynyl, or a substituted version of the aforementioned groups and R3-R6 are independently a halogen, H, an alkyl, a cycloalkyl,
15 an aryl, an alkenyl, a cycloalkyl, alkynyl, or a substituted version of the aforementioned groups. In some embodiments, the inhibitors have the structure

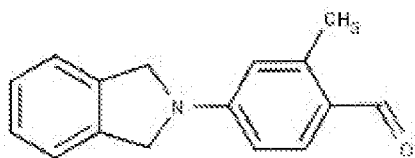
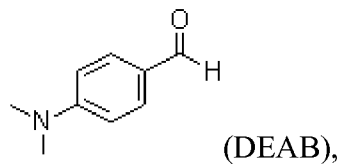


, wherein and R3-R6 are independently a halogen, H, an alkyl, a cycloalkyl, an aryl, an alkenyl, a cycloalkyl, alkynyl, or a substituted version of the aforementioned groups. In some embodiments, the compounds have the structure

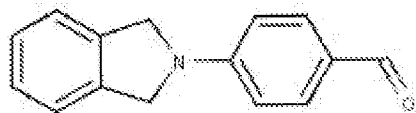


, wherein R₃ is a halogen, H, an alkyl, a cycloalkyl, an aryl, an alkenyl, a cycloalkyl, alkynyl, or a substituted version of the aforementioned groups.

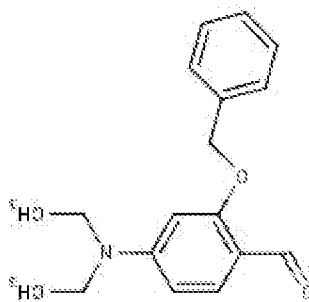
In some embodiments, the inhibitor is, for example,



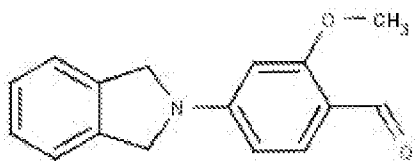
(673A),



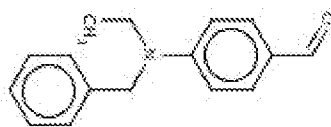
(673B)



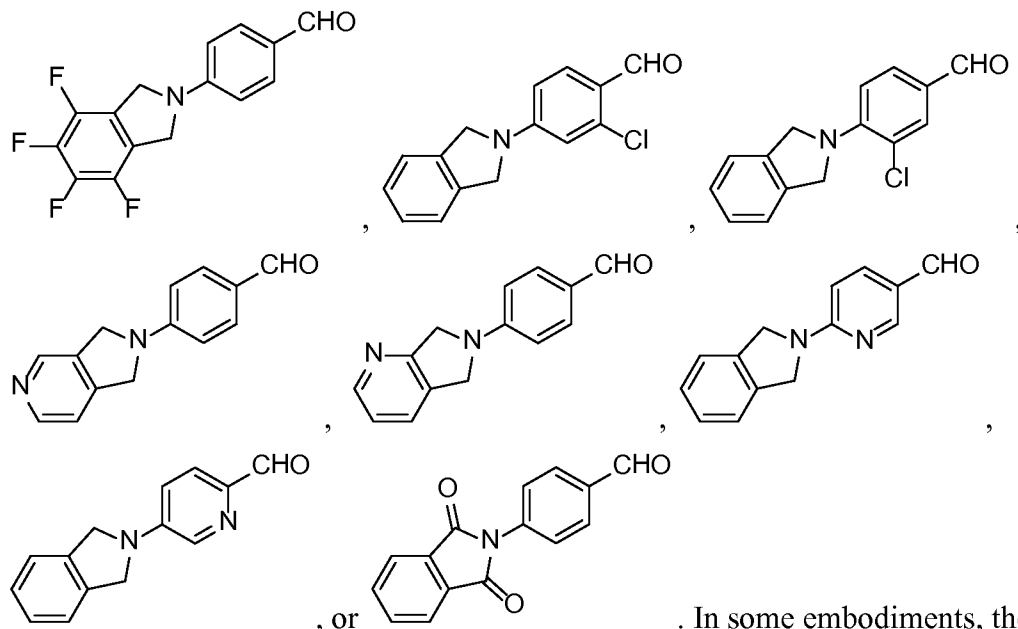
(773),



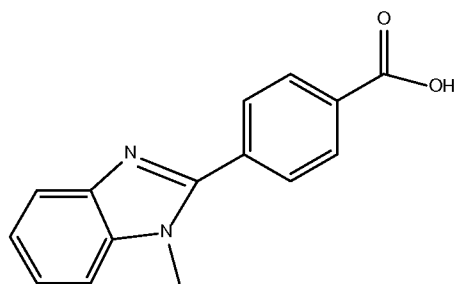
(670),



(AKO),



, or . In some embodiments, the inhibitor is a metabolite of any of the aforementioned compounds (e. g.,



or a variant thereof). In some embodiments, the composition comprises two or more of the aforementioned compounds. In some embodiments, the composition is a pharmaceutical composition (e.g., comprising a pharmaceutically acceptable carrier). In some embodiments, the composition inhibits ALDH1A1. In some embodiments, the composition kills or inhibits the growth of a cancer stem cell (e.g. an ovarian cancer stem cell). In some embodiments, the composition further comprises a known chemotherapeutic agent (e.g., platinum containing drugs (e.g. cisplatin, carboplatin or oxaliplatin) or taxane drugs (e.g., paclitaxel, docetaxel, and Abraxane). In some embodiments, the composition further comprises an agent that targets cancer stem cells (e.g., an agent that blocks BMP2, IL-6 (e.g., tocilizumab), NOTCH, or DLL4).

The further embodiments, the present invention provides the use of any one of the aforementioned compounds in the treatment of ovarian cancer.

In additional embodiments, the present invention provides a kit comprising a) any one of the aforementioned compounds; and b) a known chemotherapeutic agent (e.g., cisplatin).

In some embodiments, the ALDH inhibitor and the known chemotherapeutic agent are in the same or different compositions.

The present invention also provides a method of inhibiting ALDH in a cell, comprising: contacting a cell with any one of the aforementioned compositions under conditions such that the composition kills or inhibits the growth of the cell. In some embodiments, the cell is in vivo. In some embodiments, the cell is a cancer cell (e.g., a cancer stem cells such as an ovarian cancer stem cell). In some embodiments, the cell is in a subject diagnosed with ovarian cancer. In some embodiments, the compound radiosensitizes cancer cells to radiation therapy.

The present invention additionally provides a method of treating ovarian cancer, comprising: administering any one of the aforementioned compounds to a subject diagnosed with ovarian cancer under conditions such that the compound kills or inhibits the growth of the ovarian cancer. In some embodiments, the administering prevents recurrence or metastasis of the ovarian cancer.

Additional embodiments are described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows that ALDH inhibitors specifically kill CSC. A. FACS analysis of CD133 and ALDH demonstrating novel ALDH inhibitors lead to a loss of CD133 expressing cells. B. Summation of viability of isolated ALDH+ cells treated with cisplatin or indicated ALDH inhibitors.

Figure 2 shows effect of single agent ALDH inhibitors. A. MTT assay of cytotoxicity of indicated compounds at increasing doses 72 hours after treatment of ovarian cancer cell. B. MTT assay for pancreatic (ASPC and PANC1) and breast cancer (MCF7) cell lines. C. MTT assays of 673 for normal cells (HOSE and MSC) and ovarian cancer (OvCAR8) controls.

Figure 3 shows that ALDH inhibitors synergize with chemotherapy. A. Time to recovery for SKOV3 ovarian cancer cells treated with cisplatin or combined cisplatin and ALDH inhibitor. B. Time to recovery for purified ALDH+ and ALDH(-) cells treated with cisplatin alone or combined with ALDH inhibitor

Figure 4 shows that ALDH inhibitors functionally deplete CSC activity. A. tumor spheres formed from primary patient derived ovarian cancer cells with DMSO (control) and

673 ALDH inhibitor treatment. B. tumor growth curves from 5000 cells isolated following the indicated treatments, demonstrating 673 treatment alone or together with cisplatin reduces tumor initiation and growth.

Figure 5 shows the impact of ALDH inhibitor on tumor growth in vivo. A. tumor growth curves for control and 673 treated A2780 tumor cell xenografts. B tumor growth curves for control and 673 treated CD133+ ovarian cancer stem cell initiated tumors. C. Schematic for patient derived xenografts and tumor growth curves demonstrating ALDH inhibitor therapy reverses chemotherapy resistance in a patient derived xenografts.

Figure 6 shows that ALDH inhibition induces necrosis in CSC. A. FACs analysis for annexin-V and PI stain of control, disulfiram, and 673 treated cells demonstrating no induction of apoptosis by 673 treatment. B immunofluorescence demonstrating swollen, lytic nuclei with ALDH inhibitor treatment. C electron micrographs demonstrating ALDH Inhibitor therapy results in, cellular, mitochondrial and nuclear swelling consistent with necrosis. D. compilation of FACS demonstrating ALDH inhibitors result in intracellular calcium increases. E compilation of facts results demonstrating The calcium scavenger BAPTA can abrogate necrotic cell death of CD133+ cells.

Figure 7 shows expression changes associated with ALDH inhibitor therapy. A qRT-PCR for the indicated stem cell genes demonstrating decreased expression of CD133, SOX2, CRABP, and WNT8A. B. qRT-PCR demonstrating increased expression of necrosis associated genes MCL, UCP1 and UCP3.

Figure 8 shows the impact of ALDH inhibition on cellular metabolism. A. ALDH inhibitor therapy has no impact on extracellular acidification (ECAR)/cellular glycolysis. B. ALDH inhibitor therapy reduces oxygen Consumption suggesting mitochondrial defects.

DEFINITIONS

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

As used herein, the term "aliphatic" represents the groups including, but not limited to, alkyl, alkenyl, alkynyl, alicyclic.

As used herein, the term "alkyl" refers to an unsaturated carbon chain substituent group. In general, alkyls have the general formula C_nH_{2n+1} . Exemplary alkyls include, but are not limited to, methyl (CH_3), ethyl (C_2H_5), propyl (C_3H_7), butyl (C_4H_9), pentyl (C_5H_{11}), etc.

As used herein, the term "aryl" represents a single aromatic ring such as a phenyl ring, or two or more aromatic rings (*e.g.*, bisphenyl, naphthalene, anthracene), or an aromatic ring and one or more non-aromatic rings. The aryl group can be optionally substituted with a lower aliphatic group (*e.g.*, alkyl, alkenyl, alkynyl, or alicyclic). Additionally, the aliphatic and aryl groups can be further substituted by one or more functional groups including, but not limited to, chemical moieties comprising N, S, O, -NH₂, -NHCOCH₃, -OH, lower alkoxy (C₁-C₄), and halo (-F, -Cl, -Br, or -I).

As used herein, the term "substituted aliphatic" refers to an alkane, alkene, alkyne, or alicyclic moiety where at least one of the aliphatic hydrogen atoms has been replaced by, for example, a halogen, an amino, a hydroxy, a nitro, a thio, a ketone, an aldehyde, an ester, an amide, a lower aliphatic, a substituted lower aliphatic, or a ring (aryl, substituted aryl, cycloaliphatic, or substituted cycloaliphatic, *etc.*). Examples of such include, but are not limited to, 1-chloroethyl and the like.

As used herein, the term "substituted aryl" refers to an aromatic ring or fused aromatic ring system consisting of at least one aromatic ring, and where at least one of the hydrogen atoms on a ring carbon has been replaced by, for example, a halogen, an amino, a hydroxy, a nitro, a thio, a ketone, an aldehyde, an ester, an amide, a lower aliphatic, a substituted lower aliphatic, or a ring (aryl, substituted aryl, cycloaliphatic, or substituted cycloaliphatic). Examples of such include, but are not limited to, hydroxyphenyl and the like.

As used herein, the term "cycloaliphatic" refers to an aliphatic structure containing a fused ring system. Examples of such include, but are not limited to, decalin and the like.

As used herein, the term "substituted cycloaliphatic" refers to a cycloaliphatic structure where at least one of the aliphatic hydrogen atoms has been replaced by a halogen, a nitro, a thio, an amino, a hydroxy, a ketone, an aldehyde, an ester, an amide, a lower aliphatic, a substituted lower aliphatic, or a ring (aryl, substituted aryl, cycloaliphatic, or substituted cycloaliphatic). Examples of such include, but are not limited to, 1-chlorodecalyl, bicyclo-heptanes, octanes, and nonanes (*e.g.*, nonbornyl) and the like.

As used herein, the term "heterocyclic" represents, for example, an aromatic or nonaromatic ring containing one or more heteroatoms. The heteroatoms can be the same or different from each other. Examples of heteroatoms include, but are not limited to nitrogen, oxygen and sulfur. Aromatic and nonaromatic heterocyclic rings are well-known in the art. Some nonlimiting examples of aromatic heterocyclic rings include pyridine, pyrimidine,

indole, purine, quinoline and isoquinoline. Nonlimiting examples of nonaromatic heterocyclic compounds include piperidine, piperazine, morpholine, pyrrolidine and pyrazolidine. Examples of oxygen containing heterocyclic rings include, but not limited to furan, oxirane, 2H-pyran, 4H-pyran, 2H-chromene, and benzofuran. Examples of sulfur-containing heterocyclic rings include, but are not limited to, thiophene, benzothiophene, and parathiazine. Examples of nitrogen containing rings include, but not limited to, pyrrole, pyrrolidine, pyrazole, pyrazolidine, imidazole, imidazoline, imidazolidine, pyridine, piperidine, pyrazine, piperazine, pyrimidine, indole, purine, benzimidazole, quinoline, isoquinoline, triazole, and triazine. Examples of heterocyclic rings containing two different heteroatoms include, but are not limited to, phenothiazine, morpholine, parathiazine, oxazine, oxazole, thiazine, and thiazole. The heterocyclic ring is optionally further substituted with one or more groups selected from aliphatic, nitro, acetyl (*i.e.*, -C(=O)-CH₃), or aryl groups.

As used herein, the term “substituted heterocyclic” refers to a heterocyclic structure where at least one of the ring carbon atoms is replaced by oxygen, nitrogen or sulfur, and where at least one of the aliphatic hydrogen atoms has been replaced by a halogen, hydroxy, a thio, nitro, an amino, a ketone, an aldehyde, an ester, an amide, a lower aliphatic, a substituted lower aliphatic, or a ring (aryl, substituted aryl, cycloaliphatic, or substituted cycloaliphatic). Examples of such include, but are not limited to 2-chloropyranyl.

As used herein, the term “electron-rich heterocycle,” means cyclic compounds in which one or more ring atoms is a heteroatom (e.g., oxygen, nitrogen or sulfur), and the heteroatom has unpaired electrons which contribute to a 6- π electronic system. Exemplary electron-rich heterocycles include, but are not limited to, pyrrole, indole, furan, benzofuran, thiophene, benzothiophene and other similar structures.

As used herein, the term “linker” refers to a chain containing up to and including eight contiguous atoms connecting two different structural moieties where such atoms are, for example, carbon, nitrogen, oxygen, or sulfur. Ethylene glycol is one non-limiting example.

As used herein, the term “lower-alkyl-substituted-amino” refers to any alkyl unit containing up to and including eight carbon atoms where one of the aliphatic hydrogen atoms is replaced by an amino group. Examples of such include, but are not limited to, ethylamino and the like.

As used herein, the term “lower-alkyl-substituted-halogen” refers to any alkyl chain containing up to and including eight carbon atoms where one of the aliphatic hydrogen atoms

is replaced by a halogen. Examples of such include, but are not limited to, chlorethyl and the like.

As used herein, the term “acetylamino” shall mean any primary or secondary amino that is acetylated. Examples of such include, but are not limited to, acetamide and the like.

5 As used herein, the term "a moiety that participates in hydrogen bonding" as used herein represents a group that can accept or donate a proton to form a hydrogen bond thereby. Some specific non-limiting examples of moieties that participate in hydrogen bonding include a fluoro, oxygen-containing and nitrogen-containing groups that are well-known in the art. Some examples of oxygen-containing groups that participate in hydrogen bonding
10 include: hydroxy, lower alkoxy, lower carbonyl, lower carboxyl, lower ethers and phenolic groups. The qualifier "lower" as used herein refers to lower aliphatic groups (C₁-C₄) to which the respective oxygen-containing functional group is attached. Thus, for example, the term "lower carbonyl" refers to *inter alia*, formaldehyde, acetaldehyde. Some nonlimiting examples of nitrogen-containing groups that participate in hydrogen bond formation include
15 amino and amido groups. Additionally, groups containing both an oxygen and a nitrogen atom can also participate in hydrogen bond formation. Examples of such groups include nitro, N-hydroxy and nitrous groups. It is also possible that the hydrogen-bond acceptor in the present invention can be the π electrons of an aromatic ring.

The term "derivative" of a compound, as used herein, refers to a chemically modified
20 compound wherein the chemical modification takes place either at a functional group of the compound or backbone.

As used herein, the term “subject” refers to organisms to be treated by the methods of the present invention. Such organisms preferably include, but are not limited to, mammals (*e.g.*, murines, simians, equines, bovines, porcines, canines, felines, and the like), and most
25 preferably includes humans. In the context of the invention, the term “subject” generally refers to an individual who will receive or who has received treatment (*e.g.*, administration of a compound of the present invention and optionally one or more other agents) for a condition characterized by bacterial infection.

The term “diagnosed,” as used herein, refers to the recognition of a disease by its
30 signs and symptoms (*e.g.*, resistance to conventional therapies), or genetic analysis, pathological analysis, histological analysis, and the like.

As used herein the term, "*in vitro*" refers to an artificial environment and to processes or reactions that occur within an artificial environment. *In vitro* environments include, but are not limited to, test tubes and cell cultures. The term "*in vivo*" refers to the natural environment (e.g., an animal or a cell) and to processes or reaction that occur within a natural environment.

As used herein, the term "host cell" refers to any eukaryotic or prokaryotic cell (e.g., mammalian cells, avian cells, amphibian cells, plant cells, fish cells, and insect cells), whether located *in vitro* or *in vivo*.

As used herein, the term "cell culture" refers to any *in vitro* culture of cells. Included within this term are continuous cell lines (e.g., with an immortal phenotype), primary cell cultures, finite cell lines (e.g., non-transformed cells), and any other cell population maintained *in vitro*, including oocytes and embryos.

As used herein, the term "subject is suspected of having cancer" refers to a subject that presents one or more signs or symptoms indicative of a cancer (e.g., a noticeable lump or mass) or is being screened for a cancer (e.g., during a routine physical). A subject suspected of having cancer may also have one or more risk factors. A subject suspected of having cancer has generally not been tested for cancer. However, a "subject suspected of having cancer" encompasses an individual who has received a preliminary diagnosis (e.g., a CT scan showing a mass) but for whom a confirmatory test (e.g., biopsy and/or histology) has not been done or for whom the stage of cancer is not known. The term further includes people who once had cancer (e.g., an individual in remission). A "subject suspected of having cancer" is sometimes diagnosed with cancer and is sometimes found to not have cancer.

As used herein, the term "subject diagnosed with a cancer" refers to a subject who has been tested and found to have cancerous cells. The cancer may be diagnosed using any suitable method, including but not limited to, biopsy, x-ray, blood test, and the diagnostic methods of the present invention. A "preliminary diagnosis" is one based only on visual (e.g., CT scan or the presence of a lump) and antigen tests.

As used herein, the term "cancer cells" refers to individual cells of a cancer. Such cells may include, for example, tumorigenic cells (e.g., capable of generating a tumor), leukemogenic cells (e.g., capable of generating leukemia), cancer stem cells (e.g., capable of forming new tumors or transferring disease upon transplantation into an immunocompromised host), as well as cells that are not tumorigenic, leukemogenic or that

are capable of forming new tumors or transferring disease upon transplantation (e.g., mesenchymal and endothelial cells).

“Metastasis” as used herein refers to the process by which a cancer spreads or transfers from the site of origin to other regions of the body with the development of a similar cancerous lesion at the new location. A “metastatic” or “metastasizing” cell is one that loses adhesive contacts with neighboring cells and migrates via the bloodstream or lymph from the primary site of disease to invade neighboring body structures.

The terms “cancer stem cell,” “tumor stem cell,” or “solid tumor stem cell” are used interchangeably herein and refer to a population of cells from a solid tumor that: (1) have extensive proliferative capacity; (2) are capable of asymmetric cell division to generate one or more kinds of differentiated progeny with reduced proliferative or developmental potential; and (3) are capable of symmetric cell divisions for self-renewal or self-maintenance. These properties of “cancer stem cells,” “tumor stem cells” or “solid tumor stem cells” confer on those cancer stem cells the ability to form palpable tumors upon serial transplantation into an immunocompromised mouse compared to the majority of tumor cells that fail to form tumors. Cancer stem cells undergo self-renewal versus differentiation in a chaotic manner to form tumors with abnormal cell types that can change over time as mutations occur.

As used herein, the terms “stem cell cancer marker(s),” “cancer stem cell marker(s),” “tumor stem cell marker(s),” or “solid tumor stem cell marker(s)” refer to a gene or genes or a protein, polypeptide, or peptide expressed by the gene or genes whose expression level, alone or in combination with other genes, is correlated with the presence of tumorigenic cancer cells compared to non-tumorigenic cells. The correlation can relate to either an increased or decreased expression of the gene (e.g. increased or decreased levels of mRNA or the peptide encoded by the gene).

As used herein, the term “effective amount” refers to the amount of a compound (e.g., a compound of the present invention) sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages and is not limited intended to be limited to a particular formulation or administration route.

As used herein, the term “co-administration” refers to the administration of at least two agent(s) (e.g., a compound of the present invention) or therapies to a subject. In some embodiments, the co-administration of two or more agents/therapies is concurrent. In some

embodiments, a first agent/therapy is administered prior to a second agent/therapy. Those of skill in the art understand that the formulations and/or routes of administration of the various agents/therapies used may vary. The appropriate dosage for co-administration can be readily determined by one skilled in the art. In some embodiments, when agents/therapies are co-administered, the respective agents/therapies are administered at lower dosages than appropriate for their administration alone. Thus, co-administration is especially desirable in embodiments where the co-administration of the agents/therapies lowers the requisite dosage of a known potentially harmful (*e.g.*, toxic) agent(s).

