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(54) Title: CYCLODEXTRIN COMPOSITIONS ENCAPSULATING ALKYLATING AGENTS AND USES THEREOF

(57) Abstract: The disclosure provides compositions comprising cyclodextrins encapsulating an alkylating agent, as well as kits, methods and uses of the same. Alkylating agents encapsulated by cyclodextrins as described herein include 3-halopyruvates, e.g. 3-bromopyruvate, and other agents that inhibit ATP production, e.g., by inhibiting Monocarboxylate Transporter 1 (MCT-1).

***CYCLODEXTRIN COMPOSITIONS ENCAPSULATING ALKYLATING AGENTS
AND USES THEREOF***

Cross-Reference to Related Applications

This application claims priority to U.S. Provisional Application No. 63/468,187,
5 filed May 22, 2023, the disclosure of which is hereby incorporated by reference in its
entirety.

Government Support

This invention was made with government support under grant CA006973
awarded by the National Institutes of Health. The government has certain rights in the
10 invention.

Background

Pancreatic ductal adenocarcinoma (PDAC) confers a dismal 5-year survival.[1, 2]
Even after aggressive multimodality treatment, a minority of patients achieve long term
survival. The mainstay of treatment remains combinations of Fluoracil, Irinotecan,
15 Oxaliplatin (FOLFIRINOX), or Gemcitabine/nab-Paclitaxel, which can cause severe side
effects and offer only modest improvements in survival [3-5]. Therefore, the development
of new rationally-designed therapeutic agents are critical for improved outcomes in
patients with this horrific disease.

Metabolic reprogramming is one of the hallmarks of PDACs. A major effector of
20 metabolic re-wiring in cancer is the monocarboxylate transporter (MCT) family. Several
studies have highlighted the importance of increased MCT-1 expression in various
cancers.[1, 6-13] These transmembrane proteins mediate the transport of pyruvate,
lactate, short-chain fatty acids, and ketones in and out of cells.[14-16] Though MCT-1 is
an intriguing biological target, a small molecule inhibitor of MCT-1 (AZD3965) was not
25 found to be effective in solid tumors. [17, 18]

Accordingly, there is a great need in the art to identify safer and more effective
chemotherapeutics, suitable for systemic administration.

Summary

The present invention is based, at least in part, on the discoveries that
30 encapsulating selective inhibitors of ATP production, such as 3-halopyruvates (*e.g.*, 3-
BrPA), within cyclodextrins can both a) stabilize the alkylating agent *in vivo* by
protecting the halogen moiety away from aqueous and nucleophilic environments that

would deactivate the compound and b) provide a steady release of the compound necessary to maintain a reasonable half-life of the compound *in vivo*.

In some embodiments, provided herein are compositions comprising a cyclodextrin and an alkylating agent represented in the general formula:

- 5
$$\text{XH}_2\text{C}-\overset{\text{O}}{\overset{\parallel}{\text{C}}}-\overset{\text{O}}{\overset{\parallel}{\text{C}}}-\text{R}_1$$
 wherein, independently of each occurrence: X represents a halide, a sulfonate, a carboxylate, an alkoxide, or an amine oxide; R_1 represents OR, H, $\text{N}(\text{R}'')$, C1-C6 alkyl, C6-C12 aryl, C1-C6 heteroalkyl, or C6-C12 heteroaryl; R'' represents H, C1-C6 alkyl, or C6-C12 aryl; R represents H, alkali metal, C1-C6 alkyl, C6-C12 aryl or $\text{C}(\text{O})\text{R}'$; and R' represents H, C1-C20 alkyl or C6-C12 aryl, wherein the cyclodextrin
- 10 encapsulates the alkylating agent. In some embodiments, at least one α -D-glucopyranoside unit of the cyclodextrin has at least one hydroxyl chemical group replaced with an ionizable chemical group. In some embodiments, the at least one hydroxyl chemical group of the at least one α -D-glucopyranoside unit is selected from the group consisting of C2, C3, and C6 hydroxyl chemical groups. In some embodiments,
- 15 the C2, C3, and C6 hydroxyl chemical groups of at least one α -D-glucopyranoside unit of the cyclodextrin that are replaced with ionizable chemical groups. In some embodiments, the at least one α -D-glucopyranoside unit of the cyclodextrin is selected from the group consisting of two, three, four, five, six, seven, eight, and all α -D-glucopyranoside units of the cyclodextrin. In some embodiments, the ionizable chemical group is the same at all
- 20 replaced positions. In some embodiments, the ionizable chemical group is a weakly basic functional group or a weakly acidic functional group. For example, the weakly basic functional group (X) can have a pK_a between 6.5 and 8.5 according to $\text{CH}_3\text{-X}^-$ or the weakly acidic functional group (Y) can have a pK_a between 4.0 and 6.5 according to $\text{CH}_3\text{-Y}$. In some embodiments, the weakly basic or weakly acidic functional groups are
- 25 selected from the group consisting of amino, ethylene diamino, dimethyl ethylene diamino, dimethyl anilino, dimethyl naphthylamino, succinyl, carboxyl, sulfonyl, and sulphate functional groups. In some embodiments, the cyclodextrin has a pK_{a1} of between 4.0 and 8.5. In some embodiments, the composition is a liquid or solid pharmaceutical formulation. In some embodiments, the alkylating agent is neutrally
- 30 charged or hydrophobic. In some embodiments, the cyclodextrin is selected from the group consisting of β -cyclodextrin, α -cyclodextrin, and γ -cyclodextrin. In some embodiments, the cyclodextrin is β -cyclodextrin. In some embodiments, the alkylating

agent is 3-halopyruvate. In some embodiments, the alkylating agent is 3-bromopyruvate. In some embodiments, the composition is formulated for systemic administration. In some embodiments, the composition further comprises an anti-cancer therapeutic agent.

5 In some embodiments, provided herein are kits comprising a composition described herein and instructions for use.

In some embodiments, provided herein are methods of treating a subject having a cancer comprising administering to the subject a therapeutically effective amount of a composition described herein. In some embodiments, the composition is administered systemically. In some embodiments, the systemic administration is selected from the
10 group consisting of oral, intravenous, intraperitoneal, subcutaneous, and intramuscular administration. In some embodiments, the subject is treated with at least one additional anti-cancer therapy. In some embodiments, the at least one additional anti-cancer therapy is radiation therapy. In some embodiments, the cancer is a solid tumor. In some embodiments, the cancer is selected from the group consisting of liver cancer, pancreatic
15 cancer, lung cancer and breast cancer. In some embodiments, the cancer is liver cancer. In some embodiments, the subject is a mammal. In some embodiments, the mammal is a human.

Brief Description of Figures

20 The Figures included herein are for illustration purposes only and not for limitation.

FIG. 1A – FIG. 1C show sensitivity of pancreatic ductal adenocarcinoma cell lines to 3BP. FIG. 1A shows response of six different PDAC cell lines to 3-BP. The indicated cell lines were exposed to increasing doses of 3BP for 72 h and evaluated by
25 SYBR green growth assay. Data indicates mean +/- SD of three technical replicates and is normalized to untreated controls. FIG. 1B shows expression levels of MCT-1, MCT-4 and GLUT-1 (TPM) and corresponding IC50s of 3BP. FIG. 1C shows immunohistochemistry of PDAC cell lines performed with monoclonal mouse antibody against MCT-1 (1:2000 dilution).

30 **FIG. 2A – FIG. 2G** depict that MCT-1 is essential for 3BP activity. FIG. 2A shows a strategy for Knockout of *SLC16A1* in MiaPaCa-2 cells. FIG. 2B provides a table of knockout (KO) clones. FIG. 2C shows immunohistochemistry of representative KO clones performed with monoclonal mouse antibody against MCT-1 (1:2000 dilution).

FIG. 2D shows IHC of mixed monoclonal MCT-1 KO cells used in subsequent assessments of MCT-1 specific activity of 3BP and ME3BP-7. FIG. 2E and FIG. 2F show comparisons of cell growth over time of MiaPaCa-2 and MiaPaCa-2 MCT1-KO in absence and presence of 3BP (50 μ M), respectively, each normalized to time point 0h.

- 5 Data indicates mean $\pm$ SD of two technical replicates. FIG. 2G shows dose-response curves of MiaPaCa-2 cells and MiaPaCa-2 MCT1-KO at 36 h. Cell viability normalized to the number of cells at 0h. Data indicates mean $\pm$ SD of two technical replicates.

FIG. 3A – FIG. 3C show evaluation of new formulations and serum stability.

- FIG. 3A shows HPLC: Evaluation of different microencapsulated β -cyclodextrin complexes using size-exclusion chromatography (SEC). The agents examined were: i. 3BP (1 mg/mL), ii. succinyl- β -CD (20 mg/mL), iii. a mixture of 10 μ L of 3BP and 10 μ L succinyl- β -CD, and iv ME3BP-7 (10 mg/mL). Samples were monitored at 220 nm. Fig. 3B shows serum stability assay using DLD-1 cells. FIG. 3C shows ME3BP-7 specificity on MIA PaCa-2 parental and MIA PaCa-2 MCT-1 KO cells.

- 15 **FIG. 4** shows comparisons of MCT-1 specific cytotoxicity of 3BP, ME3BP-7 and current standard of care agents for PDAC upon short exposures. Viability of MCT-1 isogenic panel after (A) drug exposure for 30 minutes at 200 μ M; (B) drug exposure for 2 hours at 200 μ M; (C) drug exposure for 30 minutes at 100 μ M; and (D) drug exposure for 2 hours at 100 μ M.

- 20 **FIG. 5** shows linear scatter plots comparing the growth (or death) of MiaPaCa-2 parental after short exposure to 3BP, ME3BP-7 and current standard PDAC chemotherapeutic agents after (A) drug exposure for 30 minutes at 200 μ M; (B) drug exposure for 2 hours at 200 μ M; (C) drug exposure for 30 minutes at 100 μ M; and (D) drug exposure for 2 hours at 100 μ M.

- 25 **FIG. 6A – FIG. 6D** show ME3BP-7 halts tumor growth in orthotopically implanted pancreatic cancer cell line (Panc 02.13) with high MCT-1 expression. FIG. 6A shows an example timeline and design of *in vivo* tumor experiments. FIG. 6B shows bioluminescence images of nude mice bearing orthotopic Panc 02.13 tumors. FIG. 6C shows mean fold change in radiance from day of treatment initiation (** $P < 0.01$, *** $P < 0.001$ 1 way ANOVA. FIG. 6D shows weights of residual tumors harvested at end of therapy (** $P < 0.01$ Mann-Whitney U test).

FIG. 7A – FIG. 7D show ME3BP-7 reduces tumor burden in orthotopically implanted human patient derived xenograft with diffuse expression of MCT-1

(TM01212). FIG. 7A shows an example timeline and design of *in vivo* therapeutic study. FIG. 7B shows IHC of orthotopic PDx (TM01212) showing diffuse but uniform expression of MCT-1. 7C shows representative ultrasound image of orthotopically implanted tumors. FIG. 7D shows mean fold change in tumor volume (n=10) from day of treatment initiation. FIG. 7E shows weights of residual tumors harvested at end of therapy. FIG. 7F and FIG. 7G show H&E of Lung and Liver, respectively, of untreated animals with clearly visible metastases. FIG. 7 H shows metastatic lesions harvested from control vs treated mice at end of therapy. (** P < 0.01 Mann-Whitney U test).

FIG. 8A – FIG. 8E show ME3BP-7 reduces tumor burden in orthotopically implanted human patient derived xenografts from a PDAC metastatic site with focally expressed MCT-1 (TM01098). FIG. 8A shows an example timeline and design of *in vivo* therapeutic study. FIG. 8B shows IHC sections of orthotopic PDx (TM01098) showing focal expression of MCT-1. FIG. 8C shows representative Ultrasound image of orthotopically implanted tumors. FIG. 8D shows mean tumor volumes of 7 and 10 NSG mice in each group as determined by ultrasound measurements on indicated days. FIG. 8E shows weights of residual tumors harvested at end of therapy. (** P < 0.01 Mann-Whitney U test).

FIG. 9 shows representative tumor microarray with IHC. (A) Overview (1x) of TMA (HPanA150CS03, BioMax U.S) exemplifying IHC immunostaining in a random set of pancreatic carcinoma cases. (B) IHC of normal pancreas (10x) from the same microarray. (C) Representative examples of uniform high, uniform moderate and focal high expression in human PDAC samples (10x).

FIG. 10 depicts images of specific killing of MCT1 expressing cells in a mixed population of cells over time for untreated controls and 3-BP-treated (50 uM).

FIG. 11 shows Incucyte data, for 15 min exposure for higher doses of various drugs. Lower panel shows still from time lapse movie of MCT WT vs KO exposed to oxaliplatin (200 uM) for 2h.

FIG. 12A – FIG. 12C depict body weight changes over the course of ME3BP-7 administration in various murine models: (FIG. 12A) Panc 02.13 in nude mice, (FIG. 12B) TM01212 in NSG mice and (FIG. 12C) TM01098 in NCG mice.

FIG. 13 shows representative H&E images of organs of NSG mice treated with ME3BP-7 for 4 weeks: (A) heart, (B) lung, (C) kidney, (D) pancreas, (E) liver, (F) spleen x10.

FIG. 14 shows a violin plot of TCGA data for PDACs.

FIG. 15 depicts GTEx dataset showing expression of MCT-1 across 51 tissue types.

FIG. 16 shows IHC of normal tissues from human TMA BC001130 (10x) in (A) pancreas, (B) lung, (C) esophagus, (D) heart, (E) kidney (F) ovary, (G) brain, (H) intestinal lymph node, (I) liver and (J) testis.

Detailed Description of the Invention

Alkylating agents have been mainstays of cancer treatment for decades but the reason for their cancer selectivity is unknown. Monocarboxylate transporter (MCT) inhibition leads to disruption in glycolysis, induces cell death and decreases cell invasion, revealing the importance of MCT activity in intracellular pH homeostasis and tumor aggressiveness. There are 14 MCTs corresponding to 14 solute carrier 16A transporters. Without being bound by theory, malignant tumors rely heavily on anaerobic glycolysis (a/k/a the Warburg effect) and therefore need to output lactic acid via MCTs to the tumor micro-environment to maintain a robust glycolytic flux. As noted herein, ~25% of Pancreatic Ductal Adenocarcinomas (PDACs) overexpress Monocarboxylate Transporter 1 (MCT-1), a known pyruvate/lactate transporter. 3-Bromopyruvate (3BP) is a potent cytotoxic pyruvate/lactate analog with alkylating activity with a unique mode of action, and has previously been shown to be transported by MCT-1.[19] The anti-cancer effects of 3BP was initially attributed to its effect on inhibiting glycolysis.[20-22] However, recent studies have clarified that 3BP acts as an alkylating agent of many intracellular proteins.[19, 23] However, clinical development of 3BP has been hampered by its poor serum stability, poor pharmacokinetics, and excessive *in vivo* toxicity despite substantial pre-clinical and clinical efforts.[24, 25] The biochemical basis for these poor pharmacokinetic features is that 3BP alkylates free sulfhydryl groups in plasma proteins, including albumin, resulting in drug inactivation resulting in rapid loss of activity[19]. To overcome rapid loss of 3BP activity, large doses must be administered systemically, or delivered locally to tumors, such as through the hepatic artery. Thus, even though MCT-1 has been known to be essential for 3BP activity for over a decade [26], effective ways to systemically deliver 3BP remain a challenge.

As disclosed herein, 3-BP kills pancreatic cancer cells expressing high levels of MCT-1 in rapid fashion. Exposure to 3-BP for as little as 30 minutes kills pancreatic

cancer cells, unlike commonly used chemotherapeutic agents such as gemcitabine, 5-FU, irinotecan and oxaliplatin. Disclosed herein are novel microencapsulated formulations of 3BP (ME3BP-7) that are stable in serum and active against a variety of PDAC cells but had tolerable toxicity in mice when systemically administered. ME3BP-7 can thereby be
5 considered an alkylating agent that targets cells overexpressing a protein that is unique in that the targeting moiety and the cytotoxic moiety are both contained within the same single small molecule.

It has been determined herein that cyclodextrins can encapsulate selective inhibitors of ATP production such as 3-halopyruvates, including 3BP ($\alpha/k/a$ 3-BrPA), in
10 order to stabilize the alkylating compound in an aqueous environment, as well as reduce the ability of nucleophilic entities in proteins to access it, thereby lowering its systemic toxicity and maintaining its alkylating ability. Such compositions are demonstrated herein in multiple *in vitro* cell lines, using different forms of cyclodextrins (*e.g.*, beta and
15 alpha) and at different ratios of active agent encapsulation relative to the cyclodextrin, and in *in vivo* animal tumor models. For example, such compositions are demonstrated herein to maintain the functional characteristics of the selective inhibitors of ATP production to kill cancer cells both *in vitro* and *in vivo* such that their activity can be preserved and protected for systemic administration until it reaches the target tissue,
20 organ, and/or tumor while minimizing toxicity. This determination was unexpected because cyclodextrins are known to have a destabilizing effect on many compounds through direct catalysis, particularly with increasing pH (Rasheed *et al.* (2008) *Sci. Pharm.* 76: 567-598). Although this catalytic effect of cyclodextrins would have been expected to be great for 3-halopyruvates since they are halogenated derivatives of pyruvic acid, it was surprisingly determined that cyclodextrins actually protected and stabilized 3-
25 BrPA. It was further surprisingly determined that cyclodextrins modified to replace one or more hydroxyl groups on one or more of its α -D-glucopyranoside units with ionizable groups resulting in negative charges (anions) stabilizes the 3-halopyruvates better than those having ionizable groups resulting in positive charges (cations) or unmodified cyclodextrins, such as unmodified alpha- or beta-cyclodextrin. Without being bound by
30 theory, it is believed that anionic moieties on cyclodextrins force the halogen atom (*e.g.*, bromine) of a halopyruvate (*e.g.*, 3-BrPA) to sit in the cavity. It was also surprisingly determined that β -cyclodextrins encapsulate 3-BrPA in a form that protects and stabilizes

3-BrPA for *in vivo* efficacy especially and also *in vitro* efficacy significantly better than α -cyclodextrins.

Described herein are novel encapsulated formulations of 3BP that reduce its degradation in serum. Efficacy of exemplary formulations of 3BP against pancreatic cancer cells were investigated, and demonstrated killing of pancreatic cancer cells with notable speed and efficiency *in vitro* and has activity without excessive toxicity in mice.

Thus, the present invention provides compositions and kits comprising such encapsulated formulations, as well as methods of making and using such compositions and kits.

A. Definitions

In order for the present invention to be more readily understood, certain terms and phrases are defined below and throughout the specification.

The articles “a” and “an” are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

The terms “3-bromopyruvate”, “3-BrPA”, “3-BP”, or “3BP” refer to 3-bromopyruvate, analogs and derivatives of 3-bromopyruvate, prodrugs of 3-bromopyruvate, metabolites of 3-bromopyruvate and salts thereof.

The term “administering” means providing a pharmaceutical agent (*e.g.*, the alkylating agent) or composition to a subject, and includes, but is not limited to, administering by a medical professional and self-administering.

The term “cancer” includes, but is not limited to, solid tumors and blood borne tumors. The term cancer includes diseases of the skin, tissues, organs, bone, cartilage, blood and vessels. The term “cancer” further encompasses primary and metastatic cancers.

The term “inhibit” or “inhibits” means to decrease, suppress, attenuate, diminish, arrest, or stabilize the development or progression of a disease, disorder, or condition, the activity of a biological pathway, or a biological activity, such as the growth of a solid malignancy, *e.g.*, by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or even 100% compared to an untreated control subject, cell, biological pathway, or biological activity or compared to the target, such as a growth of a solid malignancy, in a subject before the subject is treated. By the term “decrease” is meant to

inhibit, suppress, attenuate, diminish, arrest, or stabilize a symptom of a cancer disease, disorder, or condition. It will be appreciated that, although not precluded, treating a disease, disorder or condition does not require that the disease, disorder, condition or symptoms associated therewith be completely eliminated.

5 The term “modulation” refers to upregulation (*i.e.*, activation or stimulation), downregulation (*i.e.*, inhibition or suppression) of a response, or the two in combination or apart.

 The term “pharmaceutically acceptable” is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of
10 sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

 The term “pharmaceutically-acceptable carrier” as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid
15 filler, diluent, excipient, or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be “acceptable” in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers
20 include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil;
25 (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates and/or
30 polyanhydrides; and (22) other non-toxic compatible substances employed in pharmaceutical formulations.

 The term “pharmaceutically-acceptable salts” refers to the relatively non-toxic, inorganic and organic salts of compounds.

A “subject” can include a human subject for medical purposes, such as for the treatment of an existing disease, disorder, condition or the prophylactic treatment for preventing the onset of a disease, disorder, or condition or an animal subject for medical, veterinary purposes, or developmental purposes. Suitable animal subjects include mammals including, but not limited to, primates, e.g., humans, monkeys, apes, gibbons, chimpanzees, orangutans, macaques and the like; bovines, e.g., cattle, oxen, and the like; ovines, e.g., sheep and the like; caprines, e.g., goats and the like; porcines, e.g., pigs, hogs, and the like; equines, e.g., horses, donkeys, zebras, and the like; felines, including wild and domestic cats; canines, including dogs; lagomorphs, including rabbits, hares, and the like; and rodents, including mice, rats, guinea pigs, and the like. An animal may be a transgenic animal. In some embodiments, the subject is a human including, but not limited to, fetal, neonatal, infant, juvenile, and adult subjects. Further, a “subject” can include a patient afflicted with or suspected of being afflicted with a disease, disorder, or condition. Thus, the terms “subject” and “patient” are used interchangeably herein. Subjects also include animal disease models (e.g., rats or mice used in experiments, and the like).

The terms “prevent,” “preventing,” “prevention,” “prophylactic treatment,” and the like refer to reducing the probability of developing a disease, disorder, or condition in a subject, who does not have, but is at risk of or susceptible to developing a disease, disorder, or condition.

The term “subject suspected of having” means a subject exhibiting one or more clinical indicators of a disease or condition. In certain embodiments, the disease or condition is cancer. In certain embodiments, the cancer is a pancreatic cancer, mesothelioma, leukemia or lymphoma.

The term “subject in need thereof” means a subject identified as in need of a therapy or treatment.

The terms “systemic administration,” “administered systemically,” “peripheral administration,” and “administered peripherally” mean the administration of a compound, drug or other material other than directly into the central nervous system, such that it enters the patient's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

The term “therapeutic agent” or “pharmaceutical agent” refers to an agent capable of having a desired biological effect on a host, such as the alkylating agent disclosed

herein. Chemotherapeutic and genotoxic agents are examples of therapeutic agents that are generally known to be chemical in origin, as opposed to biological, or cause a therapeutic effect by a particular mechanism of action, respectively. Examples of therapeutic agents of biological origin include growth factors, hormones, and cytokines.

5 A variety of therapeutic agents is known in the art and may be identified by their effects. Certain therapeutic agents are capable of regulating red cell proliferation and differentiation. Examples include chemotherapeutic nucleotides, drugs, hormones, non-specific (*e.g.* non-antibody) proteins, oligonucleotides (*e.g.*, antisense oligonucleotides that bind to a target nucleic acid sequence (*e.g.*, mRNA sequence)), peptides, and
10 peptidomimetics.

The term “therapeutic effect” refers to a local or systemic effect in animals, particularly mammals, and more particularly humans, caused by a pharmacologically active substance. The term thus means any substance intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease or in the enhancement of desirable
15 physical or mental development and conditions in an animal or human. The phrase “therapeutically-effective amount” means that amount of such a substance that produces some desired local or systemic effect at a reasonable benefit/risk ratio applicable to any treatment. In certain embodiments, a therapeutically effective amount of a compound will depend on its therapeutic index, solubility, and the like. For example, certain
20 compounds discovered by the methods of the present invention may be administered in a sufficient amount to produce a reasonable benefit/risk ratio applicable to such treatment.

The terms “therapeutically-effective amount” and “effective amount” as used herein means that amount of a compound, material, or composition comprising a compound of the present invention which is effective for producing some desired
25 therapeutic effect in at least a sub-population of cells in an animal at a reasonable benefit/risk ratio applicable to any medical treatment.

The term “treating” a disease in a subject or “treating” a subject having a disease refers to subjecting the subject to a pharmaceutical treatment, *e.g.*, the administration of a drug, such that at least one symptom of the disease is decreased or prevented from
30 worsening.

The terms “tumor,” “solid malignancy,” or “neoplasm” refer to a lesion that is formed by an abnormal or unregulated growth of cells. Preferably, the tumor is malignant, such as that formed by a cancer.

B. Cyclodextrins

The term “cyclodextrin” refers to a family of cyclic oligosaccharides composed of 5 or more α -D-glucopyranoside units linked together by C1-C4 bonds having a toroidal topological structure, wherein the larger and the smaller openings of the toroid expose certain hydroxyl groups of the α -D-glucopyranoside units to the surrounding environment (*e.g.*, solvent) (see, for examples, Structure 1). The term “inert cyclodextrin” refers to a cyclodextrin containing α -D-glucopyranoside units having the basic formula $C_6H_{12}O_6$ and glucose structure without any additional chemical substitutions (*e.g.*, α -cyclodextrin having 6 glucose monomers, β -cyclodextrin having 7 glucose monomers, and γ -cyclodextrin having 8 glucose monomers). The term “cyclodextrin internal phase” refers to the relatively less hydrophilic region enclosed within (*i.e.*, encapsulated by) the toroid topology of the cyclodextrin structure. The term “cyclodextrin external phase” refers to the region not enclosed by the toroid topology of the cyclodextrin structure and can include, for example, the aqueous environment present during systemic administration *in vivo* or to the internal phase of a structure that itself encapsulates the selective ATP production inhibitor/cyclodextrin complex. Cyclodextrins are useful for solubilizing hydrophobic compositions (see, for example, Albers and Muller (1995) *Crit. Rev. Therap. Drug Carrier Syst.* 12:311-337; Zhang and Ma (2013) *Adv. Drug Delivery Rev.* 65:1215-1233; Laza-Knoerr *et al.* (2010) *J. Drug Targ.* 18:645-656; Challa *et al.* (2005) *AAPS PharmSci. Tech.* 6:E329-357; Uekama *et al.* (1998) *Chem. Rev.* 98:2045-2076; Szejtli (1998) *Chem. Rev.* 98:1743-1754; Stella and He (2008) *Toxicol. Pathol.* 36:30-42; Rajewski and Stella (1996) *J. Pharm. Sci.* 85:1142-1169; Thompson (1997) *Crit. Rev. Therap. Drug Carrier Sys.* 14:1-104; and Irie and Uekama (1997) *J. Pharm. Sci.* 86:147-162). Any substance located within the cyclodextrin internal phase is said to be “encapsulated.”

As used herein, a cyclodextrin is useful according to the present invention so long as the cyclodextrins can encapsulate a selective ATP production inhibitor. In some embodiments, the cyclodextrin further bears ionizable (*e.g.*, weakly basic and/or weakly acidic) functional groups to enhance the stabilization of the selective ATP production inhibitor. By protecting the stability of the selective ATP production inhibitor, it is meant that the selective ATP production inhibitor /cyclodextrin complex makes the selective ATP production inhibitor molecule more stable as seen by photo stability, shelf life

stability, thermal stability, stability against intramolecular cyclization, stability to acid hydrolysis, stability against general degradation, and the like, as compared to the stability of a selective ATP production inhibitor molecule that is not in a complex with cyclodextrin.

5 For encapsulating a desired therapeutic agent, cyclodextrins can be selected and/or chemically modified according to the characteristics of the desired therapeutic agent and parameters for efficient, high-concentration loading therein. For example, it is preferable that the cyclodextrin itself have high solubility in water in order to facilitate loading of a therapeutic agent, such as a 3-halopyruvate. In some embodiments, the water solubility
10 of the cyclodextrin is at least 10 mg/mL, 20 mg/mL, 30 mg/mL, 40 mg/mL, 50 mg/mL, 60 mg/mL, 70 mg/mL, 80 mg/mL, 90 mg/mL, 100 mg/mL or higher. Methods for achieving such enhanced water solubility are well known in the art.

In some embodiments, a large association constant with the therapeutic agent is preferable and can be obtained by selecting the number of glucose units in the
15 cyclodextrin based on the size of the therapeutic agent (see, for example, Albers and Muller (1995) *Crit. Rev. Therap. Drug Carrier Syst.* 12:311-337; Stella and He (2008) *Toxicol. Pathol.* 36:30-42; and Rajewski and Stella (1996) *J. Pharm. Sci.* 85:1142-1169). As a result, the solubility (nominal solubility) of the therapeutic agent in the presence of cyclodextrin can be further improved. For example, the association constant of the
20 cyclodextrin with the therapeutic agent can be 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000, or higher.

Derivatives formed by reaction with cyclodextrin hydroxyl groups (*e.g.*, those lining the upper and lower ridges of the toroid of an inert cyclodextrin) are readily prepared and offer a means of modifying the physicochemical properties of the parent
25 (inert) cyclodextrin. In some embodiments, the physicochemical properties of the inert cyclodextrin molecule or cyclodextrin molecule that is not complexed with a selective ATP production inhibitor differ from the properties of a cyclodextrin molecule complexed with the selective ATP production inhibitor. Accordingly, the selective ATP production inhibitor molecules complexed with cyclodextrin can be characterized by
30 observing changes in solubility, chemical reactivity, UV/VIS absorbance, drug retention, chemical stability, and the like. For example, it has been determined herein that modifying hydroxyl groups, such as those facing away from the cyclodextrin interior phase, can be replaced with ionizable chemical groups to facilitate loading of therapeutic

agents, such as poorly soluble or hydrophobic agents, within the modified cyclodextrins and stabilization thereof. In some embodiments, a modified cyclodextrin having at least one hydroxyl group substituted with an ionizable chemical group will result in a charged moiety under certain solvent (*e.g.*, pH) conditions. The term “charged cyclodextrin” refers to a cyclodextrin having one or more of its hydroxyl groups substituted with a charged moiety and the moiety bearing a charge. Such a moiety can itself be a charged group or it can comprise an organic moiety (*e.g.*, a $\text{C}_1\text{-C}_6$ alkyl or $\text{C}_1\text{-C}_6$ alkyl ether moiety) substituted with one or more charged moieties.

In some embodiments, the “ionizable” or “charged” moieties are weakly ionizable. Weakly ionizable moieties are those that are either weakly basic or weakly acidic. Weakly basic functional groups (X) have a pK_a of between about 6.0-9.0, 6.5-8.5, 7.0-8.0, 7.5-8.0, and any range in between inclusive according to $\text{CH}_3\text{-X}$. Similarly, weakly acidic functional groups (Y) have a log dissociation constant (pK_a) of between about 3.0-7.0, 4.0-6.5, 4.5-6.5, 5.0-6.0, 5.0-5.5, and any range in between inclusive according to $\text{CH}_3\text{-Y}$. The pK_a parameter is a well-known measurement of acid/base properties of a substance and methods for pK_a determination are conventional and routine in the art. For example, the pK_a values for many weak acids are tabulated in reference books of chemistry and pharmacology. See, for example, IUPAC Handbook of Pharmaceutical Salts, ed. by P. H. Stahl and C. G. Wermuth, Wiley-VCH, 2002; CRC Handbook of Chemistry and Physics, 82nd Edition, ed. by D. R. Lide, CRC Press, Florida, 2001, p. 8-44 to 8-56. Since cyclodextrins with more than one ionizable group have pK_a of the second and subsequent groups each denoted with a subscript.

Representative anionic moieties include, without any limitation, succinyl, carboxylate, carboxymethyl, sulfonyl, phosphate, sulfoalkyl ether, sulphate carbonate, thiocarbonate, thiocarbonate, phosphate, phosphonate, sulfonate, nitrate, and borate groups.

Representative cationic moieties include, without limitation, amino, guanidine, and quaternary ammonium groups.

In some embodiments, the modified cyclodextrin is a “polyanion” or “polycation.” A polyanion is a modified cyclodextrin having more than one negatively charged group resulting in net negative ionic charge of more than two units. A polycation is a modified cyclodextrin having more than one positively charged group resulting in net positive ionic charge of more than two units.