As used herein, the term “toxic” refers to any detrimental or harmful effects on a cell or tissue as compared to the same cell or tissue prior to the administration of the toxicant.

As used herein, the term “pharmaceutical composition” refers to the combination of an active agent with a carrier, inert or active, making the composition especially suitable for diagnostic or therapeutic use *in vivo*, *in vivo* or *ex vivo*.

As used herein, the term “pharmaceutically acceptable carrier” refers to any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions (*e.g.*, such as an oil/water or water/oil emulsions), and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants. (*See e.g.*, Martin, Remington's Pharmaceutical Sciences, 15th Ed., Mack Publ. Co., Easton, PA [1975]).

As used herein, the term “pharmaceutically acceptable salt” refers to any pharmaceutically acceptable salt (*e.g.*, acid or base) of a compound of the present invention which, upon administration to a subject, is capable of providing a compound of this invention or an active metabolite or residue thereof. As is known to those of skill in the art, “salts” of the compounds of the present invention may be derived from inorganic or organic acids and bases. Examples of acids include, but are not limited to, hydrochloric, hydrobromic, sulfuric, nitric, perchloric, fumaric, maleic, phosphoric, glycolic, lactic, salicylic, succinic, toluene-p-sulfonic, tartaric, acetic, citric, methanesulfonic, ethanesulfonic, formic, benzoic, malonic, naphthalene-2-sulfonic, benzenesulfonic acid, and the like. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts.

Examples of bases include, but are not limited to, alkali metals (*e.g.*, sodium) hydroxides, alkaline earth metals (*e.g.*, magnesium), hydroxides, ammonia, and compounds of formula NW_4^+ , wherein W is C_{1-4} alkyl, and the like.

Examples of salts include, but are not limited to: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, flucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, palmoate, pectinate, persulfate, phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, undecanoate, and the like. Other examples of salts include anions of the compounds of the present invention compounded with a suitable cation such as Na^+ , NH_4^+ , and NW_4^+ (wherein W is a C_{1-4} alkyl group), and the like.

For therapeutic use, salts of the compounds of the present invention are contemplated as being pharmaceutically acceptable. However, salts of acids and bases that are non-pharmaceutically acceptable may also find use, for example, in the preparation or purification of a pharmaceutically acceptable compound.

As used herein, the term "sample" is used in its broadest sense. In one sense, it is meant to include a specimen or culture obtained from any source, as well as biological and environmental samples. Biological samples may be obtained from animals (including humans) and encompass fluids, solids, tissues, and gases. Biological samples include blood products, such as plasma, serum and the like. Environmental samples include environmental material such as surface matter, soil, water, and industrial samples. Such examples are not however to be construed as limiting the sample types applicable to the present invention.

As used herein, the terms "purified" or "to purify" refer, to the removal of undesired components from a sample. As used herein, the term "substantially purified" refers to molecules that are at least 60% free, preferably 75% free, and most preferably 90%, or more, free from other components with which they usually associated.

As used herein, the term "modulate" refers to the activity of a compound (*e.g.*, a compound of the present invention) to affect (*e.g.*, to promote or retard) an aspect of cellular function, including, but not limited to, bacterial growth and the like.

The term “test compound” refers to any chemical entity, pharmaceutical, drug, and the like, that can be used to treat or prevent a disease, illness, sickness, or disorder of bodily function, or otherwise alter the physiological or cellular status of a sample (*e.g.*, bacterial infection). Test compounds comprise both known and potential therapeutic compounds. A test compound can be determined to be therapeutic by using the screening methods of the present invention. A “known therapeutic compound” refers to a therapeutic compound that has been shown (*e.g.*, through animal trials or prior experience with administration to humans) to be effective in such treatment or prevention. In some embodiments, “test compounds” are agents that modulate apoptosis in cells.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to chemical compounds, methods for their discovery, and their therapeutic and research use. In particular, the present invention provides compounds as therapeutic agents against cancer stem cells.

Genetic mouse models of cancer and lineage tracing experiments have confirmed the presence of limited subsets of chemotherapy resistant cancer stem cell (CSC) like cells with multipotent differentiation capacity capable of repopulating tumors after chemotherapy (Schepers et al., *Science*. 2012; Chen et al., *Nature*. 2012; Driessens et al., *Nature*. 2012). The majority of cells in the tumor had limited proliferation capacity (Driessens et al., *supra*).

Furthermore, therapeutics specifically targeting these CSC dramatically improved therapeutic outcome (Chen et al., *supra*). These studies indicate that CSC represent an important target for therapy.

ALDH is an excellent marker of CSC in both ovarian tumor cell lines and in primary human ovarian cancer tissue (Silva et al., *Cancer research*. 2011;71:3991-4001). ALDH+ cells are inherently resistant to chemotherapy and limited numbers of ALDH+ cells initiate tumors, while a 50-fold excess of ALDH(-) tumor cells cannot. Importantly primary human ALDH+ ovarian cancer cells replicate human tumors in mice, generating tumors with both ALDH+ and ALDH- cells. ALDH used in combination with the stem cell marker CD133 enriches CSC isolation. Several other studies support ALDH and CD133 as ovarian CSC markers (Kryczek et al., *Int J Cancer*. 2011; Deng et al., *PLoS One*. 2010;5:e10277; Landen et al., *Mol Cancer Ther*. 2010;9:3186-99).

There are 19 known ALDH family members present in the human genome (Koppaka et al., Pharmacol Rev. 2012;64:520-39; Muzio et al., Free Radic Biol Med. 2012;52:735-46). These enzymes share limited sequence identity (~30% between ALDH1A and ALDH3A) and exhibit distinct substrate preferences. These enzymes also have distinct critical cellular functions such as regulating reactive oxygen species, retinal (vitamin A) metabolism, lipid oxidation, and resistance to chemotherapy (Koppaka et al. supra). Three of the ALDH isozymes, ALDH1A, ALDH2, and ALDH3A are believed to be responsible for a large majority of measurable ALDH activity. ALDH2 is a mitochondrial enzyme primarily involved in the metabolism of ethanol-derived acetaldehyde, elimination of mitochondrial lipid-peroxidation products and the bio-activation of nitrates. ALDH3A is a cytosolic enzyme which metabolizes aryl aldehydes as well as aldehydes generated by lipid peroxidation. ALDH1A is a cytosolic enzyme that contributes to the biosynthesis of RA through oxidation of retinal. Based on the regulation of RA, ALDH1A indirectly regulates numerous critical cellular processes regulated by RA mediated transcription (Napoli et al., J Steroid Biochem Mol Biol. 1995;53:497-502). Critical ovarian cancer stem cell genes, such as Nanog and Oct4 are downstream target genes of RA regulated transcription and regulate CSC differentiation (Balmer et al., J Lipid Res. 2002;43:1773-808). The RA receptor-b (RAR-b) and ALDH3 are also RA target genes, both of which have been linked with the regulation of cellular proliferation rates (Muzio et al., supra Canuto et al., Chem Biol Interact. 2003;143-144:29-35; Sabichi et al., J Natl Cancer Inst. 1998;90:597-605).

ALDH1A1 is most strongly implicated as markers of ovarian CSC. In addition to the tumor initiation capacity discussed above, it was found that the presence of ALDH1A1+CD133+ cells in patient's primary tumor specimens correlated with poor outcome (Silva et al., Cancer research. 2011;71:3991-4001). ALDH1A1 was found to be 100 fold upregulated in ovarian cancer cells that were resistant to either cisplatin or taxanes (Landen et al., Mol Cancer Ther. 2010;9:3186-99). siRNA knockdown of ALDH1A was toxic to ovarian cancer cells and restored chemosensitivity supporting the role of ALDH1A as a ovarian cancer stem cell target. Similarly, ALDH1A expression by tumor cells was correlated with poor patient outcome (Balmer et al., J Lipid Res. 2002;43:1773-808). More recently ALDH1A3, ALDH3A2, and ALDH3B have also been reported to be upregulated in ovarian cancer compared to normal ovary (Marchitti et al., J Histochem Cytochem. 2010;58:765-83; Saw et al., BMC Cancer. 2012;12:329).

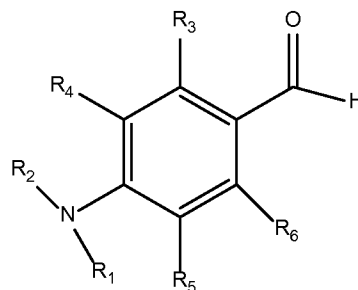
Based on its CSC specific expression and important functional role, ALDH has been supported as a CSC specific therapeutic target (Kast et al., Curr Stem Cell Res Ther. 2009;4:314-7). CSC targeted therapies are reported to have (i) up to 100-fold increase in relative therapeutic efficacy (Gupta et al., Cell. 2009;138:645-59), (ii) can reverse resistance to traditional chemotherapeutics (Wei et al., Proc Natl Acad Sci U S A. 2010;107:18874-9), and even (iii) prevent cancer recurrences (Ginestier et al., J Clin Invest. 2010;120:485-97). Indeed, ALDH inhibitors have been reported to be effective CSC therapy in breast cancer (Yip et al., Br J Cancer. 2011;104:1564-74). ALDH activators have been reported to enhance stem cell recovery in normal tissue (Banh et al., Clin Cancer Res. 2011;17:7265-72).

Despite high expression in CSC, ALDH is not essential for normal stem cells. ALDH1A knockout animals were viable with no clear defects (Levi et al., Blood. 2009;113:1670-80). ALDH1A^{-/-}ALDH3A^{-/-} mice are also viable with only modest defects; the development of cataracts and altered B cell development (Gasparetto et al., Exp Hematol. 2012;40:318-29 e2). ALDH inhibitors have proven safe for use in patients. Numerous drugs with ALDH inhibitory activity including metronidazole and chloramphenicol have safely been used in patients. Disulfiram is a potent, yet relatively non-selective, ALDH1/2 inhibitor used for the treatment of alcohol addiction (Keung et al., Proc Natl Acad Sci U S A. 1993;90:1247-51). Disulfiram has anti-cancer activity against tumor cell lines in vitro and in animal tumor models in vivo (Kast et al., Curr Stem Cell Res Ther. 2009;4:314-7, Yip et al., Br J Cancer. 2011;104:1564-74, Irving et al., Carcinogenesis. 1987;8:1309-15; Morrison et al., Melanoma Res. 2010;20:11-20; Lin et al., Prostate. 2011;71:333-43). Disulfiram has also been tested in a limited manner clinically. There are currently several ongoing new trials testing Disulfiram as an anti-CSC agent.

Accordingly, embodiments of the present invention provide compositions and methods for treating ovarian cancer (e.g., by targeting ovarian cancer stem cells) with ALDH inhibitors.

I. Inhibitors

As described herein, embodiments of the present invention provide ALDH inhibitors. In some embodiments, compounds target one or more ALDH subtypes (e.g., ALDH1A1). In

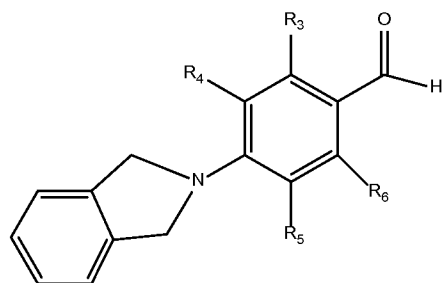


some embodiments, the inhibitors have the structure

, wherein

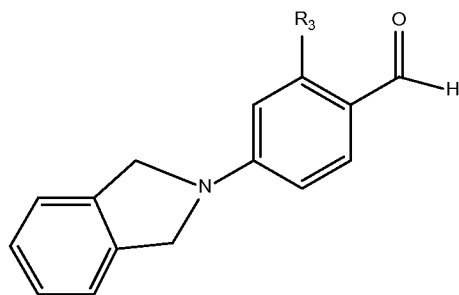
R1 and R2 are independently or together, H, an alkyl, a cycloalkyl, together form a cycloalkyl or heterocyclic alkyl or aryl, an aryl, an alkenyl, a cycloalkyl, alkynyl, or a substituted version of the aforementioned groups and R3-R6 are independently a halogen, H, an alkyl, a cycloalkyl, an aryl, an alkenyl, a cycloalkyl, alkynyl, or a substituted version of the

5



, wherein and R3-R6 are independently a halogen, H, an

alkyl, a cycloalkyl, an aryl, an alkenyl, a cycloalkyl, alkynyl, or a substituted version of the aforementioned groups. In some embodiments, the compounds have the structure

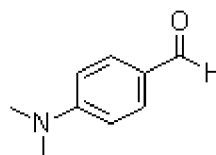


10

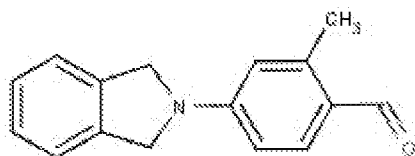
, wherein R3 is a halogen, H, an alkyl, a cycloalkyl, an

aryl, an alkenyl, a cycloalkyl, alkynyl, or a substituted version of the aforementioned groups.

In some embodiments, the inhibitor is, for example,

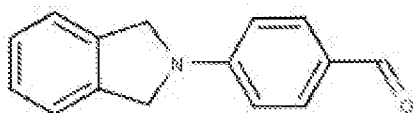


(DEAB; Sigma-



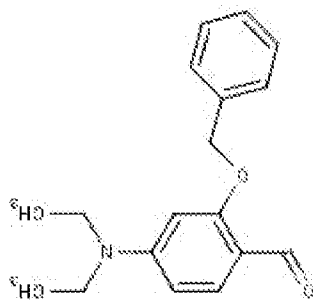
Aldrich, St. Louis, MO),

(673A; Chembridge, San Diego,



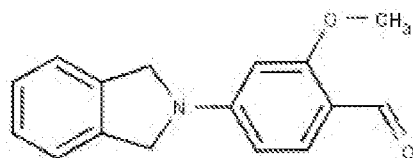
CA; Cat. No. 6737540),

(673B; Chembridge, San Diego,



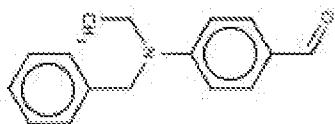
CA; Cat. No. 6730211)

(773; Chembridge, San Diego, CA;



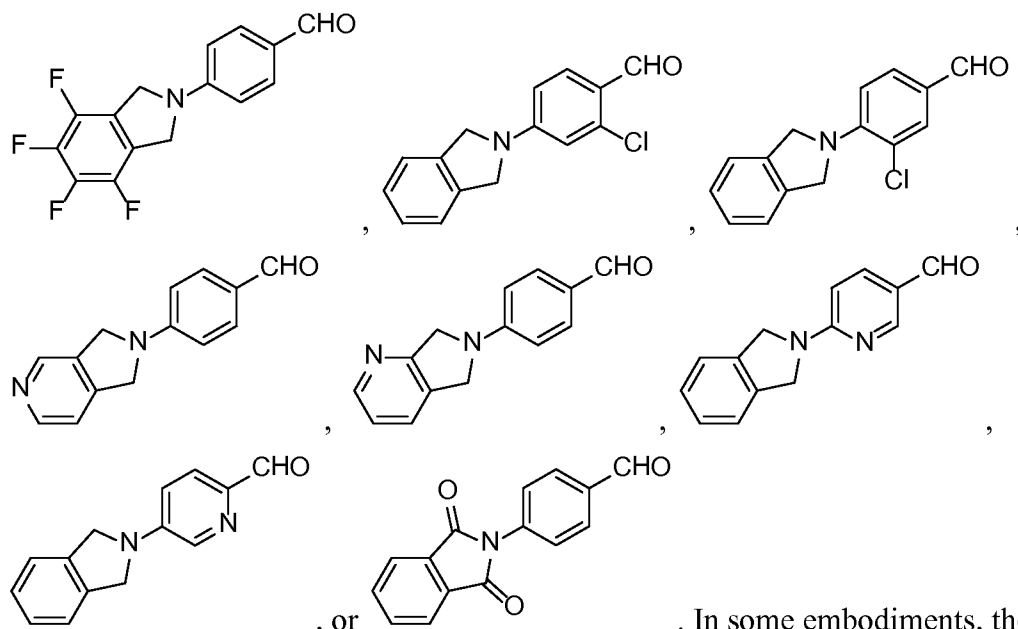
Cat. No.7735306),

(670; Chembridge, San Diego, CA; Cat.

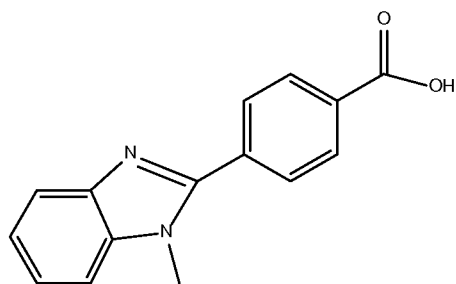


5 No.6702972),

(AKO, Molport, Riga, Latvia),



. In some embodiments, the inhibitor is a metabolite of any of the aforementioned compounds (e. g,



or a variant thereof). In some embodiments, the inhibitor is a prodrug and the metabolite of the compound is the active agent. In some embodiments, the compound is modified with a label to allow the compound to function as an imaging agent (e.g., radioactively labeled).

The present invention also provides methods of modifying and derivatizing the compositions of the present invention to increase desirable properties (e.g., binding affinity, activity, solubility and the like), or to minimize undesirable properties (e.g., nonspecific reactivity, toxicity, and the like). The principles of chemical derivatization are well understood. In some embodiments, iterative design and chemical synthesis approaches are used to produce a library of derivatized child compounds from a parent compound. In some embodiments, rational design methods are used to predict and model *in silico* ligand-receptor interactions prior to confirming results by routine experimentation.

II. Pharmaceutical compositions, formulations, and exemplary administration routes and dosing considerations

Exemplary embodiments of various contemplated medicaments and pharmaceutical compositions are provided below.

5 Embodiments of the present invention provide methods of using the aforementioned compounds in the inhibition of ALDH in cells (e.g., cancer stem cells) and in the treatment of cancers such as, for example, breast cancer, prostate cancer, lung cancer, pancreatic cancer, colon cancer, cervical cancer, uterine cancer, kidney cancer, liver cancer, stomach cancer, esophageal cancer, head and neck cancer, throat cancer, skin cancer, brain cancer (e.g.,
10 glioblastoma), testicular cancer, bone cancer, and ovarian cancer. In some embodiments, the cancer is ovarian cancer. In some embodiments, the compounds prevent the recurrence or metastasis of cancer (e.g., by targeting cancer stem cells).

A. Preparing Medicaments

15 The compounds of the present invention are useful in the preparation of medicaments to treat ovarian cancer. The methods and techniques for preparing medicaments of a compound are well-known in the art. Exemplary pharmaceutical formulations and routes of delivery are described below.

 One of skill in the art will appreciate that any one or more of the compounds
20 described herein, including the many specific embodiments, are prepared by applying standard pharmaceutical manufacturing procedures. Such medicaments can be delivered to the subject by using delivery methods that are well-known in the pharmaceutical arts.

B. Exemplary pharmaceutical compositions and formulation

25 In some embodiments of the present invention, the compositions are administered alone, while in some other embodiments, the compositions are preferably present in a pharmaceutical formulation comprising at least one active ingredient/agent (e.g., ALDH inhibitor), as defined above, together with a solid support or alternatively, together with one or more pharmaceutically acceptable carriers and optionally other therapeutic agents. Each
30 carrier should be "acceptable" in the sense that it is compatible with the other ingredients of the formulation and not injurious to the subject.

Contemplated formulations include those suitable oral, rectal, nasal, topical (including transdermal, buccal and sublingual), vaginal, parenteral (including subcutaneous, intramuscular, intravenous and intradermal) and pulmonary administration. In some embodiments, formulations are conveniently presented in unit dosage form and are prepared by any method known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association (*e.g.*, mixing) the active ingredient with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets, wherein each preferably contains a predetermined amount of the active ingredient; as a powder or granules; as a solution or suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. In some embodiments, the active ingredient is presented as a bolus, electuary, or paste, *etc.*

In some embodiments, tablets comprise at least one active ingredient and optionally one or more accessory agents/carriers are made by compressing or molding the respective agents. In some embodiments, compressed tablets are prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (*e.g.*, povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (*e.g.*, sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose) surface-active or dispersing agent. Molded tablets are made by molding in a suitable machine a mixture of the powdered compound (*e.g.*, active ingredient) moistened with an inert liquid diluent. Tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or

sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Pharmaceutical compositions for topical administration according to the present invention are optionally formulated as ointments, creams, suspensions, lotions, powders, solutions, pastes, gels, sprays, aerosols or oils. In alternatively embodiments, topical formulations comprise patches or dressings such as a bandage or adhesive plasters impregnated with active ingredient(s), and optionally one or more excipients or diluents. In some embodiments, the topical formulations include a compound(s) that enhances absorption or penetration of the active agent(s) through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethylsulfoxide (DMSO) and related analogues.

If desired, the aqueous phase of a cream base includes, for example, at least about 30% w/w of a polyhydric alcohol, *i.e.*, an alcohol having two or more hydroxyl groups such as propylene glycol, butane-1,3-diol, mannitol, sorbitol, glycerol and polyethylene glycol and mixtures thereof.

In some embodiments, oily phase emulsions of this invention are constituted from known ingredients in a known manner. This phase typically comprises an emulsifier (otherwise known as an emulgent), it is also desirable in some embodiments for this phase to further comprise a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil.

Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier so as to act as a stabilizer. In some embodiments it is also preferable to include both an oil and a fat. Together, the emulsifier(s) with or without stabilizer(s) make up the so-called emulsifying wax, and the wax together with the oil and/or fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the cream formulations.