In some embodiments, the modified cyclodextrin is a “chargeable amphiphile.” By “chargeable” is meant that the amphiphile has a pK in the range pH 4 to pH 8 or 8.5. A chargeable amphiphile may therefore be a weak acid or base. By “amphoteric” herein is meant a modified cyclodextrin having a ionizable groups of both anionic and cationic character wherein: 1) at least one, and optionally both, of the cation and anionic amphiphiles is chargeable, having at least one charged group with a pK between 4 and 8 to 8.5, 2) the cationic charge prevails at pH 4, and 3) the anionic charge prevails at pH 8 to 8.5.

In some embodiments, the “ionizable” or “charged” cyclodextrins as a whole, whether polyionic, amphiphilic, or otherwise, are weakly ionizable (*i.e.*, have a pK_{a1} of between about 4.0-8.5, 4.5-8.0, 5.0-7.5, 5.5-7.0, 6.0-6.5, and any range in between inclusive).

Any one, some, or all hydroxyl groups of any one, some or all α -D-glucopyranoside units of a cyclodextrin can be modified to an ionizable chemical group as described herein. Since each cyclodextrin hydroxyl group differs in chemical reactivity, reaction with a modifying moiety can produce an amorphous mixture of positional and optical isomers. Alternatively, certain chemistry can allow for pre-modified α -D-glucopyranoside units to be reacted to form uniform products.

The aggregate substitution that occurs is described by a term called the degree of substitution. For example, a 6-ethylenediamino- β -cyclodextrin with a degree of substitution of seven would be composed of a distribution of isomers of 6-ethylenediamino- β -cyclodextrin in which the average number of ethylenediamino groups per 6-ethylenediamino- β -cyclodextrin molecule is seven. Degree of substitution can be determined by mass spectrometry or nuclear magnetic resonance spectroscopy. Theoretically, the maximum degree of substitution is 18 for α -cyclodextrin, 21 for β , and 24 for γ -cyclodextrin, however, substituents themselves having hydroxyl groups present the possibility for additional hydroxylalkylations. The degree of substitution can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or more and can encompass complete substitution.

Another parameter is the stereochemical location of a given hydroxyl substitution. In some embodiments, at least one hydroxyl facing away from the cyclodextrin interior is substituted with an ionizable chemical group. For example, the C2, C3, C6, C2 and C3, C2 and C6, C3 and C6, and all three of C2-C3-C6 hydroxyls of at least one α -D-

glucopyranoside unit are substituted with an ionizable chemical group. Such carbon positions are well known in the art. For example, the CH₂OH moiety shown in Structure 1 of each α -D-glucopyranoside unit represents the C6 carbon. Any such combination of hydroxyls can similarly be combined with at least two, three, four, five, six, seven, eight, 5 nine, ten, eleven, up to all of the α -D-glucopyranoside units in the modified cyclodextrin as well as in combination with any degree of substitution described herein.

It is also acceptable to combine one or more of the cyclodextrins described herein.

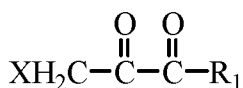
C. Selective Inhibitors of ATP Production and Related Compounds

10 Some embodiments of the present invention relate to the encapsulation of selective inhibitors of ATP production within cyclodextrins. The term “selective inhibitors of ATP production” refers to anti-metabolite agents that inhibit ATP production by interfering with the enzymatic process of generating ATP (*e.g.*, GAPDH inhibitors such as 3-halopyruvates like 3-bromopyruvate). In some embodiments, the selective 15 inhibitor of ATP production is an “antineoplastic alkylating agent,” which refers to an agent used in cancer treatment that causes replacement of hydrogen by an alkyl group.

As used herein the term “alkyl” refers to C₁₋₂₀ inclusive, linear (*i.e.*, “straight-chain”), branched, or cyclic, saturated or at least partially and in some cases fully unsaturated (*i.e.*, alkenyl and alkynyl) hydrocarbon radicals derived from a hydrocarbon 20 moiety containing between one and twenty carbon atoms by removal of a single hydrogen atom. Representative alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, sec-pentyl, iso-pentyl, neopentyl, n-hexyl, sec-hexyl, n-heptyl, n-octyl, n-decyl, n-undecyl, dodecyl, and the like, ethenyl, propenyl, butenyl, pentenyl, hexenyl, octenyl, butadienyl, propynyl, butynyl, 25 pentynyl, hexynyl, heptynyl, and allenyl groups. “Branched” refers to an alkyl group in which a lower alkyl group, such as methyl, ethyl or propyl, is attached to a linear alkyl chain. “Lower alkyl” refers to an alkyl group having 1 to about 8 carbon atoms (*i.e.*, a C₁₋₈ alkyl), *e.g.*, 1, 2, 3, 4, 5, 6, 7, or 8 carbon atoms. “Higher alkyl” refers to an alkyl group having about 10 to about 20 carbon atoms, *e.g.*, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 30 20 carbon atoms. In certain embodiments, “alkyl” refers, in particular, to C₁₋₈ straight-chain alkyls. In other embodiments, “alkyl” refers, in particular, to C₁₋₈ branched-chain alkyls.

Alkyl groups can optionally be substituted (a “substituted alkyl”) with one or more alkyl group substituents, which can be the same or different. The term “alkyl group substituent” includes but is not limited to alkyl, substituted alkyl, halo, arylamino, acyl, hydroxyl, aryloxy, alkoxy, alkylthio, arylthio, aralkyloxy, aralkylthio, carboxyl, alkoxy, carbonyl, oxo, and cycloalkyl. There can be optionally inserted along the alkyl chain one or more oxygen, sulfur or substituted or unsubstituted nitrogen atoms, wherein the nitrogen substituent is hydrogen, lower alkyl (also referred to herein as “alkylaminoalkyl”), or aryl. Thus, as used herein, the term “substituted alkyl” includes alkyl groups, as defined herein, in which one or more atoms or functional groups of the alkyl group are replaced with another atom or functional group, including for example, alkyl, substituted alkyl, halogen, aryl, substituted aryl, alkoxy, hydroxyl, nitro, amino, alkylamino, dialkylamino, sulfate, and mercapto.

In some embodiments, selective inhibitors of ATP production are generally represented by the formula:



wherein X represents a halide, a sulfonate, a carboxylate, an alkoxide, or an amine oxide. In certain embodiments, X is a halide selected from the group consisting of: fluoride, bromide, chloride, and iodide. In some embodiments, the inhibitor is a 3-halopyruvate. In certain other embodiments, the 3-halopyruvate is selected from the group consisting of: 3-fluoropyruvate, 3-chloropyruvate, 3-bromopyruvate and 3-iodopyruvate. In some embodiments, the 3-halopyruvate is 3-bromopyruvate. In other embodiments, X is a sulfonate and may be selected from the group consisting of: triflate, mesylate and tosylate. In some embodiments, X is an amine oxide is dimethylamine oxide. In certain embodiments R₁ represents OR, H, N(R’)₂, C₁-C₆ alkyl, C₆-C₁₂ aryl, C₁-C₆ heteroalkyl, or a C₆-C₁₂ heteroaryl. Independently, in other embodiments, R’ represents H, C₁-C₆ alkyl, or C₆-C₁₂ aryl. Independently, in still other embodiments, R represents H, alkali metal, C₁-C₆ alkyl, C₆-C₁₂ aryl or C(O)R’; and R’ represents H, C₁-C₂₀ alkyl or C₆-C₁₂ aryl.

In a preferred embodiment, the invention further provides inhibitors of ATP production represented by general formula:



wherein X represents a halide, a sulfonate, a carboxylate, an alkoxide, or an amine oxide. In certain embodiments, X is a halide and may be selected from the group consisting of: fluoride, bromide, chloride, and iodide. In some embodiments, the inhibitor is 3-halopyruvate. In certain embodiments, the 3-halopyruvate is selected from the group consisting of: 3-fluoropyruvate, 3-chloropyruvate, 3-bromopyruvate and 3-iodopyruvate. In some embodiments, the 3-halopyruvate is 3-bromopyruvate. In other embodiments, X is a sulfonate selected from the group consisting of: triflate, mesylate and tosylate. In some embodiments, X is an amine oxide is dimethylamine oxide.

Other analogs, derivatives, prodrugs, metabolites and salts thereof of 3-bromopyruvate can also be used, provided that these compounds or compositions have an anticancer effect that is statistically similar to that of 3-bromopyruvate. When referring herein to a treatment using 3-bromopyruvate, it should be understood that the treatment may also be conducted with analogs, derivatives, prodrugs, metabolites and salts of 3-bromopyruvate, where applicable.

D. Cyclodextrin/ATP Inhibitor Compositions

The present invention provides pharmaceutical compositions comprising selective inhibitors of ATP production, *e.g.*, 3-Bromopyruvate, described above encapsulated within inert and/or modified cyclodextrins, *e.g.*, microencapsulated 3-Bromopyruvate 7 (ME3BP-7). Such complexes are referred to herein as cyclodextrin/ATP inhibitor compositions. The ratio of selective inhibitor of ATP production to cyclodextrin may be 1:1 such that one inhibitor molecule forms a complex with one cyclodextrin molecule. Alternatively, the ratio can be 2:1, 3:1, 4:1, 5:1, or more, or any intervening fractional amount, *e.g.*, 1.1:1, 1.2:1, 1.3:1, ... 1.7:1, 1.8:1, 1.9:1.

In one aspect, the present invention provides pharmaceutically acceptable compositions which comprise a therapeutically-effective amount of one or more such cyclodextrin/ATP inhibitors described above, formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents. In another aspect the compositions can be administered as such or in admixtures with pharmaceutically acceptable carriers and can also be administered in conjunction with other anti-cancer therapies, such as chemotherapeutic agents, scavenger compounds, radiation therapy, biologic therapy, and the like. Conjunctive therapy thus includes sequential, simultaneous and separate, or co-administration of the composition, wherein the

therapeutic effects of the first administered has not entirely disappeared when the subsequent compound is administered.

As described in detail below, the pharmaceutical compositions of the present invention may be specially formulated for administration in solid or liquid form,

- 5 including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, *e.g.*, those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; (2) parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or
- 10 sustained-release formulation; (3) topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin; (4) intravaginally or intrarectally, for example, as a pessary, cream or foam; (5) sublingually; (6) ocularly; (7) transdermally; or (8) nasally.

- As set out above, certain embodiments of the selective ATP inhibitors or
- 15 cyclodextrin/ATP inhibitor compositions may contain a basic functional group, such as amino or alkylamino, and are, thus, capable of forming pharmaceutically-acceptable salts with pharmaceutically-acceptable acids. These salts can be prepared *in situ* in the administration vehicle or the dosage form manufacturing process, or by separately reacting a purified compound of the invention in its free base form with a suitable organic
- 20 or inorganic acid, and isolating the salt thus formed during subsequent purification.

- Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like (see, for example,
- 25 Berge et al. (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66:1-19).

- The pharmaceutically acceptable salts of the subject compounds include the conventional nontoxic salts or quaternary ammonium salts of the compounds, *e.g.*, from non-toxic organic or inorganic acids. For example, such conventional nontoxic salts include those derived from inorganic acids such as hydrochloride, hydrobromic, sulfuric,
- 30 sulfamic, phosphoric, nitric, and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, palmitic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-

acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isothionic, and the like.

In other cases, the selective ATP inhibitors or cyclodextrin/ATP inhibitor compositions of the present invention may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically-acceptable salts with pharmaceutically-acceptable bases. These salts can likewise be prepared *in situ* in the administration vehicle or the dosage form manufacturing process, or by separately reacting the purified compound in its free acid form with a suitable base, such as the hydroxide, carbonate or bicarbonate of a pharmaceutically-acceptable metal cation, with ammonia, or with a pharmaceutically-acceptable organic primary, secondary or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like (see, for example, Berge et al., *supra*).

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically-acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Cyclodextrin/ATP inhibitor composition formulations include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated and the particular mode of administration. The amount of active ingredient which can be combined with a carrier

material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect.

In certain embodiments, a formulation of cyclodextrin/ATP inhibitor compositions can comprise other carriers to allow more stability, to allow more stability, different releasing properties *in vivo*, targeting to a specific site, or any other desired characteristic that will allow more effective delivery of the complex to a subject or a target in a subject, such as, without limitation, liposomes, microspheres, nanospheres, nanoparticles, bubbles, micelle forming agents, *e.g.*, bile acids, and polymeric carriers, *e.g.*, polyesters and polyanhydrides. In certain embodiments, an aforementioned formulation renders orally bioavailable a compound of the present invention.

Liquid dosage formulations of cyclodextrin/ATP inhibitor compositions include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of

an active ingredient. A cyclodextrin/ATP inhibitor composition of the present invention may also be administered as a bolus, electuary or paste.

In solid dosage forms (*e.g.*, capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically-acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol, glycerol monostearate, and non-ionic surfactants; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-shelled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

The tablets, and other solid dosage forms, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also 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, other polymer matrices, liposomes and/or microspheres. Compositions may also be formulated for rapid release, *e.g.*, freeze-dried.

They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Formulations for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing one or more compounds of the invention with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active compound.

Formulations which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration of a cyclodextrin/ATP inhibitor composition of the present invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically-acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The ointments, pastes, creams and gels may contain, in addition to an active compound of this invention, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Transdermal patches have the added advantage of providing controlled delivery to the body. Such dosage forms can be made by dissolving or dispersing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the compound in a polymer matrix or gel.

Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of this invention.

Pharmaceutical compositions suitable for parenteral administration can comprise sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain sugars, alcohols, antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

In certain embodiments, the above-described pharmaceutical compositions can be combined with other pharmacologically active compounds ("second active agents") known in the art according to the methods and compositions provided herein. Second active agents can be large molecules (*e.g.*, proteins) or small molecules (*e.g.*, synthetic inorganic, organometallic, or organic molecules). In some embodiments, second active agents independently or synergistically help to treat cancer.

For example, chemotherapeutic agents are anti-cancer agents. The term chemotherapeutic agent includes, without limitation, platinum-based agents, such as carboplatin and cisplatin; nitrogen mustard alkylating agents; nitrosourea alkylating agents, such as carmustine (BCNU) and other alkylating agents; antimetabolites, such as methotrexate; purine analog antimetabolites; pyrimidine analog antimetabolites, such as fluorouracil (5-FU) and gemcitabine; hormonal antineoplastics, such as goserelin, leuprolide, and tamoxifen; natural antineoplastics, such as taxanes (*e.g.*, docetaxel and

paclitaxel), aldesleukin, interleukin-2, etoposide (VP-16), interferon alfa, and tretinoin (ATRA); antibiotic natural antineoplastics, such as bleomycin, dactinomycin, daunorubicin, doxorubicin, and mitomycin; and vinca alkaloid natural antineoplastics, such as vinblastine and vincristine.

5 Further, the following drugs may also be used in combination with an antineoplastic agent, even if not considered antineoplastic agents themselves: dactinomycin; daunorubicin HCl; docetaxel; doxorubicin HCl; epoetin alfa; etoposide (VP-16); ganciclovir sodium; gentamicin sulfate; interferon alfa; leuprolide acetate; meperidine HCl; methadone HCl; ranitidine HCl; vinblastin sulfate; and zidovudine
10 (AZT). For example, fluorouracil has recently been formulated in conjunction with epinephrine and bovine collagen to form a particularly effective combination.

Still further, the following listing of amino acids, peptides, polypeptides, proteins, polysaccharides, and other large molecules may also be used: interleukins 1 through 18, including mutants and analogues; interferons or cytokines, such as interferons α , β , and γ ;
15 hormones, such as luteinizing hormone releasing hormone (LHRH) and analogues and, gonadotropin releasing hormone (GnRH); growth factors, such as transforming growth factor- β (TGF- β), fibroblast growth factor (FGF), nerve growth factor (NGF), growth hormone releasing factor (GHRF), epidermal growth factor (EGF), fibroblast growth factor homologous factor (FGFHF), hepatocyte growth factor (HGF), and insulin growth
20 factor (IGF); tumor necrosis factor- α & β (TNF- α & β); invasion inhibiting factor-2 (IIF-2); bone morphogenetic proteins 1-7 (BMP 1-7); somatostatin; thymosin- α -1; γ -globulin; superoxide dismutase (SOD); complement factors; anti-angiogenesis factors; antigenic materials; and pro-drugs.