Emulgents and emulsion stabilizers suitable for use in the formulation of the present invention include Tween 60, Span 80, cetostearyl alcohol, myristyl alcohol, glyceryl monostearate and sodium lauryl sulfate.

++

The choice of suitable oils or fats for the formulation is based on achieving the desired properties (*e.g.*, cosmetic properties), since the solubility of the active compound/agent in most oils likely to be used in pharmaceutical emulsion formulations is very low. Thus creams should preferably be a non-greasy, non-staining and washable

products with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol CAP may be used, the last three being preferred esters. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

Formulations suitable for topical administration to the eye also include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent for the agent.

Formulations for rectal administration may be presented as a suppository with suitable base comprising, for example, cocoa butter or a salicylate.

Formulations suitable for vaginal administration may be presented as pessaries, creams, gels, pastes, foams or spray formulations containing in addition to the agent, such carriers as are known in the art to be appropriate.

Formulations suitable for nasal administration, wherein the carrier is a solid, include coarse powders having a particle size, for example, in the range of about 20 to about 500 microns which are administered in the manner in which snuff is taken, *i.e.*, by rapid inhalation (*e.g.*, forced) through the nasal passage from a container of the powder held close up to the nose. Other suitable formulations wherein the carrier is a liquid for administration include, but are not limited to, nasal sprays, drops, or aerosols by nebulizer, and include aqueous or oily solutions of the agents.

Formulations suitable for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain antioxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. In some embodiments, the formulations are presented/formulated in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections,

immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage formulations are those containing a daily dose or unit, daily subdose, as herein above-recited, or an appropriate fraction thereof, of an agent.

5 It should be understood that in addition to the ingredients particularly mentioned above, the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable for oral administration may include such further agents as sweeteners, thickeners and flavoring agents. It also is intended that the agents, compositions and methods of this invention be
10 combined with other suitable compositions and therapies. Still other formulations optionally include food additives (suitable sweeteners, flavorings, colorings, *etc.*), phytonutrients (*e.g.*, flax seed oil), minerals (*e.g.*, Ca, Fe, K, *etc.*), vitamins, and other acceptable compositions (*e.g.*, conjugated linoelic acid), extenders, and stabilizers, *etc.*

15 C. Exemplary administration routes and dosing considerations

Various delivery systems are known and can be used to administer a therapeutic agent (*e.g.*, ALDH inhibitor), *e.g.*, encapsulation in liposomes, microparticles, microcapsules, receptor-mediated endocytosis, and the like. Methods of delivery include, but are not limited to, intra-arterial, intra-muscular, intravenous, intranasal, and oral routes. In specific
20 embodiments, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, injection, or by means of a catheter.

The agents identified herein as effective for their intended purpose can be administered to subjects or individuals diagnosed with ovarian cancer. When the agent is
25 administered to a subject such as a mouse, a rat or a human patient, the agent can be added to a pharmaceutically acceptable carrier and systemically or topically administered to the subject.

In some embodiments, *in vivo* administration is effected in one dose, continuously or intermittently throughout the course of treatment. Methods of determining the most effective
30 means and dosage of administration are well known to those of skill in the art and vary with the composition used for therapy, the purpose of the therapy, the target cell being treated, and

the subject being treated. Single or multiple administrations are carried out with the dose level and pattern being selected by the treating physician.

Suitable dosage formulations and methods of administering the agents are readily determined by those of skill in the art. Preferably, the compounds are administered at about 0.01 mg/kg to about 200 mg/kg, more preferably at about 0.1 mg/kg to about 100 mg/kg, even more preferably at about 0.5 mg/kg to about 50 mg/kg. When the compounds described herein are co-administered with another agent (*e.g.*, as sensitizing agents), the effective amount may be less than when the agent is used alone.

The pharmaceutical compositions can be administered orally, intranasally, parenterally or by inhalation therapy, and may take the form of tablets, lozenges, granules, capsules, pills, ampoules, suppositories or aerosol form. They may also take the form of suspensions, solutions and emulsions of the active ingredient in aqueous or nonaqueous diluents, syrups, granulates or powders. In addition to an agent of the present invention, the pharmaceutical compositions can also contain other pharmaceutically active compounds or a plurality of compounds of the invention.

More particularly, an agent of the present invention also referred to herein as the active ingredient, may be administered for therapy by any suitable route including, but not limited to, oral, rectal, nasal, topical (including, but not limited to, transdermal, aerosol, buccal and sublingual), vaginal, parental (including, but not limited to, subcutaneous, intramuscular, intravenous and intradermal) and pulmonary. It is also appreciated that the preferred route varies with the condition and age of the recipient, and the disease being treated.

In some embodiments, agents are administered intravenously. In some embodiments, agents are formulated in Cremophor (BASF, Parsippany, N.J.)

Ideally, the agent should be administered to achieve peak concentrations of the active compound at sites of disease. This may be achieved, for example, by the intravenous injection of the agent, optionally in saline, or orally administered, for example, as a tablet, capsule or syrup containing the active ingredient.

Desirable blood levels of the agent may be maintained by a continuous infusion to provide a therapeutic amount of the active ingredient within disease tissue. The use of operative combinations is contemplated to provide therapeutic combinations requiring a

lower total dosage of each component antiviral agent than may be required when each individual therapeutic compound or drug is used alone, thereby reducing adverse effects.

D. Exemplary co-administration routes and dosing considerations

5 The present invention also includes methods involving co-administration of the compounds described herein with one or more additional active agents (e.g., known chemotherapeutic agent, e.g., cisplatin). Indeed, it is a further aspect of this invention to provide methods for enhancing prior art therapies and/or pharmaceutical compositions by co-administering a compound of this invention. In co-administration procedures, the agents may
10 be administered concurrently or sequentially. In one embodiment, the compounds described herein are administered prior to the other active agent(s). The pharmaceutical formulations and modes of administration may be any of those described above. In addition, the two or more co-administered chemical agents, biological agents or other treatments may each be administered using different modes or different formulations.

15 In certain embodiments, the present invention provides method and compositions for co-administration of therapeutics, such as at least one therapeutic that targets cancer stem cells and an anti-neoplastic agent that kills cancer cells. For example, in some embodiments, combination methods provide an ALDH inhibitor in combination with a known chemotherapeutic agent (e.g., platinum containing drugs (e.g. cisplatin, carboplatin or
20 oxaliplatin) or taxane drugs (e.g., paclitaxel, docetaxel, and Abraxane) and/or an agent that targets cancer stem cells (e.g., an agent that blocks BMP2, IL-6 (e.g., tocilizumab), NOTCH, or DLL4).

A wide range of therapeutic agents find use with embodiments of the present invention. Any therapeutic agent that can be co-administered with the agents of
25 embodiments of the present invention, or associated with the agents of the present invention is suitable for use in the methods of the present invention. Some embodiments of the present invention provide methods (therapeutic methods, research methods, drug screening methods) for administering a therapeutic compound of the present invention and at least one additional therapeutic agent (e.g., including, but not limited to, chemotherapeutic
30 antineoplastics, antimicrobials, antivirals, antifungals, and anti-inflammatory agents) and/or therapeutic technique (e.g., surgical intervention, radiotherapies).

Various classes of antineoplastic (*e.g.*, anticancer) agents are contemplated for use in certain embodiments of the present invention. Anticancer agents suitable for use with the present invention include, but are not limited to, agents that induce apoptosis, agents that inhibit adenosine deaminase function, inhibit pyrimidine biosynthesis, inhibit purine ring biosynthesis, inhibit nucleotide interconversions, inhibit ribonucleotide reductase, inhibit thymidine monophosphate (TMP) synthesis, inhibit dihydrofolate reduction, inhibit DNA synthesis, form adducts with DNA, damage DNA, inhibit DNA repair, intercalate with DNA, deaminate asparagines, inhibit RNA synthesis, inhibit protein synthesis or stability, inhibit microtubule synthesis or function, and the like.

In some embodiments, exemplary anticancer agents suitable for use in compositions and methods of the present invention include, but are not limited to: 1) alkaloids, including microtubule inhibitors (*e.g.*, vincristine, vinblastine, and vindesine, *etc.*), microtubule stabilizers (*e.g.*, paclitaxel (TAXOL), and docetaxel, *etc.*), and chromatin function inhibitors, including topoisomerase inhibitors, such as epipodophyllotoxins (*e.g.*, etoposide (VP-16), and teniposide (VM-26), *etc.*), and agents that target topoisomerase I (*e.g.*, camptothecin and irinotecan (CPT-11), *etc.*); 2) covalent DNA-binding agents (alkylating agents), including nitrogen mustards (*e.g.*, mechlorethamine, chlorambucil, cyclophosphamide, ifosfamide, and busulfan (MYLERAN), *etc.*), nitrosoureas (*e.g.*, carmustine, lomustine, and semustine, *etc.*), and other alkylating agents (*e.g.*, dacarbazine, hydroxymethylmelamine, thiotepa, and mitomycin, *etc.*); 3) noncovalent DNA-binding agents (antitumor antibiotics), including nucleic acid inhibitors (*e.g.*, dactinomycin (actinomycin D), *etc.*), anthracyclines (*e.g.*, daunorubicin (daunomycin, and cerubidine), doxorubicin (adriamycin), and idarubicin (idarubicin), *etc.*), anthracenediones (*e.g.*, anthracycline analogues, such as mitoxantrone, *etc.*), bleomycins (BLENOXANE), *etc.*, and plicamycin (mithramycin), *etc.*; 4) antimetabolites, including antifolates (*e.g.*, methotrexate, FOLEX, and MEXATE, *etc.*), purine antimetabolites (*e.g.*, 6-mercaptopurine (6-MP, PURINETHOL), 6-thioguanine (6-TG), azathioprine, acyclovir, ganciclovir, chlorodeoxyadenosine, 2-chlorodeoxyadenosine (CdA), and 2'-deoxycoformycin (pentostatin), *etc.*), pyrimidine antagonists (*e.g.*, fluoropyrimidines (*e.g.*, 5-fluorouracil (ADRUCIL), 5-fluorodeoxyuridine (FdUrd) (floxuridine)) *etc.*), and cytosine arabinosides (*e.g.*, CYTOSAR (ara-C) and fludarabine, *etc.*); 5) enzymes, including L-asparaginase, and hydroxyurea, *etc.*; 6) hormones, including glucocorticoids, antiestrogens (*e.g.*, tamoxifen, *etc.*), nonsteroidal antiandrogens (*e.g.*,

flutamide, *etc.*), and aromatase inhibitors (*e.g.*, anastrozole (ARIMIDEX), *etc.*); 7) platinum compounds (*e.g.*, cisplatin and carboplatin, *etc.*); 8) monoclonal antibodies conjugated with anticancer drugs, toxins, and/or radionuclides, *etc.*; 9) biological response modifiers (*e.g.*, interferons (*e.g.*, IFN- α , *etc.*) and interleukins (*e.g.*, IL-2, *etc.*), *etc.*); 10) adoptive immunotherapy; 11) hematopoietic growth factors; 12) agents that induce tumor cell differentiation (*e.g.*, all-trans-retinoic acid, *etc.*); 13) gene therapy techniques; 14) antisense therapy techniques; 15) tumor vaccines; 16) therapies directed against tumor metastases (*e.g.*, batimastat, *etc.*); 17) angiogenesis inhibitors; 18) proteasome inhibitors (*e.g.*, VELCADE); 19) inhibitors of acetylation and/or methylation (*e.g.*, HDAC inhibitors); 20) modulators of NF kappa B; 21) inhibitors of cell cycle regulation (*e.g.*, CDK inhibitors); 22) modulators of p53 protein function; and 23) radiation.

Any oncolytic agent used in a cancer therapy context finds use in the compositions and methods of the present invention. For example, the U.S. Food and Drug Administration maintains a formulary of oncolytic agents approved for use in the United States.

International counterpart agencies to the U.S.F.D.A. maintain similar formularies. Table 1 provides a list of exemplary antineoplastic agents approved for use in the U.S. Those skilled in the art will appreciate that the “product labels” required on all U.S. approved chemotherapeutics describe approved indications, dosing information, toxicity data, and the like, for the exemplary agents.

TABLE 1

Aldesleukin (des-alanyl-1, serine-125 human interleukin-2)	Proleukin	Chiron Corp., Emeryville, CA
Alemtuzumab (IgG1 κ anti CD52 antibody)	Campath	Millennium and ILEX Partners, LP, Cambridge, MA
Alitretinoin (9-cis-retinoic acid)	Panretin	Ligand Pharmaceuticals, Inc., San Diego CA
Allopurinol (1,5-dihydro-4 H -pyrazolo[3,4-d]pyrimidin-4-one monosodium salt)	Zyloprim	GlaxoSmithKline, Research Triangle Park, NC
Altretamine	Hexalen	US Bioscience, West

(N,N,N',N',N'',N'',- hexamethyl-1,3,5-triazine-2, 4, 6-triamine)		Conshohocken, PA
Amifostine (ethanethiol, 2-[(3-aminopropyl)amino]-, dihydrogen phosphate (ester))	Ethyol	US Bioscience
Anastrozole (1,3-Benzenediacetonitrile, a, a, a', a'-tetramethyl-5-(1H-1,2,4-triazol-1-ylmethyl))	Arimidex	AstraZeneca Pharmaceuticals, LP, Wilmington, DE
Arsenic trioxide	Trisenox	Cell Therapeutic, Inc., Seattle, WA
Asparaginase (L-asparagine amidohydrolase, type EC-2)	Elspar	Merck & Co., Inc., Whitehouse Station, NJ
BCG Live (lyophilized preparation of an attenuated strain of <i>Mycobacterium bovis</i> (<i>Bacillus Calmette-Guérin</i> [BCG], substrain Montreal)	TICE BCG	Organon Teknika, Corp., Durham, NC
bexarotene capsules (4-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl) ethenyl] benzoic acid)	Targretin	Ligand Pharmaceuticals
bexarotene gel	Targretin	Ligand Pharmaceuticals
Bleomycin (cytotoxic glycopeptide antibiotics produced by <i>Streptomyces verticillus</i> ; bleomycin A ₂ and bleomycin B ₂)	Blenoxane	Bristol-Myers Squibb Co., NY, NY
Capecitabine (5'-deoxy-5-fluoro-N-[(pentyloxy)carbonyl]-cytidine)	Xeloda	Roche
Carboplatin (platinum, diammine [1,1-cyclobutanedicarboxylato(2-)-0, 0']-, (SP-4-2))	Paraplatin	Bristol-Myers Squibb
Carmustine (1,3-bis(2-chloroethyl)-1-nitrosourea)	BCNU, BiCNU	Bristol-Myers Squibb
Carmustine with Polifeprosan 20 Implant	Gliadel Wafer	Guilford Pharmaceuticals, Inc., Baltimore, MD
Celecoxib (as 4-[5-(4-methylphenyl)-3- (trifluoromethyl)-	Celebrex	Searle Pharmaceuticals, England

1H-pyrazol-1-yl] benzenesulfonamide)		
Chlorambucil (4-[bis(2chloroethyl)amino]benzenebutanoic acid)	Leukeran	GlaxoSmithKline
Cisplatin (PtCl ₂ H ₆ N ₂)	Platinol	Bristol-Myers Squibb
Cladribine (2-chloro-2'-deoxy-b-D-adenosine)	Leustatin, 2-CdA	R.W. Johnson Pharmaceutical Research Institute, Raritan, NJ
Cyclophosphamide (2-[bis(2-chloroethyl)amino] tetrahydro-2H-13,2- oxazaphosphorine 2-oxide monohydrate)	Cytoxan, Neosar	Bristol-Myers Squibb
Cytarabine (1-b-D-Arabinofuranosylcytosine, C ₉ H ₁₃ N ₃ O ₅)	Cytosar-U	Pharmacia & Upjohn Company
cytarabine liposomal	DepoCyt	Skye Pharmaceuticals, Inc., San Diego, CA
Dacarbazine (5-(3,3-dimethyl-1-triazeno)-imidazole-4- carboxamide (DTIC))	DTIC-Dome	Bayer AG, Leverkusen, Germany
Dactinomycin, actinomycin D (actinomycin produced by <i>Streptomyces parvullus</i> , C ₆₂ H ₈₆ N ₁₂ O ₁₆)	Cosmegen	Merck
Darbepoetin alfa (recombinant peptide)	Aranesp	Amgen, Inc., Thousand Oaks, CA
daunorubicin liposomal (((8S-cis)-8-acetyl-10-[(3-amino-2,3,6-trideoxy- α - L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro- 6,8,11-trihydroxy-1-methoxy-5,12- naphthacenedione hydrochloride)	DanuoXome	Nexstar Pharmaceuticals, Inc., Boulder, CO
Daunorubicin HCl, daunomycin (((1 S,3 S)-3-Acetyl-1,2,3,4,6,11-hexahydro- 3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1- naphthacenyl 3-amino-2,3,6-trideoxy-(alpha)-L- lyxo -hexopyranoside hydrochloride)	Cerubidine	Wyeth Ayerst, Madison, NJ
Denileukin diftitox (recombinant peptide)	Ontak	Seragen, Inc., Hopkinton, MA

Dexrazoxane ((S)-4,4'-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione)	Zinecard	Pharmacia & Upjohn Company
Docetaxel ((2R,3S)-N-carboxy-3-phenylisoserine, N-tert-butyl ester, 13-ester with 5b-20-epoxy-12a,4,7b,10b,13a-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate)	Taxotere	Aventis Pharmaceuticals, Inc., Bridgewater, NJ
Doxorubicin HCl (8S,10S)-10-[(3-amino-2,3,6-trideoxy-a-L-lyxohexopyranosyl)oxy]-8-glycolyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride)	Adriamycin, Rubex	Pharmacia & Upjohn Company
doxorubicin	Adriamycin PFS Intravenous injection	Pharmacia & Upjohn Company
doxorubicin liposomal	Doxil	Sequus Pharmaceuticals, Inc., Menlo park, CA
dromostanolone propionate (17b-Hydroxy-2a-methyl-5a-androstan-3-one propionate)	Dromostanolone	Eli Lilly & Company, Indianapolis, IN
dromostanolone propionate	Masterone injection	Syntex, Corp., Palo Alto, CA
Elliott's B Solution	Elliott's B Solution	Orphan Medical, Inc
Epirubicin ((8S-cis)-10-[(3-amino-2,3,6-trideoxy-a-L-arabino-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5,12-naphthacenedione hydrochloride)	Ellence	Pharmacia & Upjohn Company
Epoetin alfa (recombinant peptide)	Epogen	Amgen, Inc
Estramustine (estra-1,3,5(10)-triene-3,17-diol(17(beta))-3-[bis(2-chloroethyl)carbamate] 17-(dihydrogen phosphate), disodium salt, monohydrate, or	Emcyt	Pharmacia & Upjohn Company

estradiol 3-[bis(2-chloroethyl)carbamate] 17-(dihydrogen phosphate), disodium salt, monohydrate)		
Etoposide phosphate (4'-Demethylepipodophyllotoxin 9-[4,6-O-(R)-ethylidene-(beta)-D-glucopyranoside], 4'-(dihydrogen phosphate))	Etopophos	Bristol-Myers Squibb
etoposide, VP-16 (4'-demethylepipodophyllotoxin 9-[4,6-O-(R)-ethylidene-(beta)-D-glucopyranoside])	Vepesid	Bristol-Myers Squibb
Exemestane (6-methylenandrosta-1,4-diene-3, 17-dione)	Aromasin	Pharmacia & Upjohn Company
Filgrastim (r-metHuG-CSF)	Neupogen	Amgen, Inc
floxuridine (intraarterial) (2'-deoxy-5-fluorouridine)	FUDR	Roche
Fludarabine (fluorinated nucleotide analog of the antiviral agent vidarabine, 9-b -D-arabinofuranosyladenine (ara-A))	Fludara	Berlex Laboratories, Inc., Cedar Knolls, NJ
Fluorouracil, 5-FU (5-fluoro-2,4(1H,3H)-pyrimidinedione)	Adrucil	ICN Pharmaceuticals, Inc., Humacao, Puerto Rico
Fulvestrant (7-alpha-[9-(4,4,5,5,5-penta fluoropentylsulphinyl) nonyl]estra-1,3,5-(10)- triene-3,17-beta-diol)	Faslodex	IPR Pharmaceuticals, Guayama, Puerto Rico
Gemcitabine (2'-deoxy-2', 2'-difluorocytidine monohydrochloride (b-isomer))	Gemzar	Eli Lilly
Gemtuzumab Ozogamicin (anti-CD33 hP67.6)	Mylotarg	Wyeth Ayerst
Goserelin acetate (acetate salt of [D-Ser(But) ⁶ , Azgly ¹⁰]LHRH; pyro-Glu-His-Trp-Ser-Tyr-D-Ser(But)-Leu-Arg-Pro-Azgly-NH ₂ acetate [C ₅₉ H ₈₄ N ₁₈ O ₁₄ •(C ₂ H ₄ O ₂) _x]	Zoladex Implant	AstraZeneca Pharmaceuticals
Hydroxyurea	Hydrea	Bristol-Myers Squibb
Ibritumomab Tiuxetan	Zevalin	Biogen IDEC, Inc., Cambridge