Chemotherapeutic agents for use with the compositions and methods of treatment
25 described herein include, but are not limited to alkylating agents such as thiotepa and cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramidate, triethylenethiophosphoramidate and trimethylolomelamine; acetogenins (especially
30 bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189

and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (*e.g.*, calicheamicin, especially calicheamicin gammall and calicheamicin omegall; dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores, aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabycin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiothane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziqune; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK polysaccharide complex); razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids,

e.g., paclitaxel and doxorubicin; chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum coordination complexes such as cisplatin, oxaliplatin and carboplatin; vinorelbine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; 5
xeloda; ibandronate; irinotecan (*e.g.*, CPT-11); topoisomerase inhibitor RFS 2000; difluoromethylomithine (DMFO); retinoids such as retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

In some embodiments, the composition of the invention may comprise other biologically active substances, including therapeutic drugs or pro-drugs, for example, 10
other chemotherapeutic agents, scavenger compounds, antibiotics, anti-virals, anti-fungals, anti-inflammatories, vasoconstrictors and anticoagulants, antigens useful for cancer vaccine applications or corresponding pro-drugs.

Exemplary scavenger compounds include, but are not limited to thiol-containing compounds such as glutathione, thiourea, and cysteine; alcohols such as mannitol, 15
substituted phenols; quinones, substituted phenols, aryl amines and nitro compounds.

Various forms of the chemotherapeutic agents and/or other biologically active agents may be used. These include, without limitation, such forms as uncharged molecules, molecular complexes, salts, ethers, esters, amides, and the like, which are biologically active.

20

E. Methods of Making Cyclodextrin/ATP Inhibitor Compositions

Methods of preparing cyclodextrin/ATP inhibitor compositions and formulations thereof include the step of bringing into association a compound of the present invention with the carrier and, optionally, one or more accessory ingredients. In general, the 25
formulations are prepared by uniformly and intimately bringing into association a selective inhibitor of ATP production described herein with a cyclodextrin. Generally, such complexes can be obtained by agitating and mixing the cyclodextrin (*e.g.*, a solution containing the cyclodextrin) upon dropwise addition of the therapeutic agent (*e.g.*, a solution containing the selective inhibitor of ATP production) or *vice versa*. Many 30
mixing means are known in the art to aid in combining the inhibitor and cyclodextrin for example, without limitation, sonication, vortexing, stirring, heating, co-precipitation, neutralization, slurring, kneading, grinding, and the like. It is possible to use a substance dissolved in a solvent or a solid substance as the therapeutic agent according to the

physical properties of the therapeutic agent. There are no particular limitations on the solvent, and one can use, for example, a substance identical to the cyclodextrin external phase. The amount of the therapeutic agent that is mixed with the cyclodextrin can be equimolar quantities or in different ratios depending on the desired level of incorporation.

- 5 In some embodiments, absolute amounts of the selective inhibitor of ATP production can range between 0.001 to 10 mol equivalents, 0.01 to 1 mol equivalent, or any range inclusive relative to the amount of cyclodextrin. For example, in some embodiments the microencapsulated formulation is ME3BP-7 comprising a molar ratio of 1-2 β -cyclodextrin per 3BP, preferably a molar ratio of 1.2 β -cyclodextrin per 3BP. Also, there
- 10 are no particular limitations on the heating temperature. For example, 5°C or higher, room temperature or higher (*e.g.*, 20°C or higher is also preferable), are all acceptable.

Well-known methods exist for removing any undesired or unincorporated complexes or compositions, such as therapeutic agent not encapsulated by cyclodextrins or therapeutic agent cyclodextrin complexes not encapsulated by liposomes.

- 15 Representative examples include, without limitation, dialysis, centrifugal separation, and gel filtration. Dialysis can be conducted, for example, using a dialysis membrane. As a dialysis membrane, one may cite a membrane with molecular weight cut-off such as a cellulose tube or Spectra/Por. With respect to centrifugal separation, centrifugal acceleration may be conducted preferably at 100,000 g or higher, and more preferably at
- 20 300,000 g or higher. Gel filtration may be carried out, for example, by conducting fractionation based on molecular weight using a column such as Sephadex or Sepharose.

- In some cases, in order to prolong the effect of a drug, it is desirable to modify (*e.g.*, slow) the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous
- 25 material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug form can be accomplished by dissolving or suspending the drug in an oil vehicle. In some embodiments, the cyclodextrin-encapsulated selective ATP inhibitor compositions
- 30 described herein can be loaded into liposomes.

Injectable depot forms are made by forming microcapsule matrices of the subject compounds in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer

employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

5

F. Therapeutic Methods

The present invention further provides novel therapeutic methods of preventing, delaying, reducing, and/or treating a cancer, including a cancerous tumor. In some embodiments, a method of treatment comprises administering to a subject (*e.g.*, a subject
10 in need thereof), an effective amount of a cyclodextrin/selective ATP production inhibitor composition. A subject in need thereof may include, for example, a subject who has been diagnosed with a tumor, including a pre-cancerous tumor, a cancer, or a subject who has been treated, including subjects that have been refractory to the previous treatment.

The term “effective amount,” as in “a therapeutically effective amount,” of a
15 therapeutic agent refers to the amount of the agent necessary to elicit the desired biological response. As will be appreciated by those of ordinary skill in this art, the effective amount of an agent may vary depending on such factors as the desired biological endpoint, the agent to be delivered, the composition of the pharmaceutical composition, the target tissue or cell, and the like. More particularly, the term “effective amount”
20 refers to an amount sufficient to produce the desired effect, *e.g.*, to reduce or ameliorate the severity, duration, progression, or onset of a disease, disorder, or condition, or one or more symptoms thereof; prevent the advancement of a disease, disorder, or condition, cause the regression of a disease, disorder, or condition; prevent the recurrence, development, onset or progression of a symptom associated with a disease, disorder, or
25 condition, or enhance or improve the prophylactic or therapeutic effect(s) of another therapy.

The methods of the present invention may be used to treat any cancerous or pre-cancerous tumor. In certain embodiments, the cancerous tumor has a highly glycolytic phenotype. For example, highly glycolytic tumors may be located in a tissue selected
30 from brain, colon, urogenital, lung, renal, prostate, pancreas, liver, esophagus, stomach, hematopoietic, breast, thymus, testis, ovarian, skin, bone marrow and/or uterine tissue. In some embodiments, methods and compositions of the present invention may be used to treat any cancer. Cancers that may be treated by methods and compositions of the invention

include, but are not limited to, cancer cells from the bladder, blood, bone, bone marrow, brain, breast, colon, esophagus, gastrointestinal, gum, head, kidney, liver, lung, nasopharynx, neck, ovary, pancreas, prostate, skin, stomach, testis, tongue, or uterus. In addition, the cancer may specifically be of the following histological type, though it is not

5 limited to these: neoplasm, malignant; carcinoma; carcinoma, undifferentiated; giant and spindle cell carcinoma; small cell carcinoma; papillary carcinoma; squamous cell carcinoma; lymphoepithelial carcinoma; basal cell carcinoma; pilomatrix carcinoma; transitional cell carcinoma; papillary transitional cell carcinoma; adenocarcinoma; gastrinoma, malignant; cholangiocarcinoma; hepatocellular carcinoma; combined

10 hepatocellular carcinoma and cholangiocarcinoma; trabecular adenocarcinoma; adenoid cystic carcinoma; adenocarcinoma in adenomatous polyp; adenocarcinoma, familial polyposis coli; solid carcinoma; carcinoid tumor, malignant; branchiolo-alveolar adenocarcinoma; papillary adenocarcinoma; chromophobe carcinoma; acidophil carcinoma; oxyphilic adenocarcinoma; basophil carcinoma; clear cell adenocarcinoma;

15 granular cell carcinoma; follicular adenocarcinoma; papillary and follicular adenocarcinoma; pancreatic ductal adenocarcinomas; neuroendocrine pancreatic cancer; nonencapsulating sclerosing carcinoma; adrenal cortical carcinoma; endometroid carcinoma; skin appendage carcinoma; apocrine adenocarcinoma; sebaceous adenocarcinoma; ceruminous adenocarcinoma; mucoepidermoid carcinoma;

20 cystadenocarcinoma; papillary cystadenocarcinoma; papillary serous cystadenocarcinoma; mucinous cystadenocarcinoma; mucinous adenocarcinoma; signet ring cell carcinoma; infiltrating duct carcinoma; medullary carcinoma; lobular carcinoma; inflammatory carcinoma; paget's disease, mammary; acinar cell carcinoma; adenosquamous carcinoma; adenocarcinoma w/squamous metaplasia; thymoma,

25 malignant; ovarian stromal tumor, malignant; thecoma, malignant; granulosa cell tumor, malignant; and roblastoma, malignant; sertoli cell carcinoma; leydig cell tumor, malignant; lipid cell tumor, malignant; paraganglioma, malignant; extra-mammary paraganglioma, malignant; pheochromocytoma; glomangiosarcoma; malignant melanoma; amelanotic melanoma; superficial spreading melanoma; malig melanoma in

30 giant pigmented nevus; epithelioid cell melanoma; blue nevus, malignant; sarcoma; fibrosarcoma; fibrous histiocytoma, malignant; myxosarcoma; liposarcoma; leiomyosarcoma; rhabdomyosarcoma; embryonal rhabdomyosarcoma; alveolar rhabdomyosarcoma; stromal sarcoma; mixed tumor, malignant; mullerian mixed tumor;

nephroblastoma; hepatoblastoma; carcinosarcoma; mesenchymoma, malignant; brenner tumor, malignant; phyllodes tumor, malignant; synovial sarcoma; mesothelioma, malignant; dysgerminoma; embryonal carcinoma; teratoma, malignant; struma ovarii, malignant; choriocarcinoma; mesonephroma, malignant; hemangiosarcoma;

5 hemangioendothelioma, malignant; kaposi's sarcoma; hemangiopericytoma, malignant; lymphangiosarcoma; osteosarcoma; juxtacortical osteosarcoma; chondrosarcoma; chondroblastoma, malignant; mesenchymal chondrosarcoma; giant cell tumor of bone; ewing's sarcoma; odontogenic tumor, malignant; ameloblastic odontosarcoma; ameloblastoma, malignant; ameloblastic fibrosarcoma; pinealoma, malignant; chordoma;

10 glioma, malignant; ependymoma; astrocytoma; protoplasmic astrocytoma; fibrillary astrocytoma; astroblastoma; glioblastoma; oligodendroglioma; oligodendroblastoma; primitive neuroectodermal; cerebellar sarcoma; ganglioneuroblastoma; neuroblastoma; retinoblastoma; olfactory neurogenic tumor; meningioma, malignant; neurofibrosarcoma; neurilemmoma, malignant; granular cell tumor, malignant; malignant lymphoma;

15 Hodgkin's disease; Hodgkin's lymphoma; paragranuloma; malignant lymphoma, small lymphocytic; malignant lymphoma, large cell, diffuse; malignant lymphoma, follicular; mycosis fungoides; other specified non-Hodgkin's lymphomas; malignant histiocytosis; multiple myeloma; mast cell sarcoma; immunoproliferative small intestinal disease; leukemia; lymphoid leukemia; plasma cell leukemia; erythroleukemia; lymphosarcoma

20 cell leukemia; myeloid leukemia; basophilic leukemia; eosinophilic leukemia; monocytic leukemia; mast cell leukemia; megakaryoblastic leukemia; myeloid sarcoma; and hairy cell leukemia.

The compositions described herein may be delivered by any suitable route of administration, including orally, nasally, transmucosally, ocularly, rectally, intravaginally,

25 parenterally, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intra-articular, intra-sternal, intra-synovial, intra-hepatic, intralesional, intracranial, intraperitoneal, intranasal, or intraocular injections, intracisternally, topically, as by powders, ointments or drops (including eyedrops), including buccally and sublingually, transdermally, through an inhalation

30 spray, or other modes of delivery known in the art.

The terms “systemic administration,” “administered systemically,” “peripheral administration,” and “administered peripherally” as used herein mean the administration

of the selective ATP production inhibitor/cyclodextrin complex such that it enters the patient's system and, thus, is subject to metabolism and other like processes.

The terms “parenteral administration” and “administered parenterally” as used herein mean modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intarterial, intrathecal, intracapsular, intraorbital, intraocular, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

In certain embodiments the pharmaceutical compositions are delivered generally (e.g., via oral or parenteral administration). In certain other embodiments the pharmaceutical compositions are delivered locally through direct injection into a tumor or direct injection into the tumor's blood supply (e.g., arterial or venous blood supply). In some embodiments, the pharmaceutical compositions are delivered by both a general and a local administration. For example, a subject with a tumor may be treated through direct injection of a composition containing a composition described herein into the tumor or the tumor's blood supply in combination with oral administration of a pharmaceutical composition of the present invention. If both local and general administration is used, local administration can occur before, concurrently with and/or after general administration.

In certain embodiments, the methods of treatment of the present invention, including treating a cancerous or pre-cancerous tumor comprise administering compositions described herein in combination with a second agent and/or therapy to the subject. By “in combination with” is meant the administration of the selective ATP production inhibitor/cyclodextrin complexes with one or more therapeutic agents either simultaneously, sequentially, or a combination thereof. Therefore, a subject administered a combination of the selective ATP production inhibitor/cyclodextrin complexes and/or therapeutic agents, can receive the selective ATP production inhibitor/cyclodextrin complexes as described herein, and one or more therapeutic agents at the same time (*i.e.*, simultaneously) or at different times (*i.e.*, sequentially, in either order, on the same day or on different days), so long as the effect of the combination of both agents is achieved in the subject. When administered sequentially, the agents can be administered within 1, 5, 10, 30, 60, 120, 180, 240 mins. or longer of one another. In other embodiments, agents

administered sequentially, can be administered within 1, 5, 10, 15, 20 or more days of one another.

When administered in combination, the effective concentration of each of the agents to elicit a particular biological response may be less than the effective concentration of each agent when administered alone, thereby allowing a reduction in the dose of one or more of the agents relative to the dose that would be needed if the agent was administered as a single agent. The effects of multiple agents may, but need not be, additive or synergistic. The agents may be administered multiple times. In such combination therapies, the therapeutic effect of the first administered agent is not diminished by the sequential, simultaneous or separate administration of the subsequent agent(s).

Such methods in certain embodiments comprise administering pharmaceutical compositions comprising compositions described herein in conjunction with one or more chemotherapeutic agents and/or scavenger compounds, including chemotherapeutic agents described herein, as well as other agents known in the art. Conjunctive therapy includes sequential, simultaneous and separate, or co-administration of the composition in a way that the therapeutic effects of the first selective ATP inhibitor administered have not entirely disappeared when the subsequent compound is administered. In some embodiments, the second agent is a chemotherapeutic agent. In some embodiments, the second agent is a scavenger compound. In some embodiments, the second agent is radiation therapy. In a further embodiment, radiation therapy may be administered in addition to the composition. In certain embodiments, the second agent may be co-formulated in the separate pharmaceutical composition.

In some embodiments, the subject pharmaceutical compositions of the present invention will incorporate the substance or substances to be delivered in an amount sufficient to deliver to a patient a therapeutically effective amount of an incorporated therapeutic agent or other material as part of a prophylactic or therapeutic treatment. The desired concentration of the active compound in the particle will depend on absorption, inactivation, and excretion rates of the drug as well as the delivery rate of the compound. It is to be noted that dosage values may also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of

the compositions. Typically, dosing will be determined using techniques known to one skilled in the art.

Dosage may be based on the amount of the composition or active compound thereof (*e.g.*, selective inhibitor of ATP production) per kg body weight of the patient.

5 For example, a range of amounts of compositions or compound encapsulated therein are contemplated, including about 0.001, 0.01, 0.1, 0.5, 1, 10, 15, 20, 25, 50, 75, 100, 150, 200 or 250 mg or more of such compositions per kg body weight of the patient. Other amounts will be known to those of skill in the art and readily determined.

10 In certain embodiments, the dosage of the composition or active compound thereof (*e.g.*, selective inhibitor of ATP production) will generally be in the range of about 0.001 mg to about 250 mg per kg body weight, specifically in the range of about 50 mg to about 200 mg per kg, and more specifically in the range of about 100 mg to about 200 mg per kg. In some embodiments, the dosage is in the range of about 150 mg to about 250 mg per kg. In some embodiments, the dosage is about 200 mg per kg.

15 In some embodiments the molar concentration of the composition or active compound thereof (*e.g.*, selective inhibitor of ATP production) in a pharmaceutical composition will be less than or equal to about 2.5 M, 2.4 M, 2.3 M, 2.2 M, 2.1 M, 2 M, 1.9 M, 1.8 M, 1.7 M, 1.6 M, 1.5 M, 1.4 M, 1.3 M, 1.2 M, 1.1 M, 1 M, 0.9 M, 0.8 M, 0.7 M, 0.6 M, 0.5 M, 0.4 M, 0.3 M or 0.2 M. In some embodiments the concentration of the
20 composition or active compound thereof (*e.g.*, selective inhibitor of ATP production) will be less than or equal to about 0.10 mg/ml, 0.09 mg/ml, 0.08 mg/ml, 0.07 mg/ml, 0.06 mg/ml, 0.05 mg/ml, 0.04 mg/ml, 0.03 mg/ml or 0.02 mg/ml.

Alternatively, the dosage may be determined by reference to the plasma concentrations of the composition or active compound thereof (*e.g.*, selective inhibitor of
25 ATP production). For example, the maximum plasma concentration ($C_{\max}$) and the area under the plasma concentration-time curve from time 0 to infinity (AUC (0-4)) may be used. Dosages for the present invention include those that produce the above values for $C_{\max}$ and AUC (0-4) and other dosages resulting in larger or smaller values for those parameters.

30 Actual dosage levels of the active ingredients in the compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The selected dosage level will depend upon a variety of factors including the activity of the particular therapeutic agent in the formulation employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion or metabolism of the particular therapeutic agent being employed, the duration
5 of the treatment, other drugs, compounds and/or materials used in combination with the particular compound employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A physician or veterinarian having ordinary skill in the art can readily determine
10 and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could prescribe and/or administer doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

In general, a suitable daily dose of a compound of the invention will be that
15 amount of the compound which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above.

If desired, the effective daily dose of the active compound may be administered as
20 two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms.

The precise time of administration and amount of any particular compound that will yield the most effective treatment in a given patient will depend upon the activity, pharmacokinetics, and bioavailability of a particular compound, physiological condition of the patient (including age, sex, disease type and stage, general physical condition,
25 responsiveness to a given dosage and type of medication), route of administration, and the like. The guidelines presented herein may be used to optimize the treatment, *e.g.*, determining the optimum time and/or amount of administration, which will require no more than routine experimentation consisting of monitoring the subject and adjusting the dosage and/or timing.

While the subject is being treated, the health of the patient may be monitored by
30 measuring one or more of the relevant indices at predetermined times during a 24-hour period. All aspects of the treatment, including supplements, amounts, times of administration and formulation, may be optimized according to the results of such

monitoring. The patient may be periodically reevaluated to determine the extent of improvement by measuring the same parameters, the first such reevaluation typically occurring at the end of four weeks from the onset of therapy, and subsequent reevaluations occurring every four to eight weeks during therapy and then every three months thereafter. Therapy may continue for several months or even years, with a minimum of one month being a typical length of therapy for humans. Adjustments, for example, to the amount(s) of agent administered and to the time of administration may be made based on these reevaluations.

Treatment may be initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage may be increased by small increments until the optimum therapeutic effect is attained.

As described above, the composition or active compound thereof (*e.g.*, selective inhibitor of ATP production) may be administered in combination with radiation therapy. An optimized dose of radiation therapy may be given to a subject as a daily dose.

Optimized daily doses of radiation therapy may be, for example, from about 0.25 to 0.5 Gy, about 0.5 to 1.0 Gy, about 1.0 to 1.5 Gy, about 1.5 to 2.0 Gy, about 2.0 to 2.5 Gy, and about 2.5 to 3.0 Gy. An exemplary daily dose may be, for example, from about 2.0 to 3.0 Gy. A higher dose of radiation may be administered, for example, if a tumor is resistant to lower doses of radiation. High doses of radiation may reach, for example, 4 Gy.

Further, the total dose of radiation administered over the course of treatment may, for example, range from about 50 to 200 Gy. In an exemplary embodiment, the total dose of radiation administered over the course of treatment ranges, for example, from about 50 to 80 Gy. In certain embodiments, a dose of radiation may be given over a time interval of, for example, 1, 2, 3, 4, or 5 mins., wherein the amount of time is dependent on the dose rate of the radiation source.

In certain embodiments, a daily dose of optimized radiation may be administered, for example, 4 or 5 days a week, for approximately 4 to 8 weeks. In an alternate embodiment, a daily dose of optimized radiation may be administered daily seven days a week, for approximately 4 to 8 weeks. In certain embodiments, a daily dose of radiation may be given a single dose. Alternately, a daily dose of radiation may be given as a plurality of doses. In a further embodiment, the optimized dose of radiation may be a higher dose of radiation than can be tolerated by the patient on a daily base. As such,

high doses of radiation may be administered to a patient, but in a less frequent dosing regimen.

The types of radiation that may be used in cancer treatment are well known in the art and include electron beams, high-energy photons from a linear accelerator or from
5 radioactive sources such as cobalt or cesium, protons, and neutrons. An exemplary ionizing radiation is an x-ray radiation.

Methods of administering radiation are well known in the art. Exemplary methods include, but are not limited to, external beam radiation, internal beam radiation, and radiopharmaceuticals. In external beam radiation, a linear accelerator is used to
10 deliver high-energy x-rays to the area of the body affected by cancer. Since the source of radiation originates outside of the body, external beam radiation can be used to treat large areas of the body with a uniform dose of radiation. Internal radiation therapy, also known as brachytherapy, involves delivery of a high dose of radiation to a specific site in the body. The two main types of internal radiation therapy include interstitial radiation,
15 wherein a source of radiation is placed in the effected tissue, and intracavity radiation, wherein the source of radiation is placed in an internal body cavity a short distance from the affected area. Radioactive material may also be delivered to tumor cells by attachment to tumor-specific antibodies. The radioactive material used in internal radiation therapy is typically contained in a small capsule, pellet, wire, tube, or implant.
20 In contrast, radiopharmaceuticals are unsealed sources of radiation that may be given orally, intravenously or directly into a body cavity.

Radiation therapy may also include stereotactic surgery or stereotactic radiation therapy, wherein a precise amount of radiation can be delivered to a small tumor area using a linear accelerator or gamma knife and three dimensional conformal radiation
25 therapy (3DCRT), which is a computer assisted therapy to map the location of the tumor prior to radiation treatment.

Toxicity and therapeutic efficacy of subject compounds may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD₅₀ and the ED₅₀. Compositions that exhibit large therapeutic indices
30 are preferred. In some embodiments, the LD₅₀ (lethal dosage) can be measured and can be, for example, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000% or more reduced for the cyclodextrin-encapsulated selective ATP inhibitor compositions described herein relative

to the selective ATP inhibitor without any cyclodextrin encapsulation. Similarly, the ED₅₀ (*i.e.*, the concentration which achieves a half-maximal inhibition of symptoms) can be measured and can be, for example, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000% or more increased for the cyclodextrin-encapsulated selective ATP inhibitor compositions described herein relative to the selective ATP inhibitor without any cyclodextrin encapsulation. Also, Similarly, the IC₅₀ (*i.e.*, the concentration which achieves half-maximal cytotoxic or cytostatic effect on cancer cells) can be measured and can be, for example, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000% or more increased for the cyclodextrin-encapsulated selective ATP inhibitor compositions described herein relative to the selective ATP inhibitor without any cyclodextrin encapsulation. Although compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets the compounds to the desired site in order to reduce side effects.

In some embodiments, the presently disclosed methods produce at least about a 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or even 100% inhibition of cancer cell growth in an assay.

In any of the above-described methods, the administering of the selective ATP production inhibitor/cyclodextrin complexes can result in at least about a 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or even 100% decrease in a solid malignancy in a subject, compared to the solid malignancy before administration of the selective ATP production inhibitor/cyclodextrin complexes.

In some embodiments, the therapeutically effective amount of a complex of a selective ATP production inhibitor/cyclodextrin is administered prophylactically to prevent a solid malignancy from forming in the subject.

In some embodiments, the subject is human. In other embodiments, the subject is non-human, such as a mammal.

The data obtained from the cell culture assays and animal studies may be used in formulating a range of dosage for use in humans. The dosage of any supplement, or alternatively of any components therein, lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary

within this range depending upon the dosage form employed and the route of administration utilized. For agents of the present invention, the therapeutically effective dose may be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ as determined in cell culture. Such information may be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

G. Kits

The selective ATP production inhibitor/cyclodextrin complexes and compositions described herein can be assembled into kits or pharmaceutical systems for use in treating or preventing a disease, such as cancer. In some embodiments, the 3-BrPA-cyclodextrin complex and compositions can be used to prevent or treat solid malignancies caused by a cancer. In general, a presently disclosed kit contains some or all of the components, reagents, supplies, and the like to practice a method according to the presently disclosed subject matter. A kit typically comprises an effective amount of complex to prevent, delay, reduce, or treat an unwanted disease (*e.g.*, a solid malignancy). In some embodiments, a kit comprises at least one container (*e.g.*, a carton, bottle, vial, tube, or ampoule) comprising a selective ATP production inhibitor/cyclodextrin complex and/or compositions thereof described herein. Typically, the complex and/or compositions will be supplied in one or more container, each container containing an effective amount of complex to allow a solid malignancy to regress, slow, or be arrested.

Accordingly, in some embodiments, the presently disclosed subject matter provides a kit comprising at least one selective ATP production inhibitor encapsulated within at least one cyclodextrin carrier. In other embodiments, the kit further comprises a set of instructions for using the at least one selective ATP production inhibitor encapsulated within the at least one cyclodextrin carrier.

It may be desirable to store the selective ATP production inhibitor and cyclodextrin separately and then combine them before use. Accordingly, in still other embodiments, the kit comprises at least one selective ATP production inhibitor in one container and at least one cyclodextrin carrier in another container.

Exemplification

The following Examples have been included to provide guidance to one of ordinary skill in the art for practicing representative embodiments of the presently disclosed subject matter. In light of the present disclosure and the general level of skill in the art, those of skill can appreciate that the following Examples are intended to be exemplary only and that numerous changes, modifications, and alterations can be employed without departing from the scope of the presently disclosed subject matter. The following Examples are offered by way of illustration and not by way of limitation.

10 **Example 1: Materials and Methods**

A. Study Design

A formulation of the alkylating agent 3BP that would be resistant to serum degradation and be systemically administered to mice was designed and tested. A particular formulation in which ME3BP-7 was encapsulated in a modified cyclodextrin and proved superior to the others tested, and its characteristics were evaluated *in vitro* and *in vivo*. For this purpose, pancreatic cancer cells were established in which MCT-1, the cell membrane receptor for 3BP, was genetically inactivated using CRISPR-mediated technologies. Pancreatic cancer cell lines that expressed various fluorescent or bioluminescent markers were also established to track the responses of cells to ME3BP-7 as well as other drugs commonly used in the clinic to treat patients with PDAC. The MCT-1-dependent killing potential of ME3BP-7 was measured with time-lapse recordings using an IncuCyte® Live Cell-Analysis System (Essen Bioscience). Tumor cells or tumor fragments were orthotopically implanted in the pancreas for all animal experiments. Appropriate implantation was confirmed with luciferase expression in cell lines or ultrasound, followed by stratification and randomization into treatment and control groups. The identities of the treatment groups in the experiments with patient-derived xenografts were blinded to the ultrasound technician. Necropsies were performed for tumor resection and organ evaluation at the end of the experiment. Sample sizes for animal experiments were selected based on previous experience with the animal models but were not predetermined by power analysis. No animals were excluded because of illness from the study unless indicated. The number of replicates in each experiment is noted in the figure legends.

B. General method for synthesis of modified β -cyclodextrins

Succinyl- β -cyclodextrins can be purchased from Sigma Chemical (St. Louis, MO, USA; Catalog No. 85990). Unmodified β -cyclodextrin and α -cyclodextrin were purchased (Sigma-Aldrich, St. Louis, MO). However, succinylated cyclodextrins can also be synthesized. For example, β -cyclodextrin (Sigma-Aldrich, St. Louis, MO) was mono-
5 tosylated with 0.9 molar equivalent of tosyl chloride in pyridine at the primary 6' hydroxyl group to afford the corresponding tosylate, which was converted to the iodo-derivative by treatment with sodium iodide in acetone. The iodo derivative was converted to the desired 6' aminated cyclodextrin by heating at 80°C for 8-12 hours with the appropriate amine (Tang and Ng (2008) *Nat. Protocol.* 3:691-697). 6' mono-
10 succinyl- β -cyclodextrin was synthesized by treatment of parent β -cyclodextrin with 0.9 equivalents of succinic anhydride in DMF (Cucinotta *et al.* (2005) *J. Pharmaceut. Biomed. Anal.* 37:1009-1014). The product was precipitated in acetone and purified by HPLC before use.

The pH range with optimal stability is pH 4-9.

C. General procedures of preparation of encapsulated complexes

Drug batches for the study disclosed herein were made as described in WO 2015/108933, incorporated by reference in its entirety. Briefly, a solid sample of 3BP (1 mmol, 167 mg) was added in small portions to a solution of the corresponding cyclodextrin (1.0-1.2 mmol) in 25 ml of deionized (DI) water under constant agitation.
20 After complete addition over 15-20 minutes, the samples were further agitated for 1-4 hours at 25°C. Finally, the sample was flash-frozen using liquid nitrogen or dry ice/acetone and lyophilized overnight. The lyophilized reagent was diluted into PBS within 15 and 30 minutes prior to in vitro and in vivo experiments, respectively.

Alternatively, a 1:1 ratio of 3-BrPA encapsulated within succinyl- β -cyclodextrins may be prepared as follows. 3-BrPA (150 mg, 1 mmol) was added in small portions (10 mg each) to a stirring solution of succinyl-beta-cyclodextrin (1,500 mg in distilled water). After complete addition, the solution was sonicated for 1 hour at room temperature. The sonicated solution was then allowed to shake overnight on a thermomixer at 25°C, flash frozen in a dry ice-acetone bath, and lyophilized.

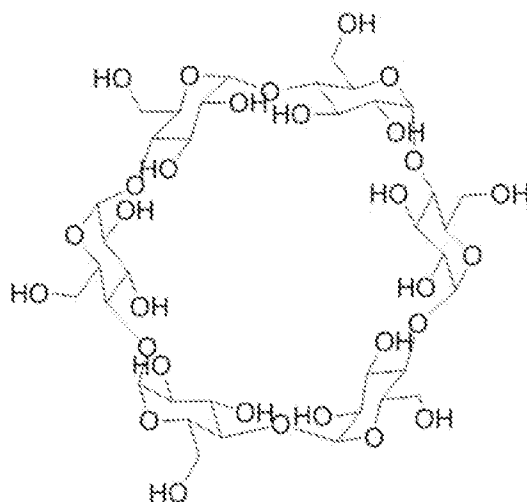
Similarly, a 2:1 ratio of 3-BrPA encapsulated within succinyl- β -cyclodextrins may be prepared. 3-BrPA (166 mg, 1 mmol) was added in small portions (10 mg each) to a stirring solution of succinyl-beta-cyclodextrin (918 mg in 20 ml distilled water). After complete addition, the solution was sonicated for 1 hour at room temperature. The

sonicated solution was then allowed to shake overnight on a thermomixer at 25°C, flash frozen in a dry ice-acetone bath, and lyophilized.