(immunoconjugate resulting from a thiourea covalent bond between the monoclonal antibody Ibritumomab and the linker-chelator tiuxetan [N-[2-bis(carboxymethyl)amino]-3-(p-isothiocyanatophenyl)- propyl]-[N-[2-bis(carboxymethyl)amino]-2-(methyl) - ethyl]glycine)		MA
Idarubicin (5, 12-Naphthacenedione, 9-acetyl-7-[(3-amino-2,3,6-trideoxy-(alpha)-L- lyxo - hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,9,11-trihydroxyhydrochloride, (7S- cis))	Idamycin	Pharmacia & Upjohn Company
Ifosfamide (3-(2-chloroethyl)-2-[(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide)	IFEX	Bristol-Myers Squibb
Imatinib Mesilate (4-[(4-Methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-phenyl]benzamide methanesulfonate)	Gleevec	Novartis AG, Basel, Switzerland
Interferon alfa-2a (recombinant peptide)	Roferon-A	Hoffmann-La Roche, Inc., Nutley, NJ
Interferon alfa-2b (recombinant peptide)	Intron A (Lyophilized Betaseron)	Schering AG, Berlin, Germany
Irinotecan HCl (((4S)-4,11-diethyl-4-hydroxy-9-[(4- piperidinopiperidino)carbonyloxy]-1H-pyrano[3', 4': 6,7] indolizino[1,2-b] quinoline-3,14(4H, 12H) dione hydrochloride trihydrate)	Camptosar	Pharmacia & Upjohn Company
Letrozole (4,4'-(1H-1,2,4 -Triazol-1-ylmethylene) dibenzonitrile)	Femara	Novartis
Leucovorin (L-Glutamic acid, N[4[[[(2-amino-5-formyl-1,4,5,6,7,8 hexahydro4oxo6-pteridiny]methyl]amino]benzoyl], calcium salt	Wellcovorin, Leucovorin	Immunex, Corp., Seattle, WA

(1:1))		
Levamisole HCl ((S)-2,3,5, 6-tetrahydro-6-phenylimidazo [2,1-b] thiazole monohydrochloride C ₁₁ H ₁₂ N ₂ S·HCl)	Ergamisol	Janssen Research Foundation, Titusville, NJ
Lomustine (1-(2-chloro-ethyl)-3-cyclohexyl-1-nitrosourea)	CeeNU	Bristol-Myers Squibb
Meclorothamine, nitrogen mustard (2-chloro-N-(2-chloroethyl)-N-methylethanamine hydrochloride)	Mustargen	Merck
Megestrol acetate 17 α (acetyloxy)- 6- methylpregna- 4,6- diene- 3,20- dione	Megace	Bristol-Myers Squibb
Melphalan, L-PAM (4-[bis(2-chloroethyl) amino]-L-phenylalanine)	Alkeran	GlaxoSmithKline
Mercaptopurine, 6-MP (1,7-dihydro-6 H -purine-6-thione monohydrate)	Purinethol	GlaxoSmithKline
Mesna (sodium 2-mercaptoethane sulfonate)	Mesnex	Asta Medica
Methotrexate (N-[4-[(2,4-diamino-6-pteridiny)methyl]methylamino]benzoyl]-L-glutamic acid)	Methotrexate	Lederle Laboratories
Methoxsalen (9-methoxy-7H-furo[3,2-g][1]-benzopyran-7-one)	Uvadex	Therakos, Inc., Way Exton, Pa
Mitomycin C	Mutamycin	Bristol-Myers Squibb
mitomycin C	Mitozytrex	SuperGen, Inc., Dublin, CA
Mitotane (1,1-dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl) ethane)	Lysodren	Bristol-Myers Squibb
Mitoxantrone (1,4-dihydroxy-5,8-bis[[2- [(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione dihydrochloride)	Novantrone	Immunex Corporation
Nandrolone phenpropionate	Durabolin-50	Organon, Inc., West Orange, NJ
Nofetumomab	Verluma	Boehringer Ingelheim Pharma

		KG, Germany
Oprelvekin (IL-11)	Neumega	Genetics Institute, Inc., Alexandria, VA
Oxaliplatin (cis-[(1R,2R)-1,2-cyclohexanediamine-N,N'] [oxalato(2-)-O,O'] platinum)	Eloxatin	Sanofi Synthelabo, Inc., NY, NY
Paclitaxel (5 β , 20-Epoxy-1,2a, 4,7 β , 10 β , 13a- hexahydroxytax-11-en-9-one 4,10-diacetate 2- benzoate 13-ester with (2R, 3 S)- N-benzoyl-3- phenylisoserine)	TAXOL	Bristol-Myers Squibb
Pamidronate (phosphonic acid (3-amino-1-hydroxypropylidene) bis-, disodium salt, pentahydrate, (APD))	Aredia	Novartis
Pegademase ((monomethoxypolyethylene glycol succinimidyl) 11 - 17 -adenosine deaminase)	Adagen (Pegademase Bovine)	Enzon Pharmaceuticals, Inc., Bridgewater, NJ
Pegaspargase (monomethoxypolyethylene glycol succinimidyl L-asparaginase)	Oncaspar	Enzon
Pegfilgrastim (covalent conjugate of recombinant methionyl human G-CSF (Filgrastim) and monomethoxypolyethylene glycol)	Neulasta	Amgen, Inc
Pentostatin	Nipent	Parke-Davis Pharmaceutical Co., Rockville, MD
Pipobroman	Vercyte	Abbott Laboratories, Abbott Park, IL
Plicamycin, Mithramycin (antibiotic produced by <i>Streptomyces plicatus</i>)	Mithracin	Pfizer, Inc., NY, NY
Porfimer sodium	Photofrin	QLT Phototherapeutics, Inc., Vancouver, Canada
Procarbazine (N-isopropyl- μ -(2-methylhydrazino)-p-toluamide	Matulane	Sigma Tau Pharmaceuticals, Inc., Gaithersburg, MD

monohydrochloride)		
Quinacrine (6-chloro-9-(1-methyl-4-diethyl-amine) butylamino-2-methoxyacridine)	Atabrine	Abbott Labs
Rasburicase (recombinant peptide)	Elitek	Sanofi-Synthelabo, Inc.,
Rituximab (recombinant anti-CD20 antibody)	Rituxan	Genentech, Inc., South San Francisco, CA
Sargramostim (recombinant peptide)	Prokine	Immunex Corp
Streptozocin (streptozocin 2-deoxy-2- [[[(methylnitrosoamino)carbonyl]amino]-a(and b)- D-glucopyranose and 220 mg citric acid anhydrous)	Zanosar	Pharmacia & Upjohn Company
Talc (Mg ₃ Si ₄ O ₁₀ (OH) ₂)	Sclerosol	Bryan, Corp., Woburn, MA
Tamoxifen ((Z)-2-[4-(1,2-diphenyl-1-butenyl) phenoxy]-N,N- dimethylethanamine 2-hydroxy-1,2,3- propanetricarboxylate (1:1))	Nolvadex	AstraZeneca Pharmaceuticals
Temozolomide (3,4-dihydro-3-methyl-4-oxoimidazo[5,1-d]-as- tetrazine-8-carboxamide)	Temodar	Schering
teniposide, VM-26 (4'-demethylepipodophyllotoxin 9-[4,6-O-(R)-2- thenylidene-(beta)-D-glucopyranoside])	Vumon	Bristol-Myers Squibb
Testolactone (13-hydroxy-3-oxo-13,17-secoandrost-1,4-dien- 17-oic acid [dgr]-lactone)	Teslac	Bristol-Myers Squibb
Thioguanine, 6-TG (2-amino-1,7-dihydro-6H-purine-6-thione)	Thioguanine	GlaxoSmithKline
Thiotepa (Aziridine, 1,1',1''-phosphinothioylidynetris-, or Tris (1-aziridiny) phosphine sulfide)	Thioplex	Immunex Corporation
Topotecan HCl	Hycamtin	GlaxoSmithKline

((S)-10-[(dimethylamino) methyl]-4-ethyl-4,9-dihydroxy-1H-pyrano[3', 4': 6,7] indolizino [1,2-b]quinoline-3,14-(4H,12H)-dione monohydrochloride)		
Toremifene (2-(p-[(Z)-4-chloro-1,2-diphenyl-1-butenyl]-phenoxy)-N,N-dimethylethylamine citrate (1:1))	Fareston	Roberts Pharmaceutical Corp., Eatontown, NJ
Tositumomab, I 131 Tositumomab (recombinant murine immunotherapeutic monoclonal IgG _{2a} lambda anti-CD20 antibody (I 131 is a radioimmunotherapeutic antibody))	Bexxar	Corixa Corp., Seattle, WA
Trastuzumab (recombinant monoclonal IgG ₁ kappa anti-HER2 antibody)	Herceptin	Genentech, Inc
Tretinoin, ATRA (all-trans retinoic acid)	Vesanoid	Roche
Uracil Mustard	Uracil Mustard Capsules	Roberts Labs
Valrubicin, N-trifluoroacetyladiamycin-14-valerate (((2S-cis)-2- [1,2,3,4,6,11-hexahydro-2,5,12-trihydroxy-7 methoxy-6,11-dioxo-[[4 2,3,6-trideoxy-3- [(trifluoroacetyl)-amino- α -L-lyxo-hexopyranosyl]oxyl]-2-naphthacenyl]-2-oxoethyl pentanoate)	Valstar	Anthra --> Medeva
Vinblastine, Leurocristine (C ₄₆ H ₅₆ N ₄ O ₁₀ •H ₂ SO ₄)	Velban	Eli Lilly
Vincristine (C ₄₆ H ₅₆ N ₄ O ₁₀ •H ₂ SO ₄)	Oncovin	Eli Lilly
Vinorelbine (3', 4'-didehydro-4'-deoxy-C'-norvincal leukoblastine [R-(R*,R*)-2,3-dihydroxybutanedioate (1:2)(salt)])	Navelbine	GlaxoSmithKline
Zoledronate, Zoledronic acid	Zometa	Novartis

((1-Hydroxy-2-imidazol-1-yl-phosphonoethyl) phosphonic acid monohydrate)		
---	--	--

III. Drug screens

In some embodiments of the present invention, the compounds of the present invention, and other potentially useful compounds, are screened for their biological activity (e.g., ability to block ALDH).

In some embodiments, structure-based virtual screening methodologies are contemplated for identifying ALDH inhibitors. For example, in some embodiments, molecular modeling is used to identify inhibitors. In some embodiments, modeling is used to identify compounds that inhibit the activity of ALDH or ALDH pathway components.

In some embodiments, compounds are screened in cell culture or *in vivo* (e.g., non-human or human mammals) for their ability to inhibit ALDH. In some embodiments, screens detecting expression or inhibition of expression of downstream signaling molecules.

In some embodiments, the present invention provides high throughput screening of test compounds. For example, in some embodiments, large numbers of different test compounds (e.g., from a test compound library) are provided (e.g. attached to or synthesized) on a solid substrate. Test compounds can be reacted with cancer stem cells, or portions thereof, and washed. Bound cancer stem cells are then detected by methods well known in the art, using commercially available machinery and methods (e.g., the Automated Assay Optimization (AAO) software platforms (Beckman, USA) that interface with liquid handlers to enable direct statistical analysis that optimizes the assays; modular systems from CRS Robotics Corp. Burlington, Ontario), liquid handling systems, readers, and incubators, from various companies using POLARA (CRS), an open architecture laboratory automation software for a Ultra High Throughput Screening System; 3P (Plug&Play Peripherals) technology, which is designed to allow the user to reconfigure the automation platform by plugging in new instruments (ROBOCON, Vienna, Austria); the Allegro system or STACCATO workstation (Zymark), which enables a wide range of discovery applications, including HTS, ultra HTS, and high-speed plate preparation; MICROLAB Vector software (Hamilton Co., Reno, Nev., USA) for laboratory automation programming and integration; and others).

In some embodiments, assays measure a response the target cells (cancer stem cells or genetically modified cancer stem cells) provide (e.g., detectable evidence that a test compound may be efficacious). In some embodiments, the detectable signal is compared to control cells and the detectable signal identified by subtraction analysis. The relative abundance of the differences between the "targeted" and "untargeted" aliquots can be simultaneously compared (e.g., using a "subtraction" analysis (differential analysis) technique such as differential display, representational difference analysis (RDA), GEM-Gene Expression Microarrays (U.S. Pat. No. 5,545,531), suppressive subtraction hybridization (SSH) and direct sequencing (PCT patent application WO 96/17957). The subtraction analysis can include the methods of differential display, representational differential analysis (RDA), suppressive subtraction hybridization (SSH), serial analysis of gene expression (SAGE), gene expression microarray (GEM), nucleic acid chip technology, or direct sequencing).

EXAMPLES

The following examples are provided to demonstrate and further illustrate certain embodiments of the present invention and are not to be construed as limiting the scope thereof.

Example 1

Material and methods

Cell lines and cytotoxic assays

A2780 and OVCAR8 ovarian cancer cell line was obtained from Dr. Susan Murphy (Duke University, Durham, NC) and SKOV3 cells were obtained from Dr. Rebecca Liu (University of Michigan, Ann Arbor, MI). 2×10^5 A2780 cells were plated in 6-well plates in RMPI-10 (10% fetal bovine and 1% streptomycin/penicillin (Invitrogen)) and incubated in 37 °C 5% CO₂ in air mixture for 24 hours. Cells were then treated with incremental doses of 673 or 12.5 μ M for 24-72 hours. Evaluation of viability was performed with tryptan blue and the Countess.

Flow cytometry and fluorescence-activated cell sorting (FACS)

Cells from human ovarian cancer cell lines (A2780, OVCAR8, or SKOV3), human ascites, or primary ovarian tumors were counted in single cell suspensions. All results were analyzed using Summit 6.0 (Beckman Coulter, Inc.)

Tumor processing

Informed consent was obtained from patients for tissue procurement in accordance with the protocol approved by the University of Michigan's Institutional Review Board. All tumors obtained were stage III or IV epithelial ovarian, fallopian tube, or primary peritoneal cancer of serous histology. Fresh tumor specimens were minced and processed into single-cell suspensions, and isolated on a ficol gradient as previously described [Pulaski 2009]. For ascites samples, cells were isolated via centrifugation, and red cells lysed using ACK buffer (Lonza Walkersville Inc.).

Tumor sphere assays

Tumor associated ascites cells were isolated by centrifugation and treated with ACK lysis buffer (Fischer Scientific). 5000 cells were placed in each well of the 6-well ultra-low adherence plates (Corning, Acton, MA, USA) with 3 ml of supplemented MEBM (Lonza). After 24 hours, cells were treated with either media alone or UM #673. Media was added every 3-4 days, and all tumor sphere counts were done at 2 weeks. Alternatively, sphere assays were also performed with cancer cell lines A2780 and OVCAR8. Cells in culture were trypsinized and 2000 cells were either directly placed or FACS isolated for CD133+ and CD133- cells before plating in wells of ultra-low adherence plates. Images of spheres were photographed using the Olympus Microsuite Biological suite Software.

Animal studies

6-8 week old nude female mice were purchased from Charles River Laboratories. All xenograft experiments were performed with approval of the University Committee on Use and Care of Animals of the University of Michigan. To generate tumors, 7.5×10^4 unsorted

A2780 cells or 7500 CD133+ sorted A2780 cells were combined with 100 μ l of DPBS and 100 μ l of Matrigel (BD Biosciences), and then implanted subcutaneously into bilateral axillae of each mouse. 3 days after inoculation with tumors, mice were given an intra-peritoneal injection of (1) control DMSO 40 μ l, (2) Cisplatin 250 μ g/ml, (3) UM #673 10 μ M, or (4) 5 Cisplatin plus UM #673 (n = 9 mice per treatment group). Tumor growth was measured using calipers, and tumor volume was calculated based on the modified ellipsoid formula ($L \times W \times W/2$), where L is the length and W is the width of the tumor. The mice were euthanized when tumors reached 1.5 to 2 cm^3 and animal safety euthanasia guidelines were met. Intraperitoneal tumors were generated using 5×10^6 OVCAR8 in 250 ml of PBS and injected 10 I.P. 3 days after inoculation, mice were given intra-peritoneal injection of Cisplatin or Cisplatin plus UM #673.

Immunohistochemistry

15 Fresh murine tumors were embedded in OCT media and placed in -80°C freezer. Tumor blocks were cut to 8 microns sections on the Microm550 cryostat and placed on slides. The slides were dried overnight in room temperature, fixed in Acetone, blocked using horse serum, and primary antibody staining with either with anti-Ki67 or mouse-CD31 antibody. CD31 + blood vessels and Ki67 + cells were counted from # high-power fields (100x) per 20 section.

MTT

7500 of HOSE, MSC, and OVCAR8 cells were plated on a 96-well plate and rested for 24 25 hours. They were then treated with incremental doses of 673 for 72 hours. They were then analyzed according to the Vybrant® MTT Cell Proliferation Assay Kit (Invitrogen).

TEM

30 2×10^6 A2780 cells were plated on 60mm^2 and rested overnight followed by no treatment, 12.5 μ M of UM #673 for 12 hours, or 25 μ M of UM #673 for 18 hours. Cells were then washed with a phosphate buffer and fixed with glutaraldehyde. The samples were then

processed, stained, and cut at the University of Michigan histology core. Images were obtained through Philips CM-100 Transmission Electron Microscope.

Statistical analysis

p values of less than 0.5 were considered statistically significant. A 2-tailed student t-test was used.

Results

UM673 is an ALDH1A1 specific inhibitor

Several compounds with molecular analogy to the ALDH inhibitor DEAB were screened for their ability to eliminate ovarian CSC. A2770 cells, a cancer cell line for which both ALDH and CD133 have been demonstrated to be markers of CSC were treated. A compound, UM673, that resulted in both reductions in not only ALDH⁺ cells, but also CD133⁺ cells (Fig 1A) was identified. In order to determine if the loss of CD133 or ALDH expressing cells is due to downregulation/loss of enzyme function or due to cell death, FACS isolated CD133⁺ or ALDH⁺ cells were treated as single cells in microfluidic culture. 673 treatment was associated with the death of ~93% of CD133⁺ cells and 65% death of ALDH⁺ cells (Fig. 1B).

In vitro enzymatic inhibition assays ALDH1A1, ALDH1A2, ALDH1A3, ALDH2, and ALDH3A1 demonstrated that compound UM 673 is a selective ALDH1A1 inhibitor. UM673 demonstrated an IC₅₀ of <230nm for all the ALDH1A isoforms with >2uM for the other ALDH isoforms (Table 1).

In order to confirm that ALDH1A1 and ALDH1A3 are primary targets of UM673, siRNA knockdown studies were performed. Similar to treatment with UM673, ALDH1A1 and ALDH1A3, siRNA knockdown was associated with a decrease in the percentage of CD133⁺ cells. Combined ALDH1A1 and ALDH1A3 knockdown was partially additive with the greatest reductions in CD133⁺ cells. Similarly, ALDH1A1 knockdown was synergistic with chemotherapy as previously reported.

Antineoplastic effects of novel ALDH inhibitor UM#673

MTT assays were first performed with increasing doses of UM673 on several ovarian cancer cell lines including A2780, SKOV3, and OvCAR8 (Fig. 2A). TD50 ranged from 12-50 μ M. Measurable effects on A2780 viability were observed as low as 2.5 μ M. Toxicity was screened versus pancreatic cancer cell lines and breast cancer cell lines. Once again
5 versus bulk populations, variable modest cytotoxicity was observed. The toxicity of these compounds versus normal human ovarian surface epithelial cells and healthy adipose derived mesenchymal stem cells was assayed. Very little toxicity was observed with TD50 of ~105 μ M for each cell line.

To further demonstrate the anti-CSC activity of UM673, isolated ALDH⁺ and ALDH⁻
10 cells were treated with either cisplatin alone or cisplatin plus UM673 (Fig. 3A). Cisplatin treated ALDH⁺ cells required approximately 6 days to recover whereas cisplatin treated ALDH⁻ cells required 14 days to recover. In contrast, ALDH⁺ cell treated concurrently with cisplatin and UM673 required 21 days to recover back to initial cell levels. ALDH⁻ cells treated concurrently with cisplatin and showed no additive benefit suggesting selectivity for
15 the ALDH⁺ cells.

UM #673 functionally restricts OvCSC activity.

In order to further characterize UM #673 activity targeting OvCSC, a functional CSC assay was performed. Tumor sphere forming assays were performed with both FACS isolated
20 CD133⁺ A2780 cells and OVCAR8 cells (a cell line with >90% CD133 expression). A single treatment with 10 μ M of UM #673 in the CD133⁺ sorted A2780 cells resulted in a 2.6 fold decrease in sphere number and a nearly 8-fold reduction in total cell number. Treatment of OVCAR8 cells resulted in a 5.6 fold decrease in sphere formation and a 3 fold reduction total cell numbers. This assay was repeated using primary human ovarian cancer associated ascites
25 cell from three patients. Treatment with 673 was associated with an average of 7.3 fold (range 4-50) reduction in the number of spheres formed (Fig. 4A).

An important functional aspect of a cancer stem cell is the ability to initiate tumors with limited cell numbers. In order to determine if UM673 could impact tumor initiation, cells were treated in vitro with a single dose of UM673 alone or in combination with
30 cisplatin. Cells were allowed to recover for three days and then PI(-)/Annexin(-) live cells were faxed isolated and injected into mice. 200, 1000, or 5000 cells from each treatment group (n=4-10/group) were injected. As a single agent neither cisplatin or UM673 affected

tumor initiation compared to untreated controls (Table 1; fig. 4B). However when used in combination cisplatin and UM673 eliminated all tumor initiating capacity (Table 1). This was seen at all cellular titrations. Taken together this data indicated that UM673 either eliminates or chemo sensitizes chemotherapy resistant OvCSC.

5

UM673 antineoplastic effect in vivo.

The impact of UM673 as a single agent was evaluated. NOD/SCID mice were inoculated with 1×10^5 unsorted A2780 cells and treatment was initiated three days after tumor inoculation with either vehicle control or UM #673. Treatment with UM673 resulted in a statistically significant reduction in tumor growth, though did not eliminate tumors (Fig. 5A). Greatest activity was seen when isolated CD133+ cancer stem cells were used to initiate tumors (Fig 5B). This is consistent with other studies of CSC targeting agents.

The experiments were repeated in combination with cisplatin. In addition, xenografts from both whole cell line and FACS isolated CD133+ cells were treated. While single agent therapy with both cisplatin and UM 673 demonstrated modest restrictions in tumor growth, concurrent therapy led a 4-fold reduction in tumors. Indicating a stem cell selective activity for UM673 greatest therapeutic benefit was observed in the tumor xenografts generated from isolated CD133+ cells.