A 1:1.2 ratio of 3-BrPA encapsulated within succinyl- β -cyclodextrins may be prepared. 3-BrPA (166 mg, 1 mmol) was added in small portions (10 mg each) to a stirring solution of succinyl-beta-cyclodextrin (1800 mg in 24 ml distilled water). After complete addition, the solution was sonicated for 1 hour at room temperature. The sonicated solution was then allowed to shake overnight on a thermomixer at 25°C, flash frozen in a dry ice-acetone bath, and lyophilized.

In addition, a 1:1 ratio of 3-BrPA encapsulated within α -cyclodextrins (see structure below) may be prepared. 3-BrPA (166 mg, 1 mmol) was added in small portions (10 mg each) to a stirring solution of alpha-cyclodextrin (972 mg, 1 mmol in 10 ml distilled water). After complete addition, the solution was sonicated for 1 hour at room temperature. The sonicated solution was then allowed to shake overnight on a thermomixer at 25°C, flash frozen in a dry ice-acetone bath, and lyophilized. Non-GRAS and GRAS versions were used with similar results.

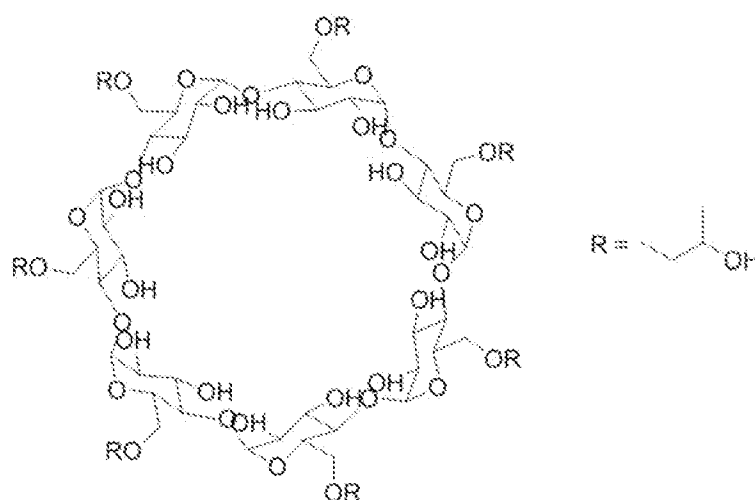
Structure 1: α -cyclodextrin structure



It was determined that cyclodextrins modified to replace one or more hydroxyl groups on one or more of its α -D-glucopyranoside units with ionizable groups resulting in negative charges (anions) stabilizes the 3-halopyruvates better than those having ionizable groups resulting in positive charges (cations) or unmodified cyclodextrins, such as unmodified alpha- or beta-cyclodextrin. It was also surprisingly determined that β -cyclodextrins encapsulate 3-BrPA in a form that protects and stabilizes 3-BrPA for *in vivo* efficacy especially and also *in vitro* efficacy significantly better than α -cyclodextrins.

In addition, *in vitro* cell culture and *in vivo* mouse treatments were prepared and performed as described above and below for succinyl- β -cyclodextrins encapsulating 3-BrPA. Using generally recognized as safe (GRAS) versions of β -cyclodextrins (*e.g.*, hydroxypropyl- β -cyclodextrin having a level of substitution of 3-5 such as that shown in chemical form below) encapsulating 3-BrPA and the results were similar to those described for succinyl- β -cyclodextrins encapsulating 3-BrPA. In some embodiments, the succinyl- β -cyclodextrins encapsulating 3-BrPA comprises an average of 3.4 succinyl groups per cyclodextrin.

Structure 2: GRAS hydroxypropyl- β -cyclodextrin structure



D. Size-exclusion HPLC chromatography

SEC chromatography using a Shodex-OH Pak was performed using elution at 1ml/min of PBS under isocratic conditions and monitored at 220 nm. The samples shown (FIG. 3A) included i) free 3BP (1 mg/mL); ii) succinyl- β -CD without 3BP (20 mg/mL); iii) a mixture of 10 μ L of free 3BP at 1 mg/mL and 10 μ L ME3BP-7 at 10 mg/mL; and iv) ME3BP-7 (10 mg/mL).

E. Cell Culture

Human PDAC cell lines, including MIA PaCa-2, PSN-1, Panc 02.13, AsPC-1, BxPC-3, and CFPAC-1 were obtained from the American Type Cell Culture (ATCC) (Manassas, VA, USA). The cancer cells were maintained at 37°C in a humidified 5% CO₂ atmosphere in T75 tissue culture flasks containing 14 mL of the medium recommended by ATCC (*i.e.*, DMEM for MIA PaCa-1 parental and MCT-1-KO, RPMI 1640 for Panc 02.13, AsPC-1, BxPC-3, DLD-1, and IMDM media for CFPAC-1. All culture media were supplemented with 10% fetal bovine serum (Mediatech, Inc, Manassas, VA, USA) and 1 % penicillin-streptomycin. Cells were passaged at the ATCC recommended ratio every 4-5 days by trypsinization and resuspended in fresh medium in a new flask. Only early passages cells harvested at 70-85% confluence was used for *in vitro* and *in vivo* experiments.

F. Lentiviral transduction

A CMV-Firefly luciferase lentivirus carrying a puromycin-selectable marker (Cellomics Tech), was used to transduce MIA PaCa-2 or Panc 02.13 cells according to the lentivirus manufacturer. Luciferase-expressing cells were selected with puromycin at 4 µg/mL in the growth medium. Lentiviral NucLight Red and Green vectors (Sartorius) carrying a puromycin-selectable marker were similarly used to transduce parental and MCT-1-KO MIA PaCa-2 cells, respectively, and also selected with puromycin at 4 µg/mL.

G. Viability assays

Three thousand to twelve thousand cells were seeded into the wells of 96-well plates and treated with various amounts of drug 24 h after plating. For MIA PaCa-2 parental and MCT-1-KO co-culture experiments, an aliquot with an equal concentration of each cell type was prepared, and 24,000 cells were seeded in the wells (12,000 of each genotype). After treatment for various time periods, cells were imaged with time-lapse fluorography using IncuCyte® Live Cell-Analysis System (Essen Bioscience). For green- and red-labeled cells, a processing definition for the IncuCyte® was created, and the green or red fluorescence channels were used for analysis. All *in vitro* experiments were conducted at least in duplicate, with consistent results. Green and red object counts were used to track parental and MCT-1 KO MIA PaCa-2 experiments, while confluence was used to track unlabeled cells. Cell viabilities are recorded as the fraction of cells that survived treatment at the indicated drug molarities relative to the control wells. Dose-response curves were fit using Prism 9 software.

H. Serum stability assays

Free 3BP, ME3BP-7, and HPCD-3BP were incubated at various concentrations (12.5 µM, 25 µM, 50 µM, 100 µM, and 200 µM) with 90 µL of human sera (Sigma, Cat No. H3667) at 37°C for up to 8 hours. Aliquots were collected at 30 minutes, 1 hour, 2 hours, 4 hours, and 8 hours and stored at -80°C until further analyses. A cell toxicity assay was used to assess the amount of biologically active 3BP at each time point. For this purpose, parental DLD-1 (MCT-1, 387 TPM) cells were plated at 35-40% confluence and 10 µL of collected sample was diluted with 190 µL of media. Cell death was measured by imaging cells after 72 hours of culture using the IncuCyte® Live Cell-Analysis System (Essen Bioscience). We estimated residual drug activity by comparison of cell death induced by samples incubated with human sera for different time points, to the cell death induced by an unincubated sample to derive % drug activity.

I. Timed exposures to the drug

Cells were seeded in 96-well plates at a density of 24,000 cells per well (12,000 parental MIA PaCa-2 red and 12,000 MCT-1-KO MIA PaCa-2 green). Twenty-four hours after plating,

cells were treated with vehicle (complete DMEM media) or a serial dilution of the drug of choice in the media described above. Each treatment condition was conducted in triplicate unless noted otherwise. Drugs were purchased from Aldrich (Bromopyruvic acid) and Selleck Chemicals (Gemcitabine HCl, Irinotecan, Oxaliplatin, 5-Fluorouracil). The drugs were removed from the plates at 30 minutes, 2 hours, or retained in the plates continuously. To ensure no residual drug was left in the wells, those were gently washed with 200 uL of PBS twice and replaced with fresh media. Residual cells were measured with IncuCyte® Live Cell-Analysis System. Cell viabilities are reported as the percentage of cells that remained after treatment at the indicated drug molarities relative to the average of three control wells without drug.

J. Genetic inactivation of MCT-1

The Alt-R CRISPR system (Integrated DNA Technologies, IDT) was used to delete SLC16A1, the gene encoding MCT-1 protein, in the DLD-1 and MIA PaCa-2 cell lines. The gRNA sequence was designed using CHOPCHOP v.3 12. Alt-R CRISPR Cas9 crRNAs (ACCATGCCATTCAGGCTAGT, IDT; SEQ ID NO:1) and Alt-R CRISPR-Cas9 tracrRNA (1072532, IDT) were re-suspended at 100 µM with Nuclease-Free Duplex Buffer (IDT). The crRNAs and tracrRNA were mixed at a 1:1 molar ratio and denatured for 5 min at 95°C, followed by slow cooling to room temperature to duplex before mixing with Cas9 Nuclease (1081059, IDT) at a 1.2:1 molar ratio for 15 min. Forty pmoles of the Cas9 ribonucleoprotein (RNP) containing tracrRNA/MCT-1 crRNA duplex was mixed with 2×10^5 cells in 20 µL of OptiMEM (31985088, ThermoFisher Scientific). This mixture was loaded into a 0.1 cm cuvette (1652089, Bio Rad) and electroporated at 120 V for 16 ms using an ECM 2001 (Harvard Apparatus). Cells were immediately transferred to complete growth medium and cultured for one week. Upon reaching confluence, cells were plated at a density of 0.5 to 2 cells per well in 96-well plates and cultured for 3 weeks. Single colonies were transferred into 2 replica 96-well plates. Genomic DNA was harvested from one of the plates using the Quick-DNA™ 96 Kit (Zymo Research) and PCR amplified using Q5® Hot Start High-Fidelity 2X Master Mix (New England BioLabs). SafeSeqS[52] was to confirm the mutation status of selected clones. Single MIA PaCa-2 clones with successful MCT-1 deletion, as evidenced by sequencing, were collected from a matched replica plate and six clones mixed together at equal ratios for further experiments, as described in the main text.

K. Optimization of MCT-1 IHC protocol

Various concentrations of five commercially available antibodies were tested for specificity using DLD-1 parental cells as positive and the isogenic DLD-1 cells in which MCT-1 was genetically inactivated as negative controls. Cells were trypsinized, washed in media to

inactivate the trypsin, and then spun at 400xg for 10 min to form a cell pellet which was subsequently fixed in 10% formalin and embedded in paraffin. Four μ m sections were cut from formalin-fixed, paraffin-embedded blocks of these cells and evaluated with various dilutions of the antibodies and species-appropriate secondary antibodies. The anti-MCT-1 mouse antibody from Santa Cruz Biotechnology (SC-365501, Lot number D2319) was found to be the most specific for MCT-1 as assessed by staining DLD-1 cells with and without MCT-1, and this antibody was used for subsequent immunostaining with a Ventana Discovery Ultra autostainer (Roche Diagnostics) at the Oncology Tissue Services Core of Johns Hopkins University School of Medicine. Briefly, following dewaxing and rehydration on board, epitope retrieval was performed using Ventana Ultra CC1 buffer (catalog# 6414575001, Roche Diagnostics) at 96°C for 64 minutes. The anti-MCT-1 antibody from Santa Cruz Biotechnology was diluted 1:2000 for cell pellets, 1:200 for patient derived xenografts grown in mice, and 1:100 for other tissues or tissue microarrays. Dilutions were all in antibody dilution buffer (catalog# 5280524001, Roche Diagnostics) solution and applied at 36°C for 60 minutes to the slides. Following standard washing in the Ventana auto-stainer, bound antibodies were detected with an anti-mouse HQ detection system (catalog# 7017936001 with 7017782001, Roche Diagnostics) and a Chromomap DAB IHC detection kit (catalog # 5266645001, Roche Diagnostics). The slides were then counterstained with Mayer's hematoxylin, dehydrated, and mounted in Toluene mounting medium (MER 7720, Mercedes Scientific).

L. Tissue microarrays

Two commercially available FFPE tissue microarrays from US Biomax, INC were used to evaluate IHC of MCT1 as described above. A 100 total cases were reviewed. BC001130 included 20 cases of pancreatic carcinoma (triplicate cores per case), and HPanA150CS03 included 80 cases with adjacent normal tissue. Two independent reviewers, including a pathologist, rated the intensity and pattern of MCT1 staining for tumor and normal tissues. Any differences in grading were reviewed and solved.

M. Animal experiments

Six- to eight-week-old female mice were maintained according to the JHU Animal Care and Use Committee-approved research protocol MO18M79. Crl:NU(NCr)-*Foxn1*tm (athymic nude mice) from Charles River (490) were used for the experiments described in FIG. 6A – FIG.6D. Patient-derived xenografts would not uniformly grow in athymic nude mice and more severely immunodeficient mice were used for these experiments. Different types of severely immunocompromised used because of the limited availability of these mice from the suppliers during the COVID pandemic. NOD.Cg-Prkdc Il2rg /SzJ (NSG) acquired from the Jackson

Laboratory (005557) were used for the experiments described in FIG. 7A-H. NOD-*Prkdc^{em26Cd52}Il2rg^{em26Cd22}*/NjuCrl (NCG) from Charles River (572) were used for the patient-derived xenografts reported in FIG. 8A-E.

Models

5 **Panc02.13:** Orthotopic tumors were generated by implanting 1.5 million luciferase-expressing Panc 02.13 cells (with 10% Matrigel) in the pancreas of 20 nude mice. The tumors were allowed to grow for 13 days, at which point tumor burdens were evaluated using IVIS Live cell imaging system. Fifteen animals with similar bioluminescence signals were then stratified and randomized into 3 cohorts. On day 16, a second baseline image was recorded, and treatment was
10 initiated on day 17 post-implantation. Animals in the control group were administered 200 uL of PBS (vehicle), while the two treatment arms received 33 and 41 mg/Kg of ME3BP-7 in 200 ul PBS, respectively. Appropriate doses were calculated and delivered based on the animals' body weights, which were measured immediately prior to dosing. Treatments were administered by a single intravenous (i.v.) bolus delivered over 30 seconds for 4 weeks on each Monday,
15 Wednesday, and Friday (total of 12 injections), with bioluminescence recorded once a week. At the end of four weeks, all animals were euthanized and their tumor weighed.

TM01212: 20 NSG mice were orthotopically transplanted with 2 x 1 x 1 mm sized pieces of PDX tumors harvested from 3 hosts bearing subcutaneous tumors of TM01212. US at Day 13 ensured that the orthotopically implanted tumors were efficiently transplanted (take rate 90%),
20 and study subjects were randomized into 2 groups of 10 mice each. The control group was infused with the inactive ingredient (270 mg/kg sCD), while the treatment group was treated with ME3BP-7 formulated at 25 mg/kg of 3BP. Bolus injections via the tail vein were administered every Monday, Wednesday, and Friday for four weeks (12 total doses) starting at Day 20 post tumor implantation. Weekly US measurements were used to track tumor growth and animals
25 euthanized on day 35 after treatment initiation to assess tumor burden in the pancreas, lung, and liver.

TM01098: 29 NCG mice (17 with surgically implanted vascular access buttons and 12 without) were transplanted with 2 x 3 x 3 mm sized pieces of xenografts harvested from 3 hosts bearing subcutaneous versions of the TM01098. US on day 9 confirmed successful transplantation (take
30 rate 76%). 5 mice died from VAB related complications and were removed from the study. Tumor bearing hosts were separated into 2 groups; the control group (n=7) was treated (200 uL of PBS) *via* tail vein i.v bolus infusion (Monday, Wednesday, and Friday), while the treatment group (n=11) received 21mg/kg 3BP as ME3BP-7 via a VAB at the same schedule (FIG. 8A). US measurements were taken weekly. Treatment was started on post-implantation day 14, and

the hosts were subjected to this regimen for 5 weeks. Following a final ultrasound on treatment day 38, the mice were euthanized on day 38 post-treatment initiation and tumor burden in the pancreas and at distal sites was assessed by necropsy.