In order to confirm in vivo activity against primary ovarian cancers, primary human tumor xenografts were generated from a patient with chemotherapy refractory ovarian cancer. Xenografts were allowed to grow until they reached $\sim 500 \text{mm}^3$, and then treatment with either vehicle, cisplatin or cisplatin and UM 673 was initiated. While cisplatin therapy alone had no effect on tumor growth, concurrent therapy resulted in tumor regression. Maintenance of UM673 therapy alone prevented tumor regrowth. After discontinuation of therapy, tumor regrowth was observed after approximately 4 weeks (Fig. 5D).

UM673 induces karyolysis and necrosis.

Next, the mechanism whereby UM673 induces OvCSC cell death was investigated.

PI/Annexin-V FACS analysis of UM673 treated cells was performed to determine if cells were undergoing apoptosis. Treatment with 673 was not associated with an increase in Annexin-V staining in cells suggesting apoptosis was not the primary mechanism of cell

death (Fig. 6A). Further supporting this, use of the pan-caspase inhibitor ZVAD-FMK had no impact on UM673 induced reductions in CD133+ cell counts.

Cellular histology was evaluated 24 hours after treatment with UM 673. Fluorescent images of DAPI labeled cells demonstrated that treatment with UM 673 results in nuclear swelling and loss of DNA in a manner consistent with karyolysis and program cell necrosis (Fig. 6B). Light microscopy images of 0.5 micron sections stained with toluidine blue helps to confirm the presence of fragmented nuclei and loss of cell architecture with treatment. In order to determine whether the nuclear findings on fluorescent microscopy images were truly karyolysis, a distinct nuclear process associated with necrosis, transmission electron microscopy was performed. Once again cells treated with UM #673 demonstrated findings consistent with necrosis (Fig. 6C). In comparison to controls, there is evidence of cell enlargement and swelling. There is an increase in the translucency of the cytoplasm, as well as mitochondria swelling and loss of architecture. In the nucleus, the presence of fragmented chromatin is seen secondary to nuclear DNase. With higher dose of UM #673, rupture of plasma membrane with spilling of cytoplasmic contents is evident.

Program cell necrosis associated with the nuclear cytoplasmic shuttling of the protein HMBG1. Immunofluorescent analysis of HMBG1 protein with 673 treated cells demonstrated clear relocalization of HMBG1.

Programmed cell necrosis is known to be linked with decreases in cellular ATP and an increase in intracellular calcium. Using fluorescent analysis of intra-cellular calcium, treatment with UM 673 was associated with a fourfold increase in intracellular calcium (Fig. 6D). Furthermore, reduction in CD 133 positive cells with UM 673 treatment could be partially abrogated by the calcium scavenging compound BAPTA (Fig. 6E). As program cell necrosis is known to be activated via the RIP kinases, the impact of the RIP kinase inhibitor necrostatin was evaluated. Necrostatin treatment could only partially abrogate the effects of UM673, indicating that UM673 may be inducing necrosis in part in a RIP kinase independent manner.

UM673 and retinoic acid signal transduction and metabolism.

ALDH1A isoforms are the primary members of the 'retinaldehyde' subgroup of aldehyde dehydrogenases. Retinaldehydes play a primary role in the biosynthesis of retinoic acid and thereby regulate retinoic acid mediated transcription. Retinoic acid transcription changes in

cells treated with UM673 were assayed. It was observed that numerous important stem cell factors including Oct4, and Sox2 were down regulated upon treatment with UM673 (Fig. 7A). 80x induction of the mitochondrial uncoupling proteins UCP1 and UCP3 was observed (Fig. 7B).

5 UCP1 and UCP3 proteins play a critical role in cellular metabolism. The impact of UM673 on cellular metabolism was evaluated. SeaHorse studies demonstrated that UM673 treatment was associated with a slow and steady decline in cellular oxygen consumption. Treatment with oligomycin, which promotes glycolysis, was associated with an appropriate increase in extracellular acidification, indicating UM673 is not primarily impacting
10 glycolysis. In contrast the addition of two deoxy glucose have minimal further reductions in oxygen consumption, indicating UM673 act primarily at the mitochondria.

To directly assess cellular metabolism of the mitochondria, LCMS was performed in control and UM673 treated cells. Consistent with reduced mitochondrial metabolism, a decrease in the ratio of ATP:ADP and ATP:AMP was observed. No significant change in the
15 ratio of NADH:NAD was observed, indicating an early blockage in the Krebs cycle. A marked decrease in the levels of citrate with UM673 treatment and a decrease in downstream molecules including a decrease in succinate and malate was observed.

Finally, to determine if UM673 induction of UCP1 or UCP3 resulted in the observed metabolic defects and program cell necrosis, UCP1 was overexpressed into several ovarian
20 cancer cell lines.

The present example describes the identification of a novel ALDH1A1 specific inhibitor which selectively induces necrotic cell death of ovarian cancer stemlike cells. This compound demonstrates minimal toxicity towards normal cells in vitro, and no toxicity in vivo. As a single agent this compound has modest antitumor activity. However this
25 compound is highly synergistic with chemotherapy and rude versus chemotherapy resistance in vivo.

siRNA knockdown of ALDH1A1 in ovarian cancer, breast cancer, and lung cancer all resulted in restoration of chemo sensitivity. The results are highly consistent with the studies with ALDH1A1 inhibitor therapy being highly synergistic with chemotherapy and
30 reversing chemotherapy resistance in vivo in primary human tumor explants. The present invention is not limited to a particular mechanism. Indeed, an understanding of the mechanism is not necessary to practice the present invention. Nonetheless, the results suggest

synergy with chemotherapy may be more than just reduction in chemotherapy cellular metabolism. Based upon the single agent activity of the compounds to eliminate cells with cancer stemlike activity synergy is likely also in part due to the elimination of these inherently chemo resistant cells.

5 Disulfiram is a potent LDH inhibitor however it has broad ALDH isoform targeting capacity, with primary activity targeting ALDH2. While disulfiram demonstrates some modest ability to deplete CD133+ cells, it did not eliminate tumor initiation capacity alone or in combination with chemotherapy. The present invention is not limited to a particular mechanism. Indeed, an understanding of the mechanism is not necessary to practice the
10 present invention. Nonetheless, it is contemplated that ?UM673 demonstrates superiority to disulfiram due to specific enzyme targeting. Drugs such as disulfiram and daidizin, with broad ALDH targeting activity can lead to the induction of expression of ALDH isoforms. As such this may overcome the drug. In contrast with UM 673, ALDH isoform induction was not observed.

15 Many anticancer therapies work via the induction of apoptosis also known as programmed cell death. However, cancer cells and cancer stem cells have evolved numerous mechanisms to escape the induction of apoptosis. The induction of program cell necrosis may be a means to overcome cancer cell resistance to apoptosis. In fact, cells which cannot apoptosis may undergo program cell necrosis as a 'backup' mechanism to undergo cell death.
20 As such it is been proposed that the induction of program cell necrosis could be a very potent means to enhance cancer therapy. The data described herein indicate that UM 673 is inducing program cell necrosis of cancer cells.

In conclusion, the present example describes small molecules that selectively inhibit ALDH1A1. This compound selectively depletes cells expressing ovarian cancer stem cell
25 markers. This compound reduces O-ring cancer stem cell functional activity in both cell lines and primary human ovarian cancer samples. This compound is highly synergistic chemotherapy and leads to regression of chemo refractory primary human tumor explants.

Table 1

	673A	673B	DEAB	B13
	<u>IC₅₀</u>	<u>IC₅₀</u>	<u>IC₅₀</u>	<u>IC₅₀</u>
ALDH1A1 (WT)	216 ± 22 nM	450 ± 35 nM	519 ± 75 nM (2 min incub)	110 ± 15 nM
ALDH1A1 (S121)	230 ± 10 nM	1240 ± 118 nM	1490 ± 240 nM	>20 µM
ALDH1A2	211 ± 36 nM	800 ± 147 nM	>20 µM	>20 µM
ALDH1A3	170 ± 28 nM (2 min incub)	1340 ± 480 nM (2 min incub)	>20 µM	400 nM
ALDH2	1680 ± 190 nM	>20 µM	>20 µM	>20 µM
ALDH3A1	>20 µM	>20 µM	>20 µM	>20 µM

Table 2: Impact of UM673 on the tumor initiation capacity of in vitro treated, PI-/Annexin-FACS isolated live cells.

In Vitro Treatment Groups				
No. of cells injected	Control	Cisplatin	UM673	Cisplatin + UM673
200	ND	1/4	2/4	0/4
1000	ND	4/4	4/4	1/4
5000	4/4	8/8	4/5	6/10

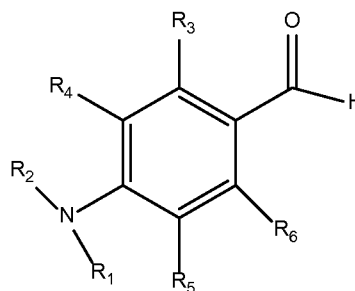
ND: Not Done.

All publications and patents mentioned in the above specification are herein incorporated by reference. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in the relevant fields are intended to be within the scope of the following claims.

CLAIMS

We claim:

- 5 1. A composition comprising a compound that inhibits aldehyde dehydrogenase

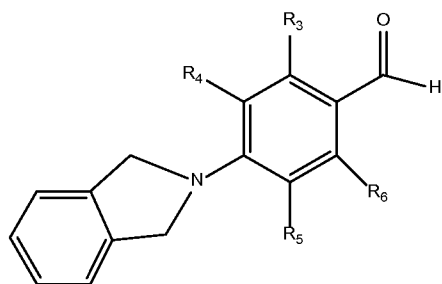


(ALDH), wherein said compound has the structure , wherein R1

and R2 are independently or together selected from the group consisting of H, an alkyl, a cycloalkyl, together form a cycloalkyl or heterocyclic alkyl or aryl, an aryl, an alkenyl, a cycloalkyl, alkynyl, and a substituted version of the aforementioned groups; and R3-R6 are

- 10 independently selected from the group consisting of a halogen, H, an alkyl, a cycloalkyl, an aryl, an alkenyl, a cycloalkyl, alkynyl, and a substituted version of the aforementioned groups.

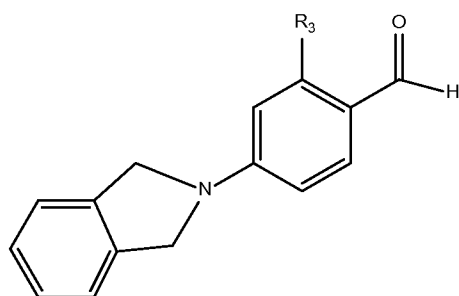
2. The composition of claim 1, wherein said compound has the structure



, wherein are independently selected from the group

- 15 consisting of a halogen, H, an alkyl, a cycloalkyl, an aryl, an alkenyl, a cycloalkyl, alkynyl, and a substituted version of the aforementioned groups.

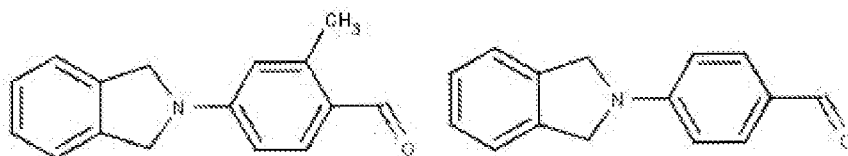
3. The composition of claim 1, wherein said compound has the structure



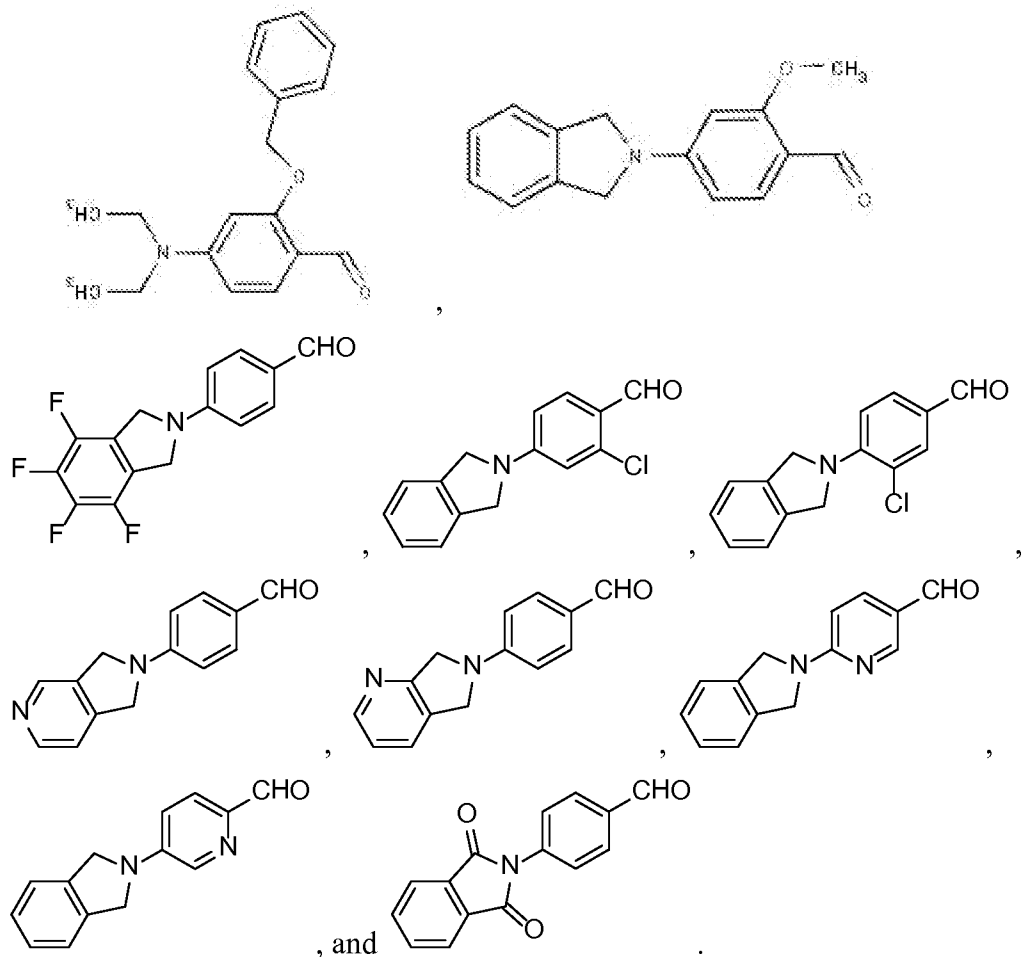
, wherein R3 is selected from the group consisting of a

halogen, H, an alkyl, a cycloalkyl, an aryl, an alkenyl, a cycloalkyl, alkynyl, and a substituted version of the aforementioned groups.

4. The composition of claim 1, wherein said compound is selected from the



5 group consisting of



5. The composition of any one of claims 1 to 4, wherein said composition is a pharmaceutical composition.

6. The composition of claim 5, wherein said composition further comprises a pharmaceutically acceptable carrier.

5 7. The composition of any one of claims 1 to 6, wherein said composition inhibits ALDH1A1.

8. The composition of any one of claims 1 to 6, wherein said composition kills or inhibits the growth of a cancer stem cell.

10 9. The composition of claim 8, wherein said cancer stem cell is an ovarian cancer stem cell.

10. The composition of any one of claims 1 to 9, wherein said composition further comprises a known chemotherapeutic agent.

15

11. The composition of claim 10, wherein said known chemotherapeutic agent is selected from the group consisting of a cancer stem cell inhibitor, a platinum containing compound, and a taxane.

20

12. The use of the composition of any one of claims 1 to 11 in the treatment of cancer.

13. The use of claim 12, wherein the cancer is ovarian cancer.

25

14. A kit comprising a) the composition of any one of claims 1 to 9; and b) a known chemotherapeutic agent.

15. The kit of claim 13, wherein said known chemotherapeutic agent is selected from the group consisting of a cancer stem cell inhibitor, a platinum containing compound, and a taxane.

30

16. A method of inhibiting ALDH in a cell, comprising:
contacting said cell with a composition of any one of claims 1 to 11 under conditions
such that said composition kills or inhibits the growth of said cell.

5 17 The method of claim 16, wherein said cell is in vivo.

18. The method of claim 16, wherein said cell is a cancer cell.

19. The method of claim 18, wherein said cell is a cancer stem cell.

10

20. The method of claim 19, wherein said cancer stem cell is an ovarian cancer
stem cell.

15 21. The method of claim 17, wherein said cell is in a subject diagnosed with
ovarian cancer.

22. A method of treating ovarian cancer, comprising:
administering a compound of any one of claims 1 to 11 to a subject diagnosed with
ovarian cancer under conditions such that said compound kills or inhibits the growth of said
20 ovarian cancer.

23. The method of claim 22, wherein said administering prevents recurrence or
metastasis of said ovarian cancer.

25

FIG. 1

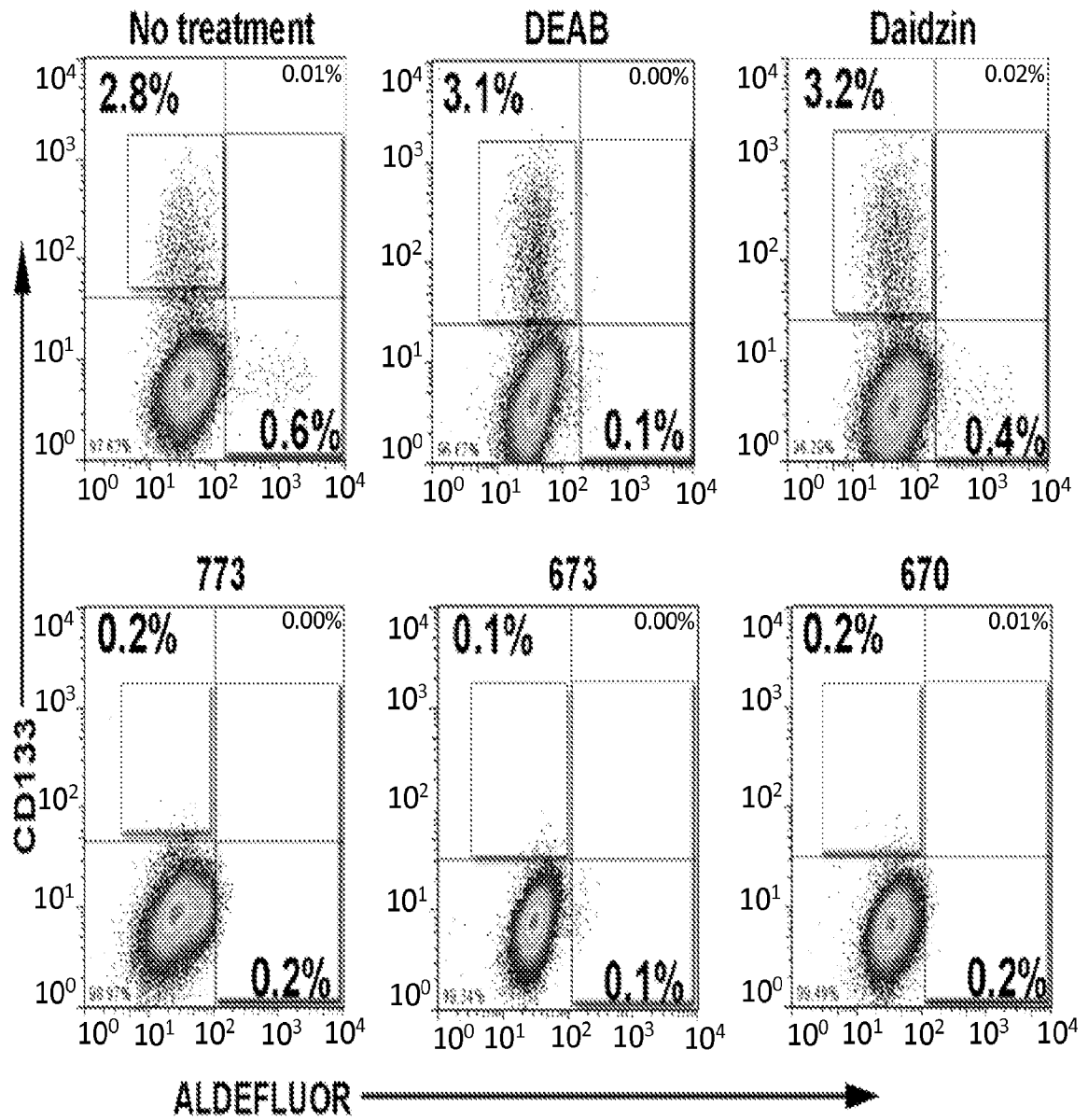
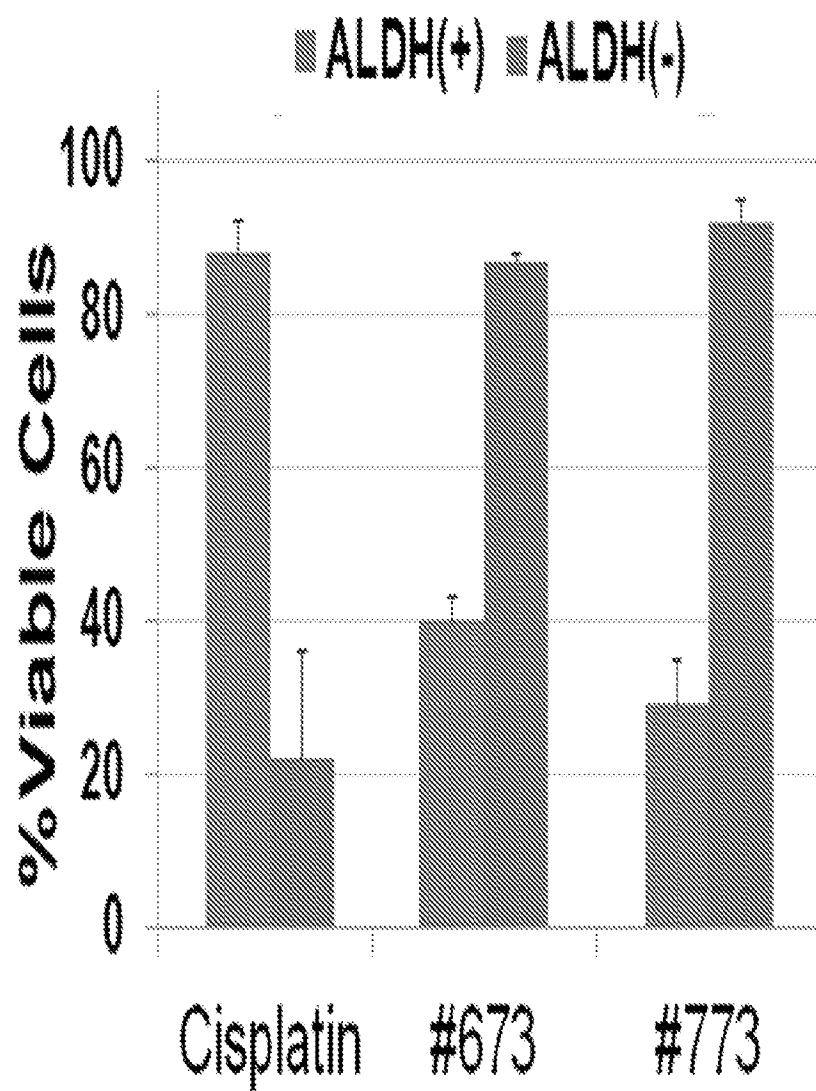


FIG. 1 (cont.)



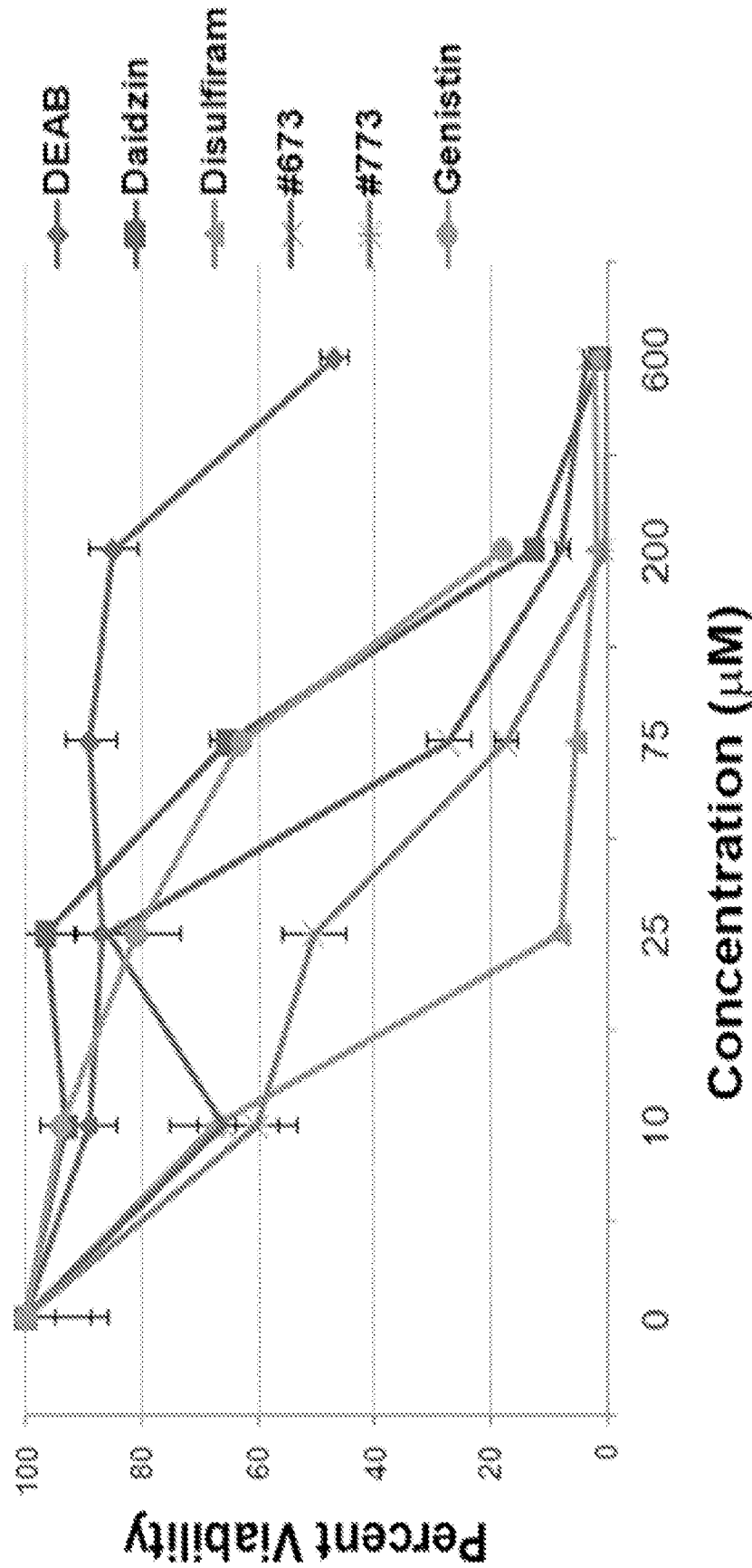


FIG. 2

FIG. 2 (cont.)

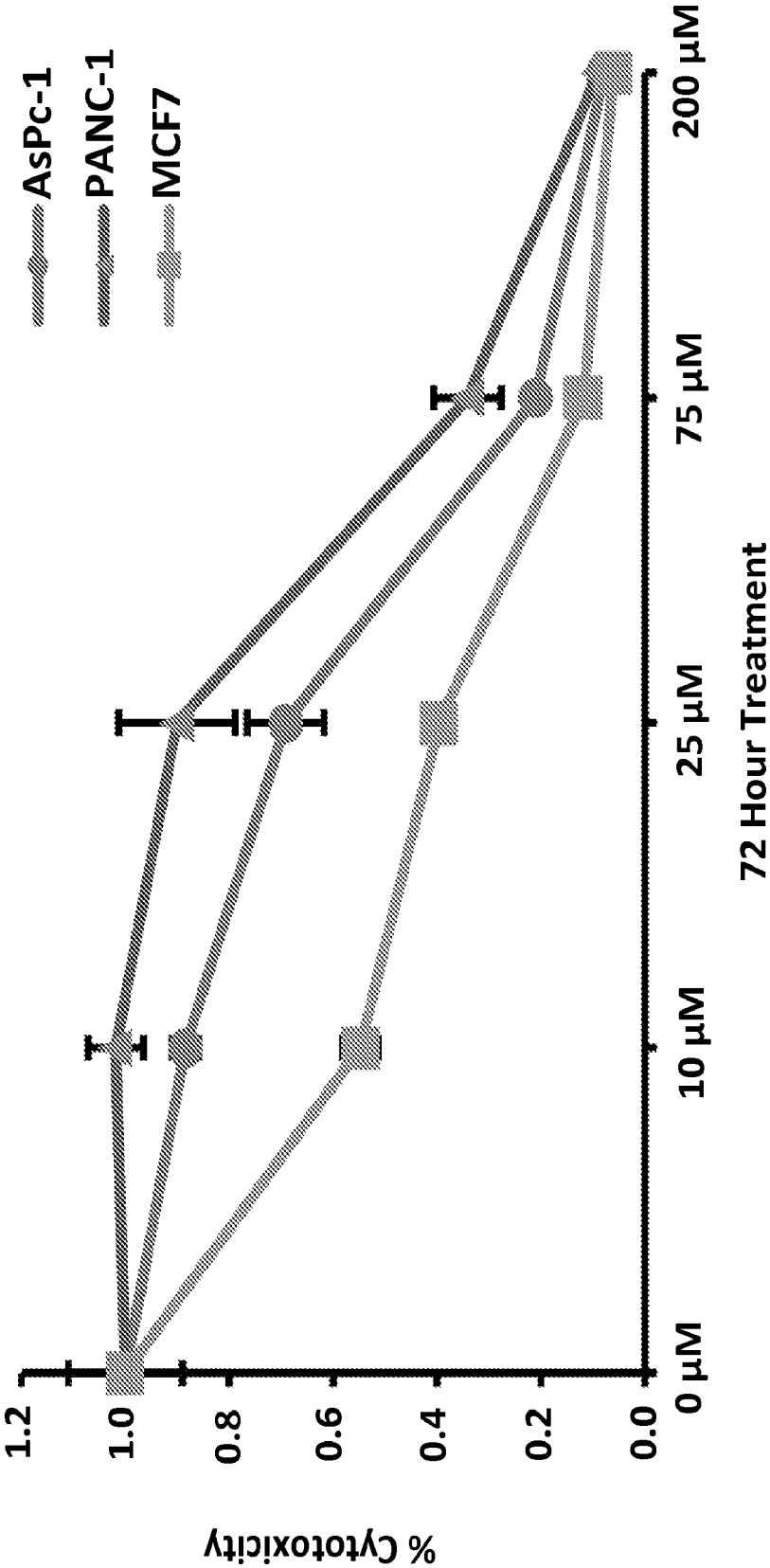


FIG. 2 (cont.)

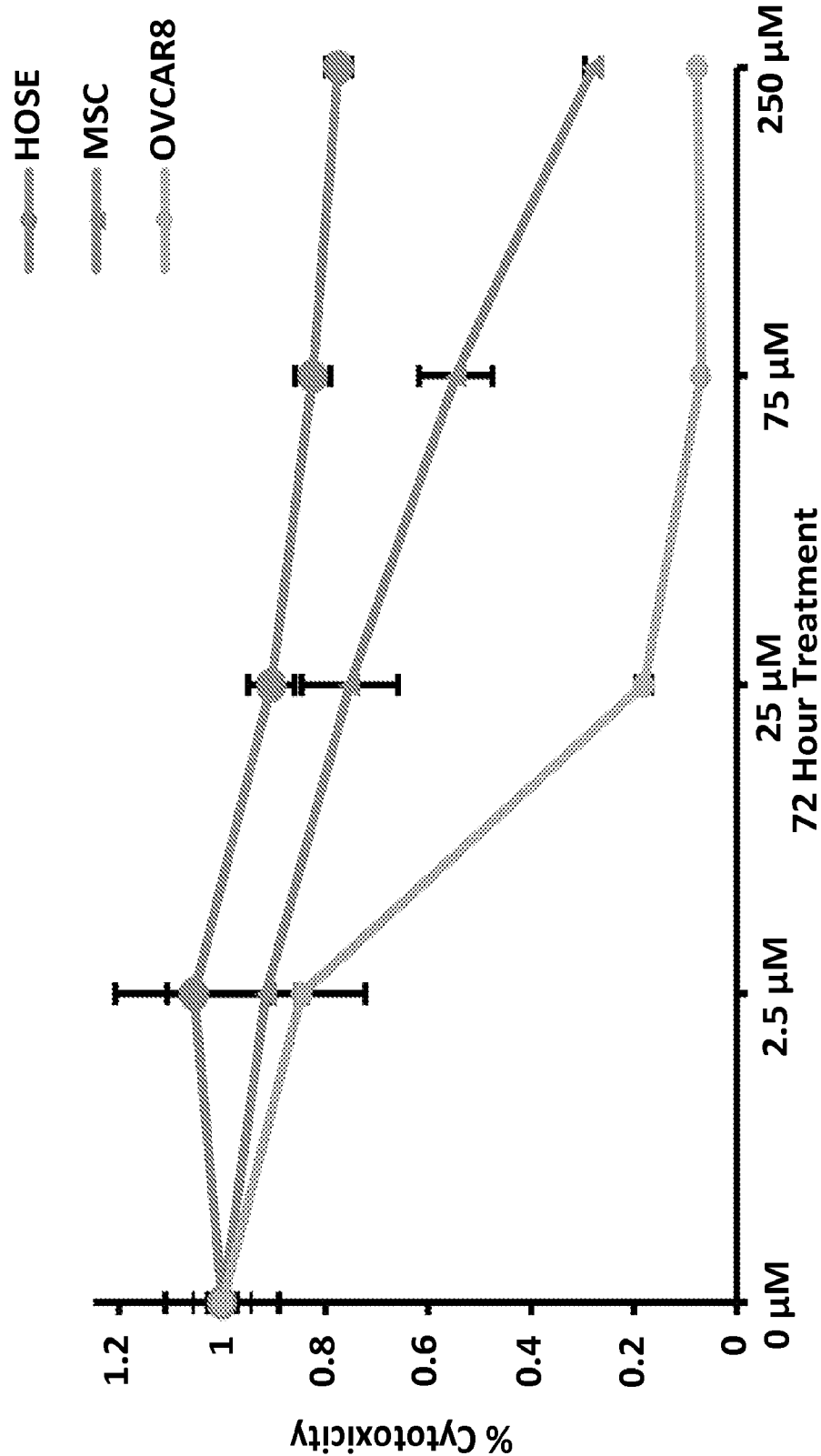


FIG. 3

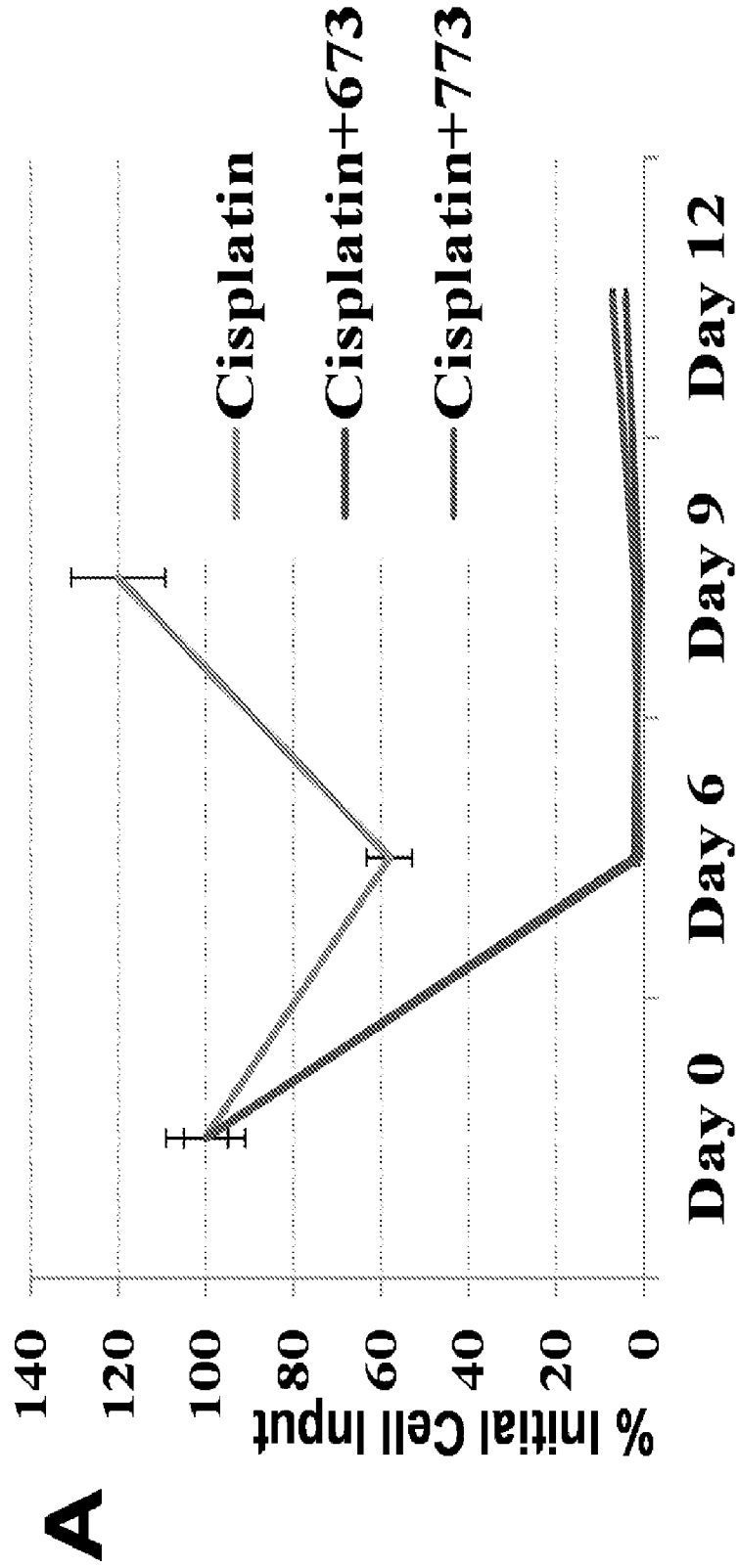


FIG. 3 (cont.)

B

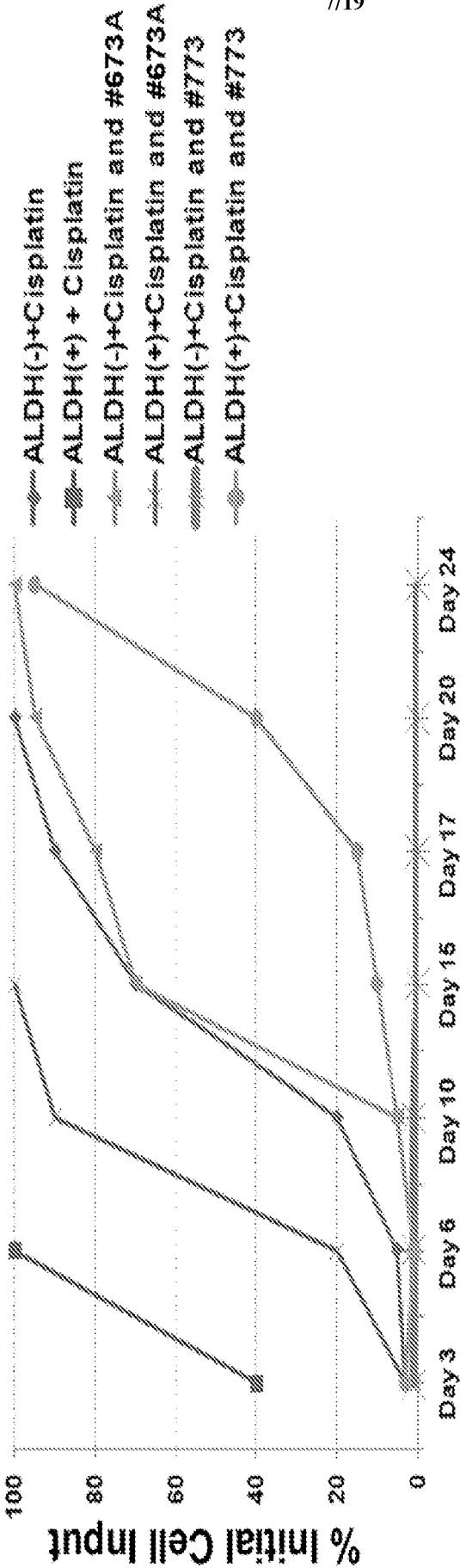
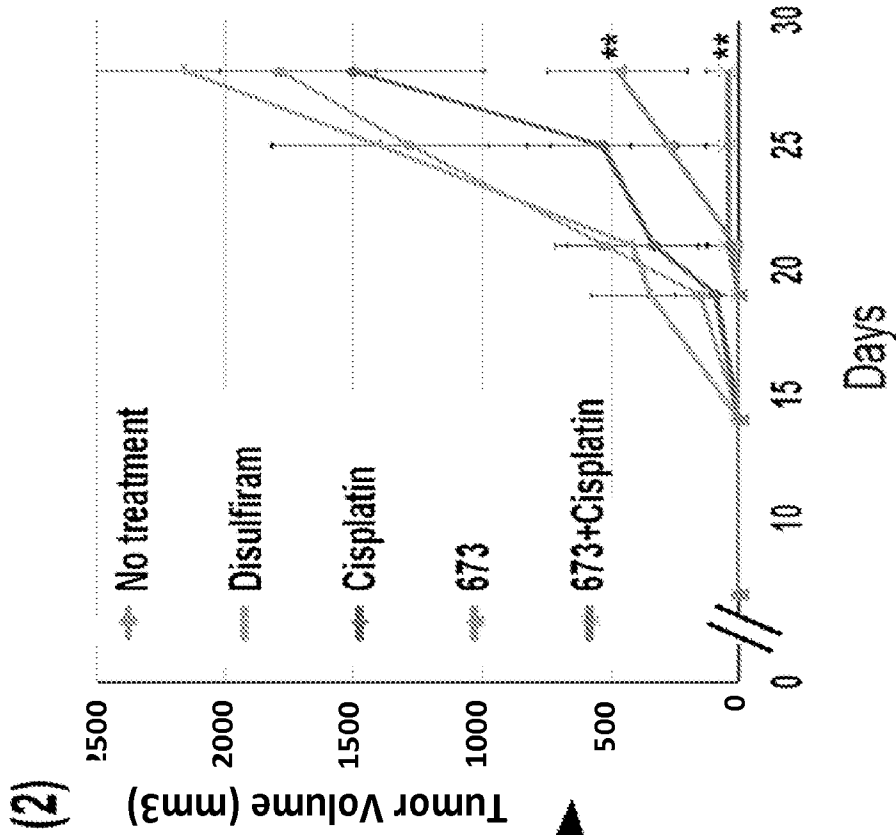
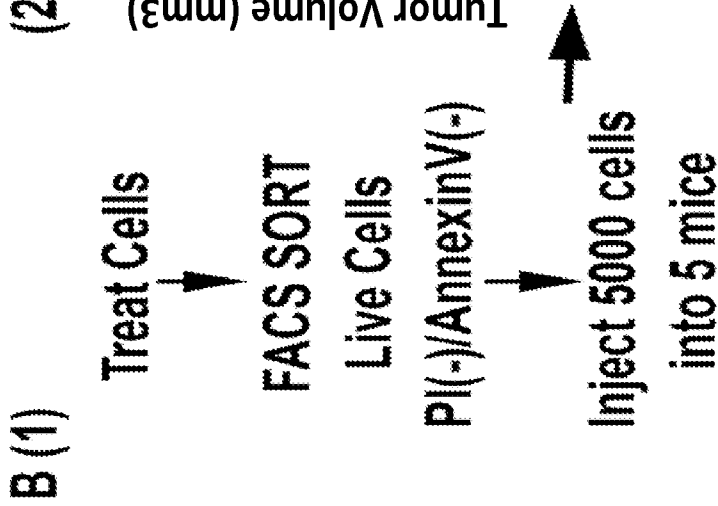
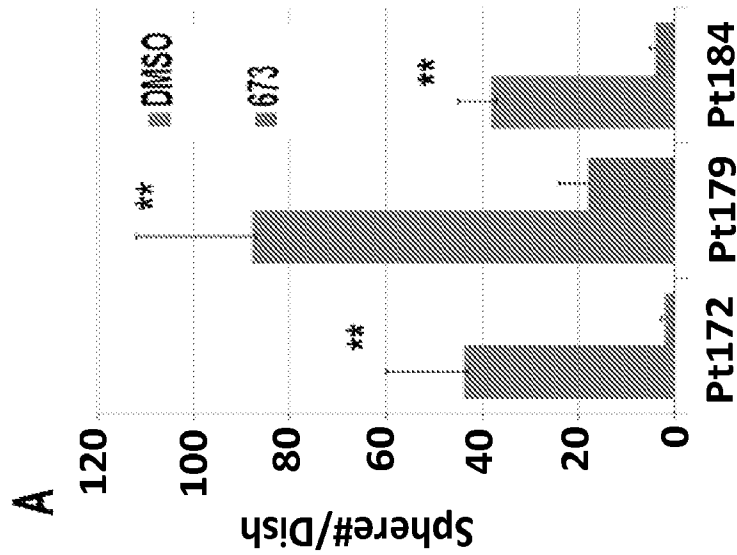
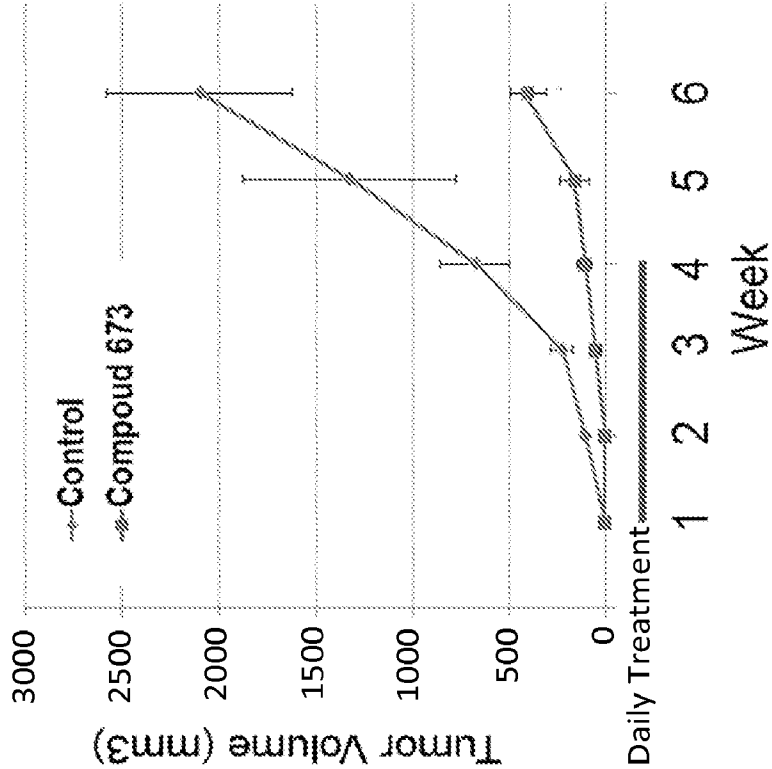