Surgeries

5 For orthotopic models, cells or small pieces of xenografts were implanted into the pancreas of each mouse as a modification of previously described techniques[53]. After anesthesia, the skin over the left abdomen was shaved if necessary and sterilized. A horizontal 1 cm skin incision was made over the left upper quadrant of the abdomen, right below the ribs. The underlying peritoneum was incised, and the spleen and pancreas were located. The pancreatic
10 tissue was gently extruded from the abdominal cavity for good visualization. At that point, either a cell aliquot was injected with a Hamilton syringe, or a small piece of tumor was stitched to the tail of the pancreas with a 5-0 prolene suture avoiding major vessels. The implantation of the tumor fragment was standardized by size, time from extraction to implantation, and implantation technique in the tail of the pancreas in all cases. The pancreas with implanted cells/tumor was
15 gently internalized in the abdominal cavity. The peritoneal layer was closed using absorbable sutures, and the skin incision was closed using wound clips. Wound clips were removed once the incision site had healed, usually ~10 days after surgery.

Mice were euthanized at the termination of the experiment or if major weight loss or toxicity was observed following JHU Animal Care and Use Committee standards. After
20 weighting, tumors and organs were placed in 10% formalin, processed, and fixed in paraffin. Standard H&E staining was performed, and an expert comparative pathologist reviewed all presented data.

In vivo-imaging for orthotopic Panc 02.13 animal model

Luminescence quantification was performed using the IVIS imaging system and Living
25 Image software (Perkin Elmer). Before imaging, mice were anesthetized at 37°C using inhaled isoflurane in an induction chamber for 5 min and received an intraperitoneal injection of luciferin (150 µl, RediJect D- Luciferin Ultra Bioluminescent Substrate, PerkinElmer, 770505). Control fluorescence images were obtained each time to confirm satisfactory intraperitoneal
Bioluminescence images were taken 13 minutes after injection.

Ultrasound protocol for orthotopic PDX animal model

A modification of an ultrasound imaging method for pancreas imaging in mice was used.²⁰ In brief, the mouse's left flanks were shaved with a clipper. Mice were injected with 2 mL of 0.9% sterile saline intraperitoneally to increase the contrast between intrabdominal organs. Anesthesia was induced with isoflurane in the induction chamber for 5 minutes. The mice were

placed in the lateral recumbent position with the left flank up on the imaging over a heated pad. Continuous anesthesia was applied through a face cone. A high-resolution ultrasound VisualSonics Vevo2100 High-Resolution Ultrasound System was then used to detect and measure pancreatic tumors. A 15 mm depth US window in B-mode acquisition was used in all cases. After applying gel over the area for US visualization, images were obtained in a standardized fashion. The spleen, liver, left kidney, and pancreas were always identified first to confirm correct positioning. Tumors were hypoechoic (dark/gray), with the surrounding pancreas being hyperechoic (bright/white). Trans-axial US images of the tumor were obtained by placing the ultrasound probe parallel to the rib cage with the notched side to the left (pointing anteriorly). Then, longitudinal US images were obtained by placing the US probe parallel to the mouse axis with the notch side pointing superiorly. Videos were acquired at the point where the maximum tumor diameter was visualized and multiple images were saved to visualize and measure the entire tumor. For those mice with several discrete tumors (e.g., at the peritoneal wall), the same process was repeated for each tumor. After imaging, mice were cleaned and allowed to wake up from anesthesia. To obtain measurements, the desired files were loaded using the ultrasound software study management function. Maximal longitudinal and transversal diameters were obtained for each tumor image. To calculate tumor volumes, the formula $V = a \cdot b^2 / 2$, where (a) is largest and (b) is the smallest diameter within the 4 obtained measurements (2 trans-axial and 2 longitudinal).²¹

N. Statistical methods

Data are presented as means $\pm$ SD unless otherwise specified. Statistical analyses were carried out using the specific tests indicated in the main text figure legends. A P value of <0.05 was used to denote statistical significance unless otherwise indicated. All analyses and graph production were performed using Prism version 9.0 (GraphPad) or Microsoft Excel.

Example 2: *MCT1 expression mediates sensitivity of PDAC cell lines to 3BP*

Careful comparison of RNAseq datasets from TCGA, the Cancer Cell Line Encyclopedia (CCLE) with The Genotype-Tissue Expression (GTEx) portal revealed that ~20-25% of PDACs exhibit a marked elevation of Monocarboxylate Transporter 1 (MCT-1) expression. The activity of 3BP as free drug was then explored in a representative panel of PDAC cell lines: MIA PaCa-2, PSN-1, Panc 02.13, AsPC-1, BxPC-3, and CFPAC-1. These cells were treated with increasing concentrations of 3BP (0 to 220 μ M) and assessed for cell death by real-time quantitative live-cell imaging. All cell lines except CFPAC-1 were sensitive to 3BP, with their IC50s ranging from 24-40 μ M (FIG. 1A and FIG. 1B). Even high concentrations (220 μ M) of 3BP did not affect

the viability CFPAC-1. The RNA expression levels of three cellular transporters[28, 29]: GLUT-1, MCT-1 and MCT-4 in these lines are presented in FIG. 1B. The expression of MCT-1 was much lower in the 3BP-resistant cell line CFPAC-1 than any of the other five cell lines, while there was no clear relationship between 3BP-resistance and the expression of the other
5 transporters. MCT-1 immunohistochemical (IHC) analyses of these cell lines using the protocol described herein showed that the expression of the MCT-1 protein on the cell surface reflected its RNA levels (FIG. 1C).

Though the observations disclosed herein are consistent with a role for MCT-1 in the cytotoxicity of 3-BP to pancreatic cancer cells, the optimum way to demonstrate specificity is to
10 genetically inactivate MCT-1 and compare the inactivated cells to their parental counterparts. This is similar to the strategy used to establish the essential nature of MCT-1 for the effects of 3BP in KBM7 cells, a unique near haploid cell line derived from a leukemia[26]. *SLC16A1*, the gene encoding MCT-1, was deleted in MIA PaCa-2. MIA PaCa-2 is sensitive to 3BP and expresses high levels of MCT-1 (FIG. 1A, FIG. 1B). The successful knockout of MCT-1 was
15 documented in six single clones by next-generation sequencing (FIG. 2A, FIG. 2B) and the results validated using IHC (FIG. 2C, FIG. 2D). The six monoclonal cell lines were then mixed at equal ratios to create an MCT-1 KO population (henceforth denoted as KO cells) thereby avoiding the potential confounders associated with the evaluation of a single clone. [30, 31]

Next, a nuclear-restricted green fluorescent protein was introduced into the KO cells and
20 a nuclear-restricted red fluorescent protein into the parental cells. Co-culture of these cells allowed simultaneous visualization of the parental and KO cells under the identical microenvironmental conditions. KO cells grew at a rate indistinguishable from the parental cells under normal culture conditions (FIG. 2E). However, in the presence of 50 μ M 3BP, the parental cells, with an IC₅₀ of 24 μ M (FIG. 2G), were almost completely eliminated while the KO cells
25 continued to proliferate (FIG. 2F and FIG. 11). These results indicated specificity of 3BP for cells expressing MCT1.

Example 3: Encapsulation of 3BP protects 3BP from degradation by human serum

As noted herein, one of the major problems with using 3BP as a chemotherapeutic
30 agent is that it is rapidly inactivated by the serum proteins that it alkylates before it has a chance to leave the circulation and enter cancer cells.[25] Encapsulation of 3BP with a cyclodextrin has shown that it can mitigate its toxicity, presumably by permitting the use of lower concentrations of the encapsulated drug compared to the free drug. The study disclosed herein represents a

microencapsulation procedure which was then tested for increased the serum stability of the drug.[27]

For microencapsulation of 3BP in cyclodextrins, β -cyclodextrins were chosen rather than alpha or gamma cyclodextrins as the base, given the structural modeling such as previously described.[27] Two types of β -cyclodextrin modifications were evaluated, one with its hydroxyls substituted with succinyl groups and the other with hydroxyls substituted with 2-hydroxypropyl groups. For those substituted with succinyl groups, the optimum number of hydroxyl substitutions was determined. Finally, the optimal ratio of the cyclodextrin to 3BP was determined.

Various formulations were first evaluated by size exclusion chromatography to ensure that the amount of free 3BP was minimized (examples in FIG. 3A). Second, a way to test their stability was designed. Briefly, the formulations were incubated with human sera at 37°C and aliquots were collected at various time points for assessment of cell toxicity on a patient derived cancer cell line (DLD-1) that has particularly high expression of MCT-1 (FIG. 1B) and is accordingly sensitive to 3BP. Because the cytotoxicity of 3BP is lost upon interaction with serum proteins, residual cell toxicity following exposure to serum is directly related to its resistance to serum degradation. Among the β -cyclodextrin formulations tested, succinyl was found to be superior to hydroxypropyl substitutions. Moreover, a succinyl-substituted cyclodextrin with an average of 3.4 succinyl groups per cyclodextrin, at a molar ratio of 1.2 β -cyclodextrin per 3BP, performed best. This MicroEncapsulated formulation was named “ME3BP-7”. Although free 3BP lost 90% of its activity by 30 minutes of exposure to serum, ME3BP-7 lost <10% of its activity in 30 minutes (FIG. 3B). Notably, even after 8 hours of incubation with serum, ME3BP-7 retained >70% of its activity (FIG. 3B). Hydroxypropyl-substituted cyclodextrin-3BP complexes were more stable than free 3BP, losing just over half of activity after 30 minutes of exposure to serum, but not nearly as stable over long periods as ME3BP-7 (FIG. 3B). Importantly, the encapsulation of 3-BP within the ME3BP-7 compound retained both its cytotoxicity as well as its specificity for cells expressing MCT-1, as evidenced by experiments with parental MiaPaCa-2 and KO cells (FIG. 3C).

Example 4: ME3BP-7 results in rapid target-cell killing

During the course of the experiments described herein, both 3BP and ME3BP-7 seemed to rapidly change the morphology of the Mia PaCa-2 cells. To extend this observation, co-cultures of WT and KO cells (plated in equal numbers) were exposed to PBS (control), or drugs at various concentrations and time periods in culture medium, then washed them extensively with

cell culture medium, then followed their growth for 48 hours in the absence of drug. Exposure to either 3BP or ME3BP-7 for as little as 30 minutes led to loss of most of the WT Mia PaCa-2 cells from the culture (FIG. 4 and FIG. 10). In contrast, short exposures of MiaPaCa-2 cells to drugs used to commonly treat pancreatic cancer, such as Gemcitabine or the components of

5 FOLFIRINOX (Irinotecan, Oxaliplatin, and 5-FU) had no differential effect on WT vs. KO cells. Moreover, unlike the dramatic effects of 3BP and ME3BP-7 on cell loss, while the other four drugs decreased the growth rate of MiaPaCa-2 cells, they did not result in any loss of cells from the culture (FIG. 5 and FIG. 11).

10 Example 5: The effects of ME3BP-7 *in vivo*

For *in vivo* comparisons, the feasibility of delivering ME3BP-7 systemically was first tested and the toxicity of free 3BP compared with that of ME3BP-7 in athymic nude mice. An escalating dose ranging from 8.0-33 mg/kg of 3BP (as ME3BP-7) was used via tail-vein injection to identify a maximum tolerated dose of ME3BP-7. Repeated tail vein injections of free 3BP

15 caused scarring of the tail, limiting the ability to use it for sustained injections of the free drug. Much less scarring was evident with injections of ME3BP-7. To provide optimal comparison with the free drug, though, implantable vascular access buttons (VAB) that provided direct access to the jugular vein were used for accurate administration of both drugs. Equivalent amounts of 3BP and ME3BP-7 were infused via VAB (33 mg/kg of 3BP) in 200 uL of PBS

20 every other day for one week. In preliminary experiments, two out of three mice in the free 3BP group became ill with severe weight loss, pallor and decreased activity, and died after only two doses. No overt toxicity was evident after even five weeks of treatment with ME3BP-7 at the same dose (33 mg/kg) as described herein (FIG. 11, lower panel, time-lapse still).

To assess efficacy, orthotopic xenografts were generated with Panc 02.13, a patient-

25 derived PDAC cell line with high expression of MCT-1 was used (RNA expression of 137 transcripts per million) (FIG. 1B). These cells were engineered to express firefly luciferase using a lentivirus vector and orthotopically implanted into the pancreata of nude mice (Methods). Three treatment arms on the basis of intra-vital multiphoton imaging (IVI) and treatment was initiated a day later (FIG. 6A). The control arm (n=4) received vehicle only (PBS), while the other two

30 groups received 33.3 (n=5) or 41 mg/kg (n=4) of 3BP in the form of ME3BP-7. Over the course of four weeks of treatment (dosed Mondays, Wednesdays, and Fridays), there were no noticeable adverse effects or discernible weight loss in the treated animals. (FIG. 12A – FIG.12C) While animals in the control group demonstrated an average 200-fold increase in luminescence signal from baseline (FIG. 6C), animals in both treatment cohorts showed significantly less progression

($p < 0.01$, 1-way ANOVA, FIG. 6B and FIG. 6C). To independently evaluate the effects of treatment on tumor size, animals were euthanized and residual tumors were weighed, confirming the significant difference between the treated vs untreated groups ($p = 0.01$, Mann Whitney U Test, FIG. 6D).

5 The efficacy of ME3BP-7 was then tested on TM01212 tumor cells, a patient-derived xenograft derived from a PDAC. This xenograft was orthotopically transferred to the pancreas of severely immune-deficient mice (methods). The severely immune-deficient mice were used instead of nude mice because orthotopic patient-derived tumors would not uniformly grow in the latter. TM0212 expressed moderately high levels of MCT-1 RNA (37 transcripts per million),
10 consistent with the expression of MCT-1 protein assessed by immunohistochemistry (FIG. 7B). Because these xenografts had never been passaged *in vitro*, they had no bioluminescent signal and non-invasive ultrasonography (US) (FIG. 7C) was used to track tumor growth (FIG. 7A). [32, 33]

Severely immune-deficient mice tolerated less ME3BP-7 (25 mg/kg) and were more
15 susceptible to scarring in the tail after repeated *i.v.* administrations. Despite a lower administered dose (~75% when compared to the dose delivered to the nude mice bearing Panc 02.13 cancers illustrated in FIG. 6A-D), treatment with ME3BP-7 ($n = 7$) resulted in significantly slower tumor growth than observed in the control mice ($n = 9$) ($p < 0.01$, Mann Whitney U test, FIG. 7D). This reduction was confirmed by weighing the tumors after euthanasia of the mice ($p < 0.01$, Mann
20 Whitney U test, FIG. 7E). Two of the treated mice received <80% of the intended dose of ME3BP-7 and were removed from the analysis. Notably, the mice unable to receive the full dose because of tail-scarring responded less well than the other mice.

Implantation of TM01212 into the pancreas resulted in development of duct-like structures within the tumor (FIG. 7A). More importantly, they developed metastasis to the lung
25 and liver, mimicking the behavior of human PDACs (FIG. 7E), ME3BP-7 had a striking effect on the metastatic behavior of these cells. Every one of the nine animals in the control group developed metastasis to the liver or harbored lung metastases. But the mice treated with ME3BP-7 had minimal to no distant metastases, and the number of metastases in the treated vs. untreated mice were strikingly different ($p < 0.01$, Mann Whitney U Test, FIG. 7F). In addition, microscopic
30 evaluation of major organs did not reveal any pathological changes of significance as a result of potential toxicity in the treated mice (FIG. 13).

ME3BP-7 were additionally evaluated in TM01098, another PDAC patient-derived xenograft model according to the timeline described in FIG. 8A. These tumor cells had a relatively high average RNA expression level (96 transcripts per million) but the protein

expression was focal (FIG. 8B), in contrast to the uniform expression in Panc 02.13 (FIG. 1C) and TM01212 (FIG. 7B). TM01098 cells were implanted orthotopically into severely immunodeficient mice. The control arm received intravenous sCD alone, the treated cohort tolerated a lower daily dose of ME3BP-7 (21 mg/kg of 3BP). This was 84% of dose tolerated by NSG mice bearing TM01212 (FIG. 7A-H) and 51-63% of the dose delivered to the nude mice bearing Panc 02.13 cancers (FIG. 6A-D). Even though MCT-1 expression was focal rather than widespread, ME3BP-7 resulted in significantly slower growth of the tumor as assessed by both US ($p < 0.01$, Mann Whitney U Test, FIG. 8D) and tumor weights following euthanasia ($p = 0.0028$, FIG. 8E). As expected, the reductions in growth observed with the TM01098 was less than that observed with either Panc02.13 (FIG. 6A-D) or TM01212 (FIG. 7A-H), presumably because of lower administered dose as well as a less uniform expression of MCT-1 in TM01098.