FIG. 4



CD133+ cells



Whole cell line

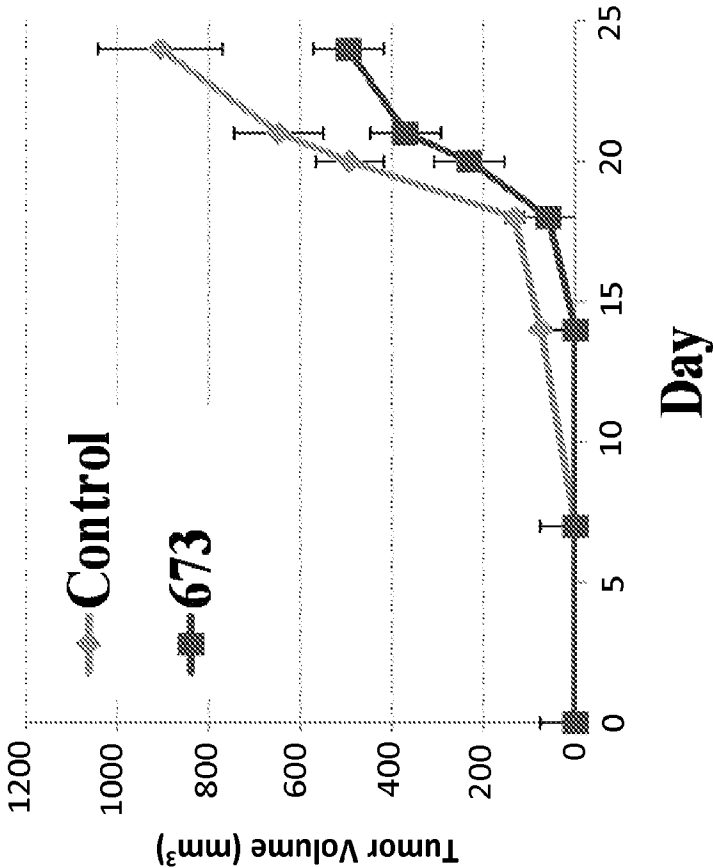


FIG. 5

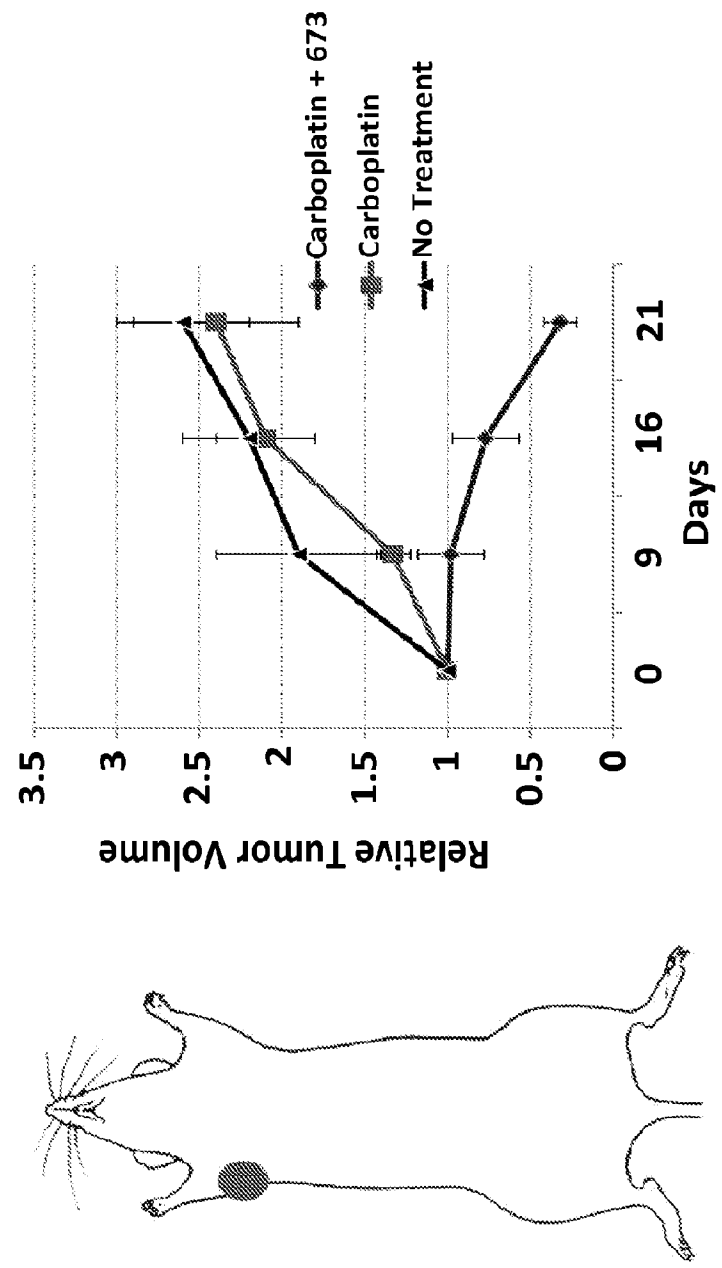


FIG. 5 (cont.)

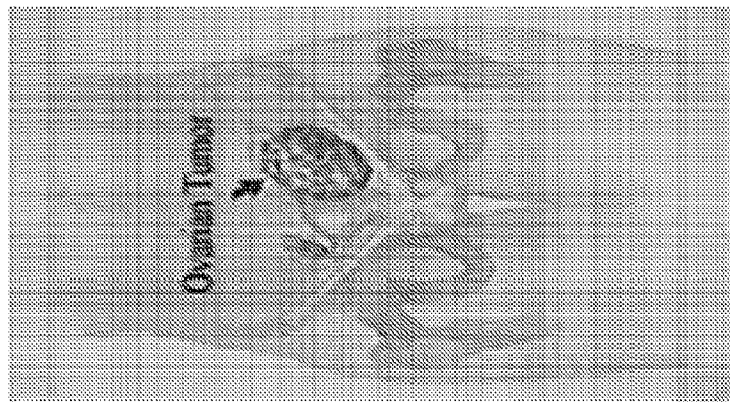


FIG. 6

A

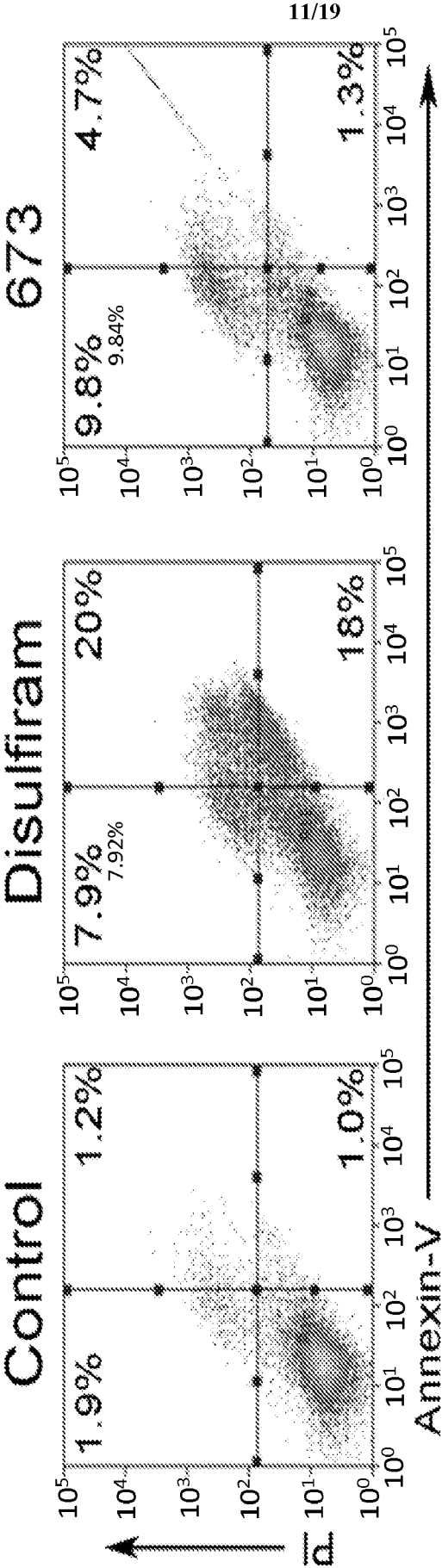
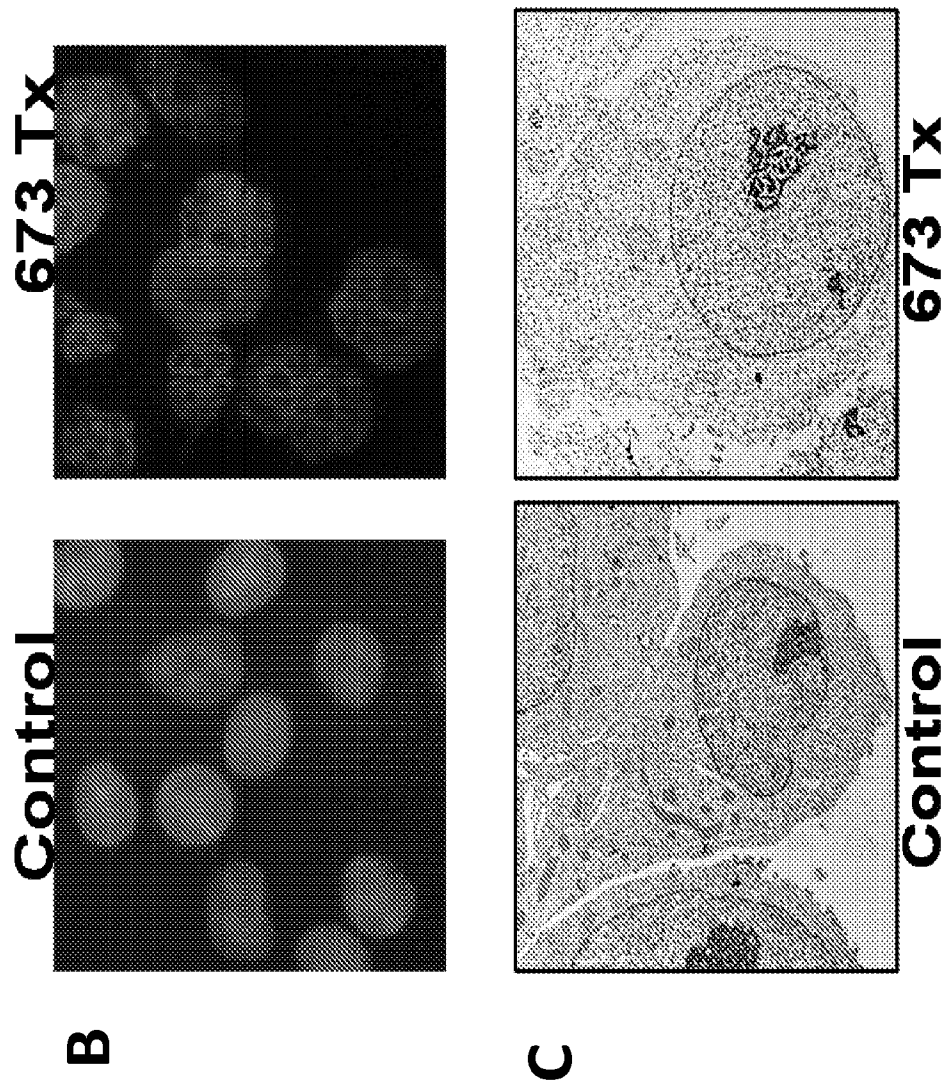


FIG. 6 (cont.)



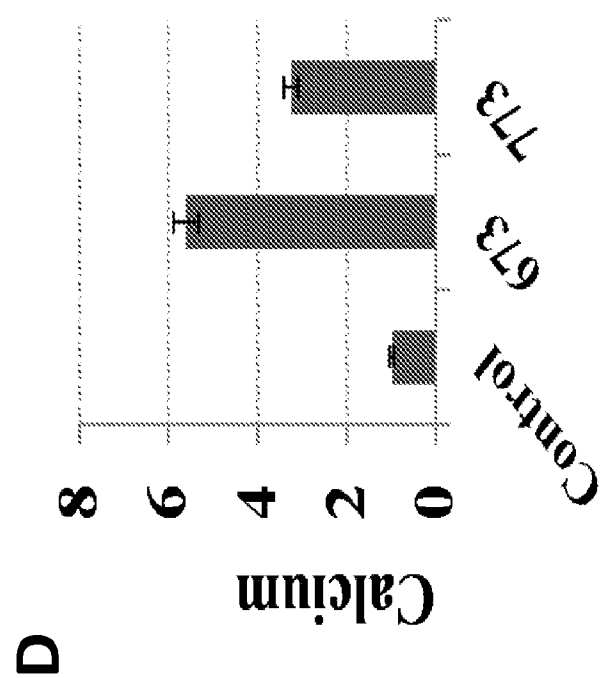
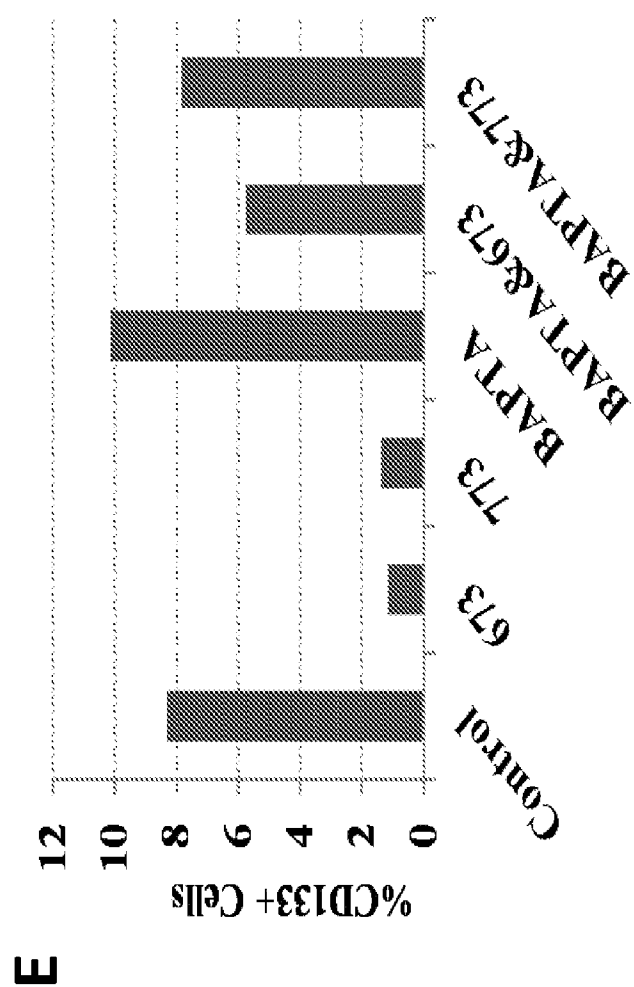


FIG. 6 (cont.)

FIG. 7

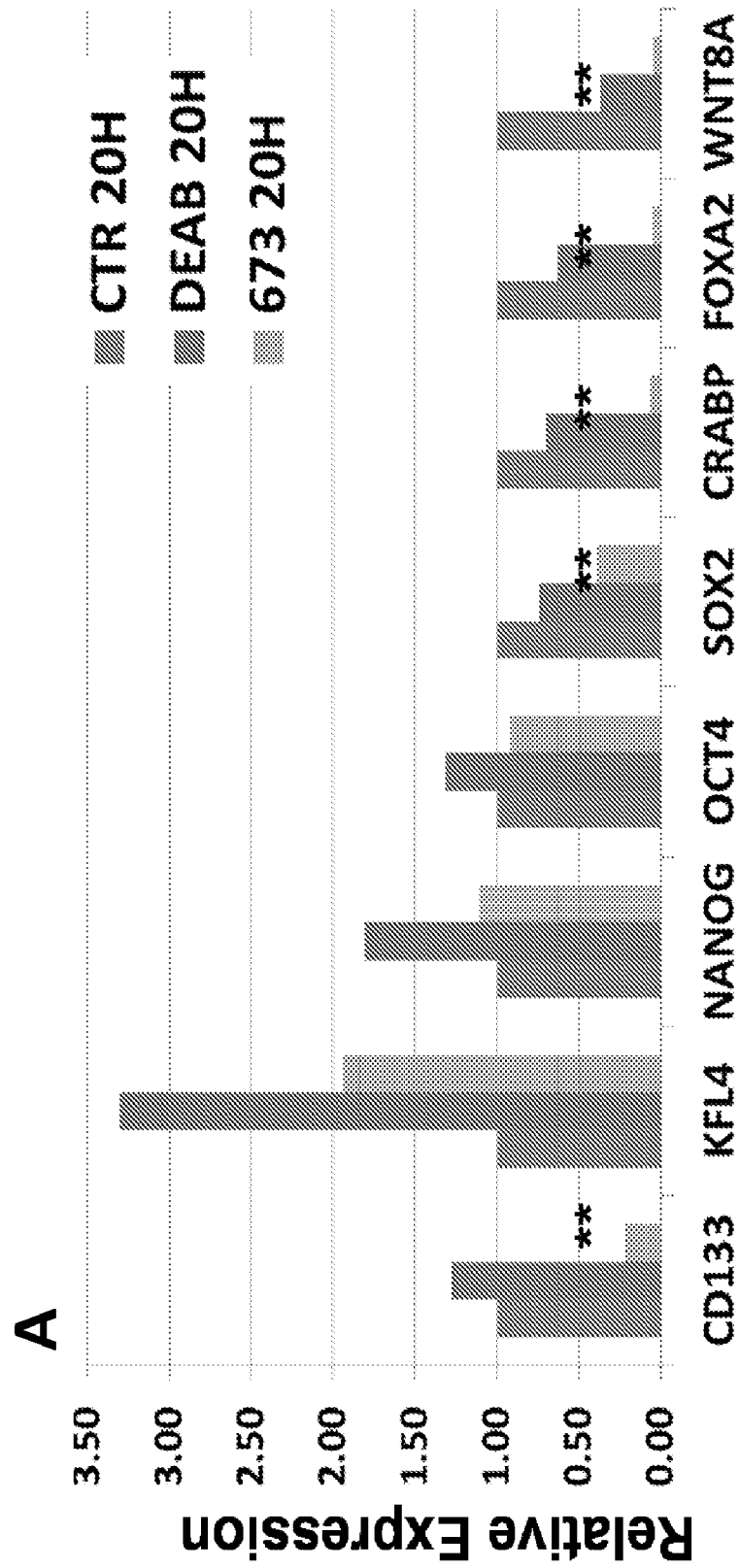


FIG. 7 (cont.)

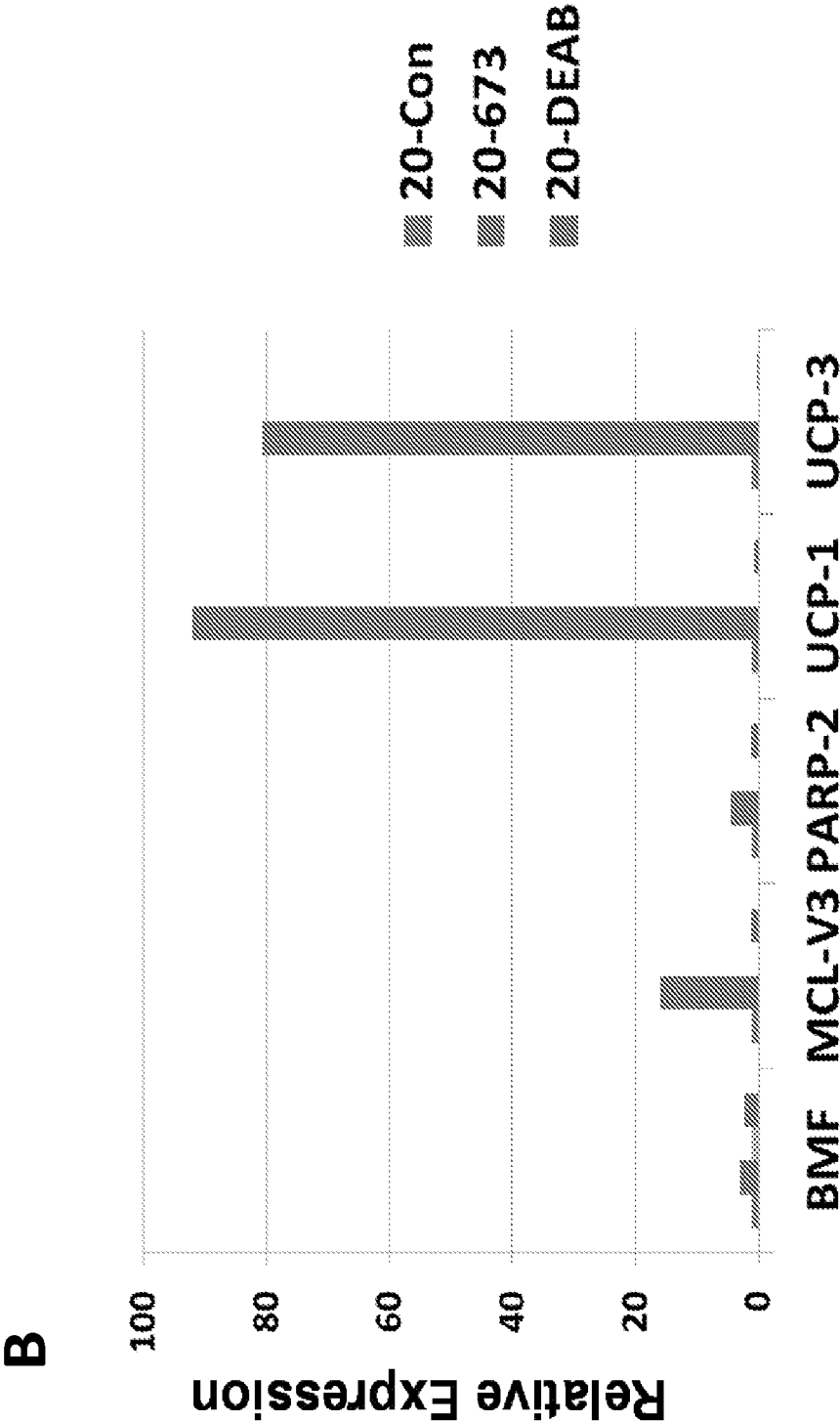


FIG. 8

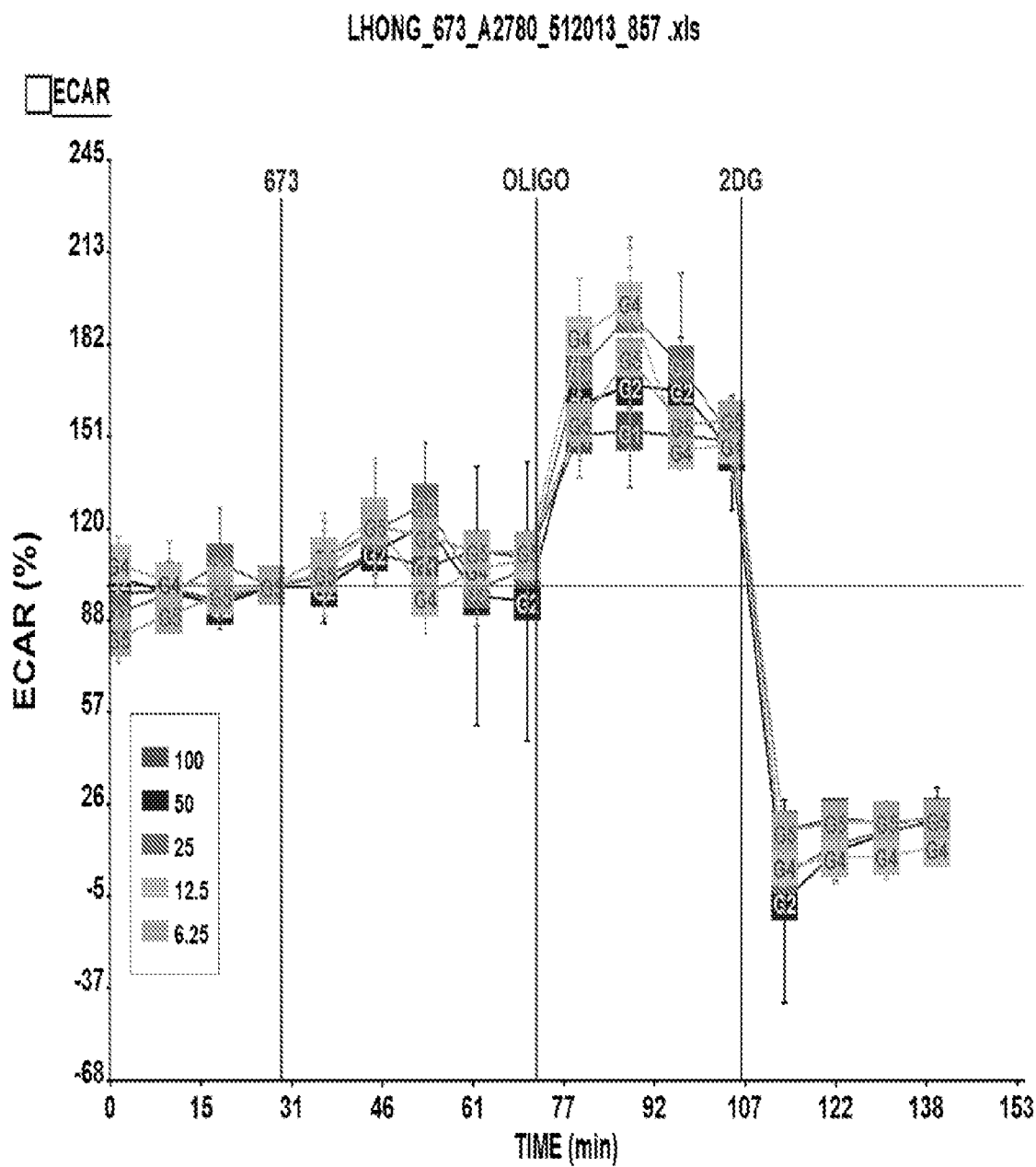


FIG. 8 (cont.)

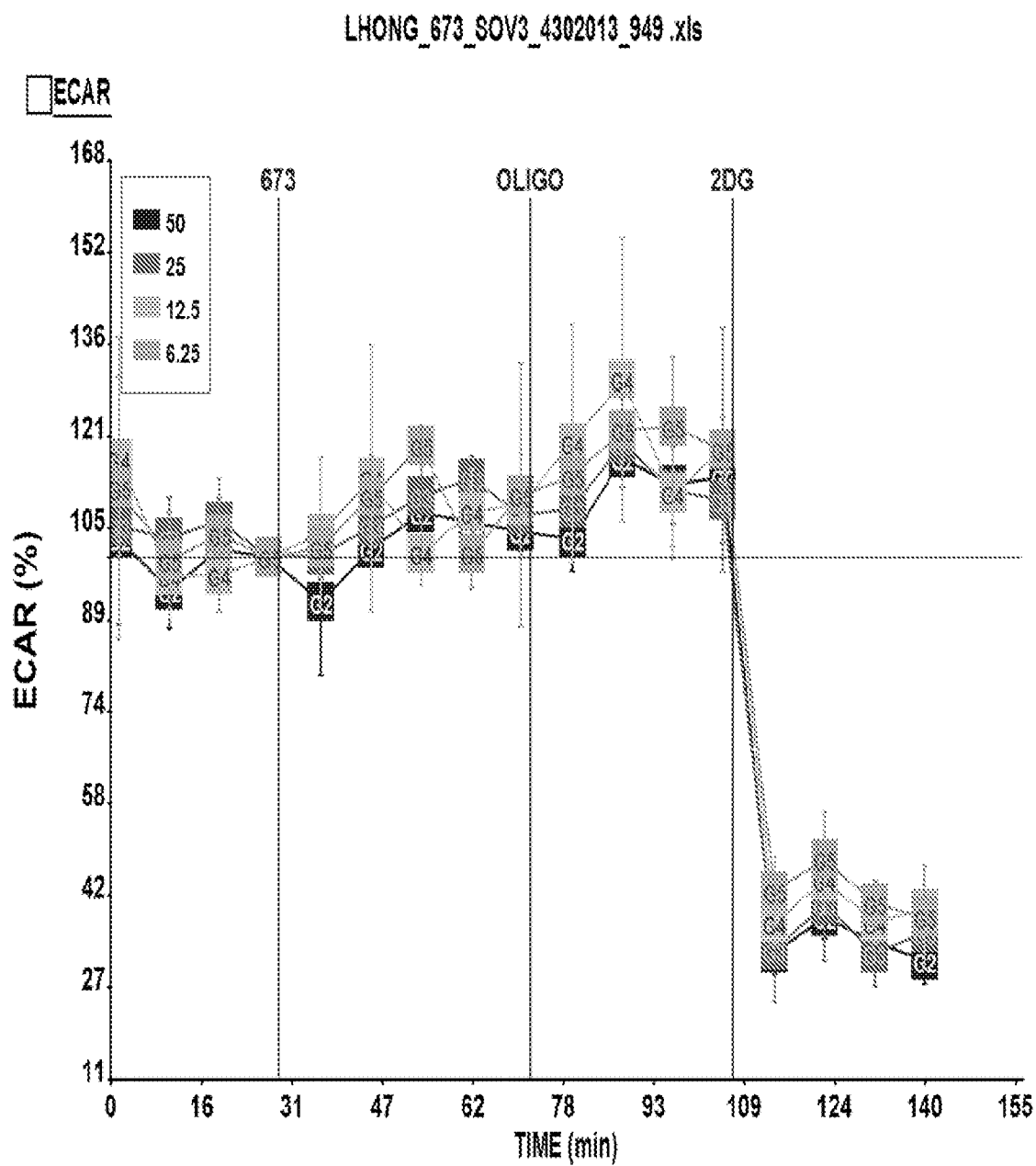


FIG. 8 (cont.)

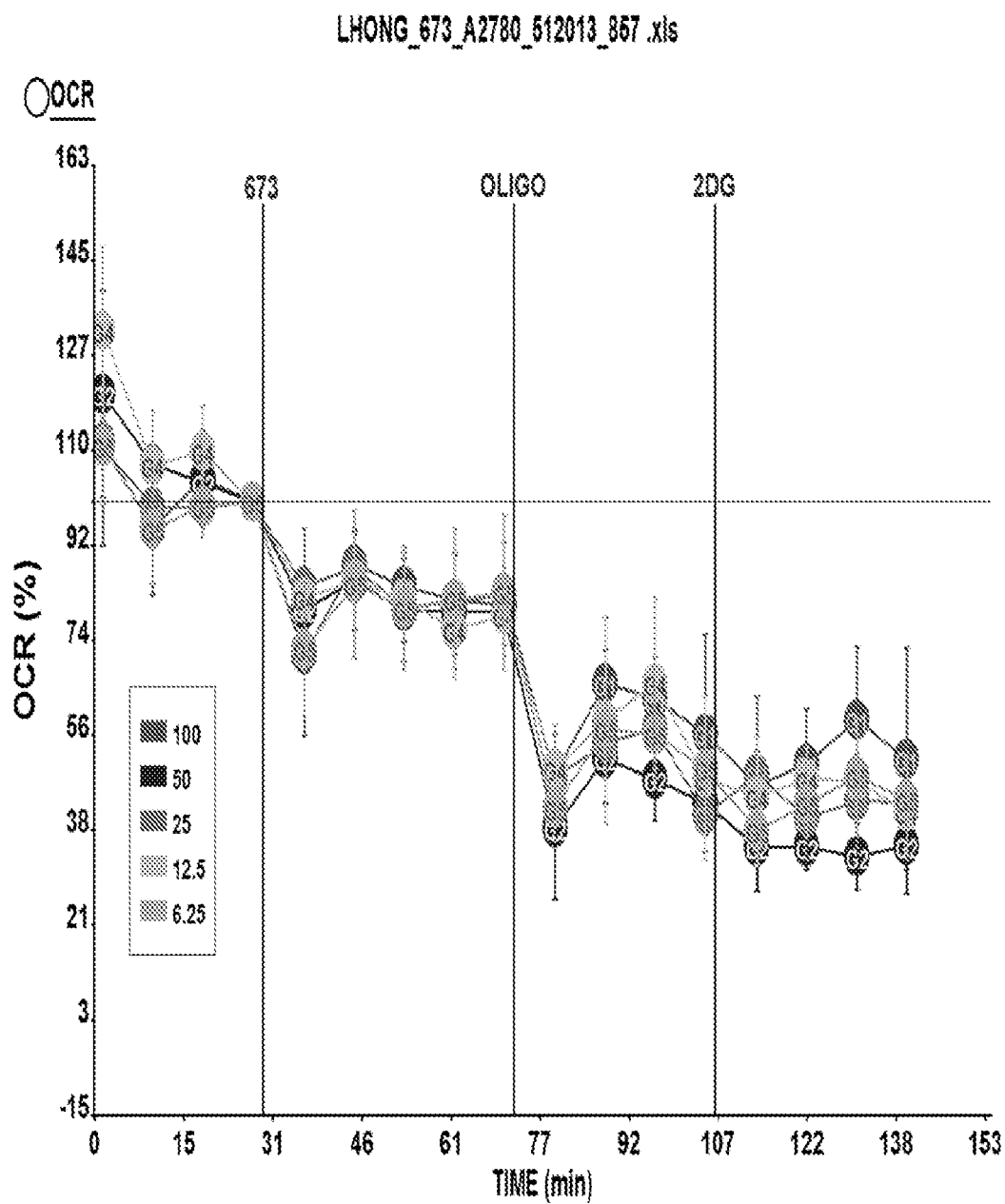
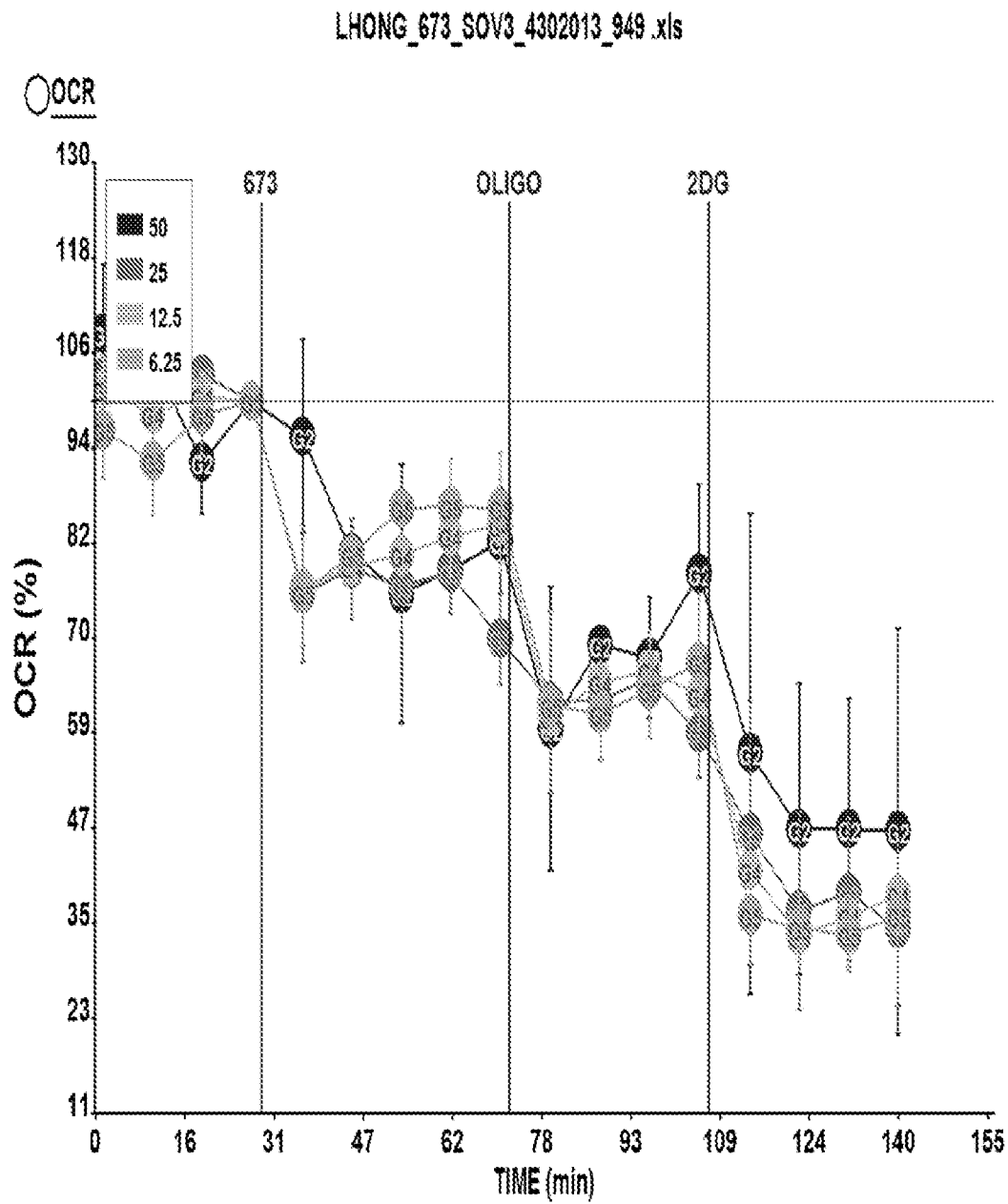


FIG. 8 (cont.)



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/072055**A. CLASSIFICATION OF SUBJECT MATTER****A61K 31/11(2006.01)i, A61K 31/404(2006.01)i, A61P 35/00(2006.01)i**

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K 31/11; C07D 213/38; C07D 403/02; A61K 31/404; A61P 35/00

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal) & Keywords: 4-aminobenzaldehyde, 4-(2-isoinoline)benzaldehyde, cancer, ovarian

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4446321 A (SCHMIDT, P. J. et al.) 01 May 1984	1-4
A	See column 10, example 17.	5,6
X	KUKIDOME, J. et al., `The mechanism of cell death in human cultured colon adenocarcinoma cell line COLO 201 induced by $\beta$ -D-N-acetylglucosaminyl-p-nitrophenol`, The Tohoku Journal of Experimental Medicine, 2001, Vol. 194, pages 23-34.	1,5,6
A	See page 29.	2-4
A	PRESCOTT, B. et al., `Long-chain thiosemicarbazones as potential anticancer and antiviral agents`, Journal of Medicinal Chemistry, 1964, Vol. 7, No. 3, pages 383-385.	1-6
A	See page 383, right-column; and tables 1 and 2.	
A	CORMA, A. et al., `Preparation of substituted anilines from nitro compounds by using supported gold catalysts`, Nature Protocols, 2006, Vol. 1, No. 6, pages 2590-2595.	1-6
A	See page 2591, left-column; and figure 1.	



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

27 February 2014 (27.02.2014)

Date of mailing of the international search report

27 February 2014 (27.02.2014)

Name and mailing address of the ISA/KR

Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City,
302-701, Republic of Korea

Facsimile No. +82-42-472-7140

Authorized officer

CHOI, Sung Hee

Telephone No. +82-42-481-8740



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2013/072055

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2008-137102 A2 (TORREYPINES THERAPEUTICS, INC.) 13 November 2008 See abstract; paragraphs [0013] and [0109]; and claim 1.	1-6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/072055

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

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

1. ☒ Claims Nos.: 16-23
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 16-23 pertain to methods for treatment of the human body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under PCT, to search.
2. ☒ Claims Nos.: 9, 11, 13, 15, 17-21, 23
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claims 9, 11, 13, 15, 17-21 and 23 do not comply with PCT Article 6, since they refer to one of claims which are not drafted in accordance with PCT Rule 6.4(a)./ Claim 15 is unclear, since it is concerned with "a kit", but claim 13, to which claim 15 refers, is directed to "a use" (PCT Article 6).
3. ☒ Claims Nos.: 7, 8, 10, 12, 14, 16, 22
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2013/072055

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 04446321 A	01/05/1984	EP 0061128 A1	29/09/1982
		EP 0061128 B1	04/09/1985
		JP 57-165457 A	12/10/1982
		US 04363503 A	14/12/1982
		US 04396767 A	02/08/1983
		US 04540790 A	10/09/1985
WO 2008-137102 A2	13/11/2008	WO 2008-137102 A3	27/05/2010