Example 6: Analysis of expression of MCT-1 in human tissues

The prevalence of MCT-1 RNA overexpression in PDACs in the TCGA database was assessed [34] and it was found that 23% had relatively high levels of expression (>13.6 FPKM, FIG. 14).[35] This expression was confirmed at the protein level through the evaluation of 95 human PDACs using tissue microarrays and the optimized immunohistochemical protocol described in Methods. About 56% of these cores showed positive MCT-1 expression relative to adjacent normal cells. Of the positive cores, 12% showed regions of high expression, 30% intermediate, and 58% had low MCT-1 expression (FIG. 9, panel A).

None of the 51 normal tissues recorded in the GTEx database expressed average levels of MCT-1 RNA higher than the approximate threshold required for sensitivity to 3BP in cancer cell lines (~ 50 transcripts per million, FIG. 15). Among the normal tissues, the highest levels were recorded in the colon, testis, and uterus (median 37, 38, and 32 transcripts per million, respectively, among 142 to 376 tissue samples assessed for each tissue type)[36]. The RNA levels were consistent with the immunohistochemical evaluation, in which little staining was observed in normal pancreas, brain, or ovary while staining was readily observed in testis, colon, and uterine tissue (FIG. 16). On the basis of this information, ME3BP-7 treated mice were carefully examined at necropsy and no discernible damage was found after 4 weeks of treatment in either the colon or uterus, while the pancreatic cancers showed extensive necrosis (Table 1, FIG. 9, and FIG. 13).

Example 7: Discussion

The results described herein show that ME3BP-7 is an example of an alkylating agent that targets a membrane protein expressed at relatively high levels in a sizable fraction of pancreatic cancers.[34] The closest successful precedents for ME3BP-7 are antibody-drug conjugates such as trastuzumab emtansine[37] or inotuzumab ozogamicin.[38] ME3BP-7 differs from antibody-drug conjugates in several important respects. First, ME3BP-7 is a small molecule, drastically reducing drug production challenges and cost. Second, ME3BP-7 itself is responsible for the targeting as well as the toxicity, while antibody-drug conjugates use an antibody for targeting and a separate, small molecule to kill cells.[39-41]

One of the most challenging aspects of the current study was delivery of ME3BP-7 to the mice. After multiple attempts to deliver various 3BP formulations through other routes, the only way to reliably deliver multiple doses of the drug was intravenous. The attempts included various forms of subcutaneous and intraperitoneal injections as well as catheterization techniques employing manual and pump-driven injections over various time periods and at various volumes. The reason that delivery is so difficult is that free 3BP is a very potent and fast-acting alkylating agent and quickly reacts with proteins to which it is exposed. This reactivity results in tissue damage and subsequent scarring to any injection site, severely limiting the ability to deliver multiple doses. Fortunately, the encapsulated 3BP in ME3BP-7 does not damage tissues as quickly as the free form (FIG. 13 and Table 1), so multiple doses could generally be administered through the tail vein. But to compare the systemic toxicity of 3BP and ME3BP-7 in an equivalent way, they both had to be delivered through vascular access buttons which are challenging to implant and maintain for long periods of time. Though ME3BP-7 protects the 3BP from degradation associated with its alkylation of other proteins (FIG. 4), it does not protect it long enough to preclude local damage to tissues when administered subcutaneously or intraperitoneally. Interestingly, this logistical issue is peculiar to small animals such as mice, in which repeated vascular access is difficult. In humans, delivery of anti-cancer drugs through pump-driven intravenous or intra-arterial catheters is routinely practiced.[42-44]

Table 1: Gross pathology and assessment of potential tissue damage in organs of mice treated for 4 weeks (12 doses) with ME3BP-7

Mouse no	Arm	Heart	Lungs	Liver	Spleen	Pancreas
1	Control	0	0	0	0	1 (edema)
2	Control	0	0	0	0	1 (edema)
3	Control	0	0	0	0	0
4	Control	0	0	0	0	0
5	Control	0	0	0	0	0
6	Control	0	0	0	0	0
7	Control	0	0	0	0	0
8	Control	0	0	0	0	1 (edema)
9	Control	0	0	0	0	0
1	ME3BP-7 (25 mg/kg)	0	0	0	0	0
2	ME3BP-7 (25 mg/kg)	0	0	0	1 (calcification)	0
3	ME3BP-7 (25 mg/kg)	0	0	1	0	1 (edema)
4	ME3BP-7 (25 mg/kg)	0	0	0	0	0
5	ME3BP-7 (25 mg/kg)	0	0	0	0	0
6	ME3BP-7 (25 mg/kg)	0	0	0	0	1 (edema)
7	ME3BP-7 (25 mg/kg)	0	0	0	0	0

Mouse no	Arm	Small Intestine	Colon	Reproductive (endometrium and ovaries)
1	Control	0	0	0
2	Control	0	0	0
3	Control	0	0	0
4	Control	1 (short villi)	0	0
5	Control	2 (short villi)	0	0
6	Control	0	0	0
7	Control	0	0	0
8	Control	0	0	0
9	Control	0	0	0
1	ME3BP-7 (25 mg/kg)	0	0	0
2	ME3BP-7 (25 mg/kg)	0	0	0
3	ME3BP-7 (25 mg/kg)	0	0	0
4	ME3BP-7 (25 mg/kg)	0	0	0
5	ME3BP-7 (25 mg/kg)	0	0	0
6	ME3BP-7 (25 mg/kg)	0	0	0
7	ME3BP-7 (25 mg/kg)	0	0	0

Mouse no	Arm	Kidney	Stomach and Duodenum
1	Control	0	0
2	Control	0	0
3	Control	0	0
4	Control	0	0
5	Control	0	0
6	Control	0	0
7	Control	0	0
8	Control	0	0
9	Control	0	0
1	ME3BP-7 (25 mg/kg)	0	0
2	ME3BP-7 (25 mg/kg)	0	0
3	ME3BP-7 (25 mg/kg)	2 (vacuolar changes in tubules with regeneration)	0
4	ME3BP-7 (25 mg/kg)	0	0
5	ME3BP-7 (25 mg/kg)	1 (vacuolar changes in tubules)	0
6	ME3BP-7 (25 mg/kg)	0	0
7	ME3BP-7 (25 mg/kg)	0	0

Table 2: continued

0	No changes
1	Minimal changes of unknown significance
2	Minimal pathological changes of unlikely significance
3	Moderate Pathological changes
4	Severe pathological changes
5	Death causing changes

Although pancreatic cancers are described herein, MCT-1 is overexpressed in subsets of mesotheliomas, leukemias, and several other tumor types. As with any targeted agent, a potential mechanism of resistance will be loss of the target. [45-47] With targets that are the products of oncogenes, simple loss won't suffice, as the oncogene protein product is necessary for cell proliferation. In such cases, mutations in other sites of the protein that make it resistant to the drug, or other genes in the same pathway, generally occur. [48-51] MCT-1 is not an oncogene, so loss of MCT-1 is conceivable. The results in FIG. 3A-C show that ME3BP-7 is uniquely fast-acting compared to other drugs now used in the clinic to treat patients with pancreatic cancer. Even brief exposure to ME3BP-7 results in effective cell killing of the great majority of the cells. On the other hand, despite these striking effects *in vitro*, true regressions of extant tumors in mice was not induced. Growth was slowed (FIG. 6A-D, FIG. 7A-H, FIG. 8A-E) and metastases were markedly reduced (FIG. 7F). Better ways to administer ME3BP-7 may improve its *in vivo* potency.

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35 **Incorporation by Reference**

The contents of all references, patent applications, patents, and published patent applications, as well as the Figures and the Sequence Listing, cited throughout this application are hereby incorporated by reference. It will be understood that, although a number of patent

applications, patents, and other references are referred to herein, such reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art.

5 **Equivalents**

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

We claim:

1. A composition comprising a cyclodextrin and an alkylating agent, wherein the cyclodextrin encapsulates the alkylating agent, wherein the alkylating agent targets a membrane protein of a cancer cell.
2. The composition of claim 1, wherein at least one α -D-glucopyranoside unit of the cyclodextrin has at least one hydroxyl chemical group replaced with an ionizable chemical group.
3. The composition of claim 2, wherein the at least one hydroxyl chemical group of the at least one α -D-glucopyranoside unit is selected from the group consisting of C2, C3, and C6 hydroxyl chemical groups.
4. The composition of claim 3, wherein the C2, C3, and C6 hydroxyl chemical groups of at least one α -D-glucopyranoside unit of the cyclodextrin that are replaced with ionizable chemical groups.
5. The composition of any one claims 1-4, wherein the at least one α -D-glucopyranoside unit of the cyclodextrin is selected from the group consisting of two, three, four, five, six, seven, eight, and all α -D-glucopyranoside units of the cyclodextrin.
6. The composition of any one of claims 2-5, wherein the ionizable chemical group is the same at all replaced positions.
7. The composition of any one of claims 2-6, wherein the ionizable chemical group is a weakly basic functional group or a weakly acidic functional group.
8. The composition of any one of claims 2-7, wherein the ionizable chemical group is selected from the group consisting of amino, ethylene diamino, dimethyl ethylene diamino, dimethyl anilino, dimethyl naphthylamino, succinyl, carboxyl, sulfonyl, and sulphate functional groups.

9. The composition of any one of claims 2-7, wherein the ionizable chemical group is a succinyl group.
10. The composition of claim 9, wherein the succinyl-substituted cyclodextrin comprises an average of 3.4 succinyl groups per cyclodextrin.
11. The composition of claim 9 or 10, wherein the succinyl-substituted cyclodextrin comprises a molar ratio of 1.2 β -cyclodextrin per alkylating agent.
12. The composition of any one of claims 1-11, wherein the cyclodextrin has a pK_{a1} of between 4.0 and 8.5.
13. The composition of any one of claims 1-12, wherein the composition is a liquid or solid pharmaceutical formulation.
14. The composition of any one of claims 1-13, wherein the alkylating agent is neutrally charged or hydrophobic.
15. The liposome composition of any one of claims 1-14, wherein the cyclodextrin is selected from the group consisting of β -cyclodextrin, α -cyclodextrin, and γ -cyclodextrin.
16. The liposome composition of claim 15, wherein the cyclodextrin is β -cyclodextrin.
17. The composition of any one of claims 1-16, wherein the alkylating agent is 3-halopyruvate.
18. The composition of any one of claims 1-17, wherein the alkylating agent is 3-bromopyruvate.
19. The composition of any one of claims 1-18, wherein the alkylating agent targets a monocarboxylate transporter (MCT) protein.

20. The composition of any one of claims 1-19, wherein the alkylating agent targets MCT-1.
21. The composition of any one of claims 1-20, wherein the composition is formulated for systemic administration.
22. The composition of any one of claims 1-21, further comprising an anti-cancer therapeutic agent.
23. A kit comprising a composition of any one of claims 1-22, and instructions for use.
24. A method of treating a subject having a cancer comprising administering to the subject a therapeutically effective amount of a composition of any one of claims 1-23.
25. The method of claim 24, wherein the composition is administered systemically.
26. The method of claim 25, wherein the systemic administration is selected from the group consisting of oral, intravenous, intraperitoneal, subcutaneous, and intramuscular administration.
27. The method of any one of claims 24-26, wherein the subject is treated with at least one additional anti-cancer therapy.
28. The method of claim 27, wherein the at least one additional anti-cancer therapy is radiation therapy.
29. The method of any one of claims 24-28, wherein the cancer is a solid tumor.
30. The method of any one of claims 24-29, wherein the cancer is selected from the group consisting of liver cancer, pancreatic cancer, lung cancer and breast cancer.
31. The method of claim 30, wherein the cancer is pancreatic cancer.

32. The method of claim 30 or 31, wherein the cancer is Pancreatic Ductal Adenocarcinoma (PDAC).
33. The method of any one of claims 24-31, wherein the subject is a mammal.
34. The method of claim 33, wherein the mammal is a human.

FIG. 1A

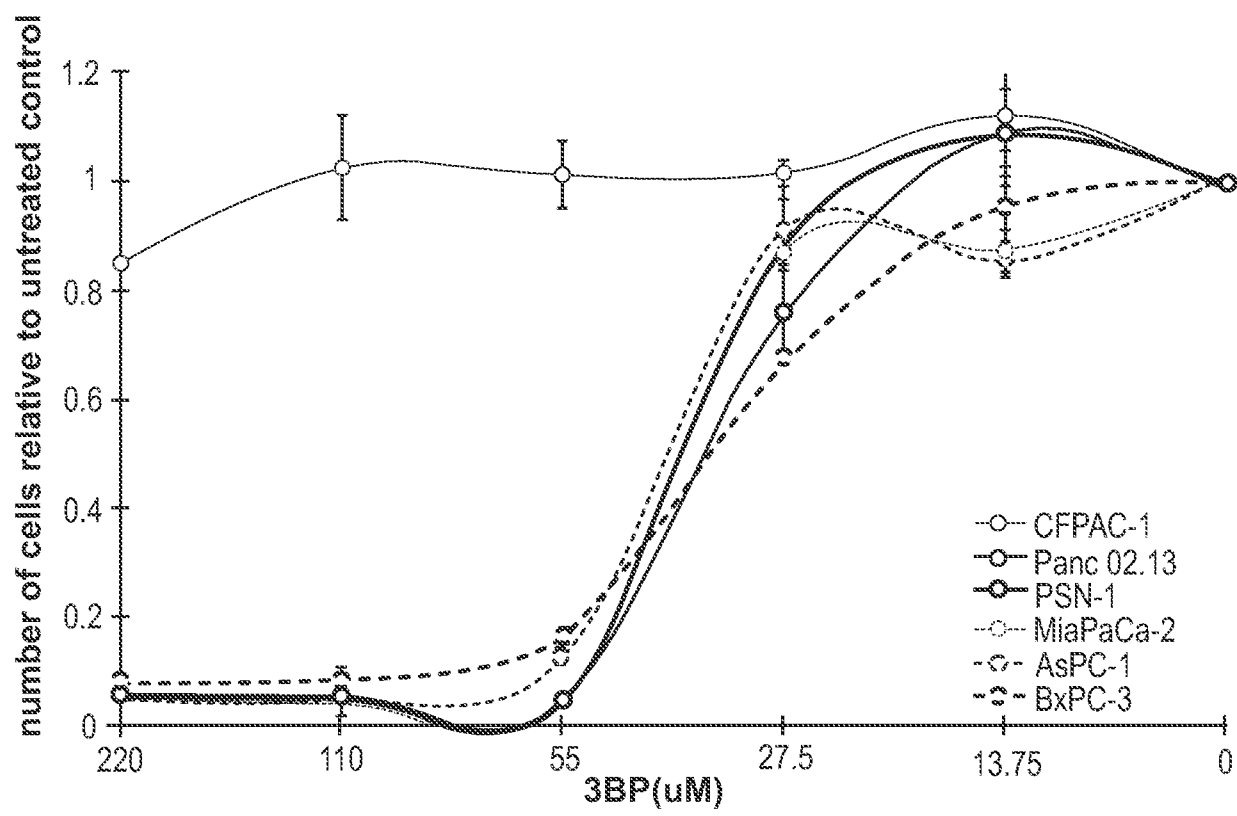


FIG. 1B

Cell Line	MCT-1 (TPM)	MCT-4 (TPM)	GLUT-1 (TPM)	3BP IC50 (uM)
MIA PaCa-2	163	42	40	24.14
PSN1	159	212	175	28.36
Panc 02.13	137	68	410	30.16
AsPC-1	65	29	168	39.72
BxPC-3	64	49	362	32.41
CFPAC-1	22	51	274	>650.00
DLD-1	387	20	409	22.64

FIG. 1C

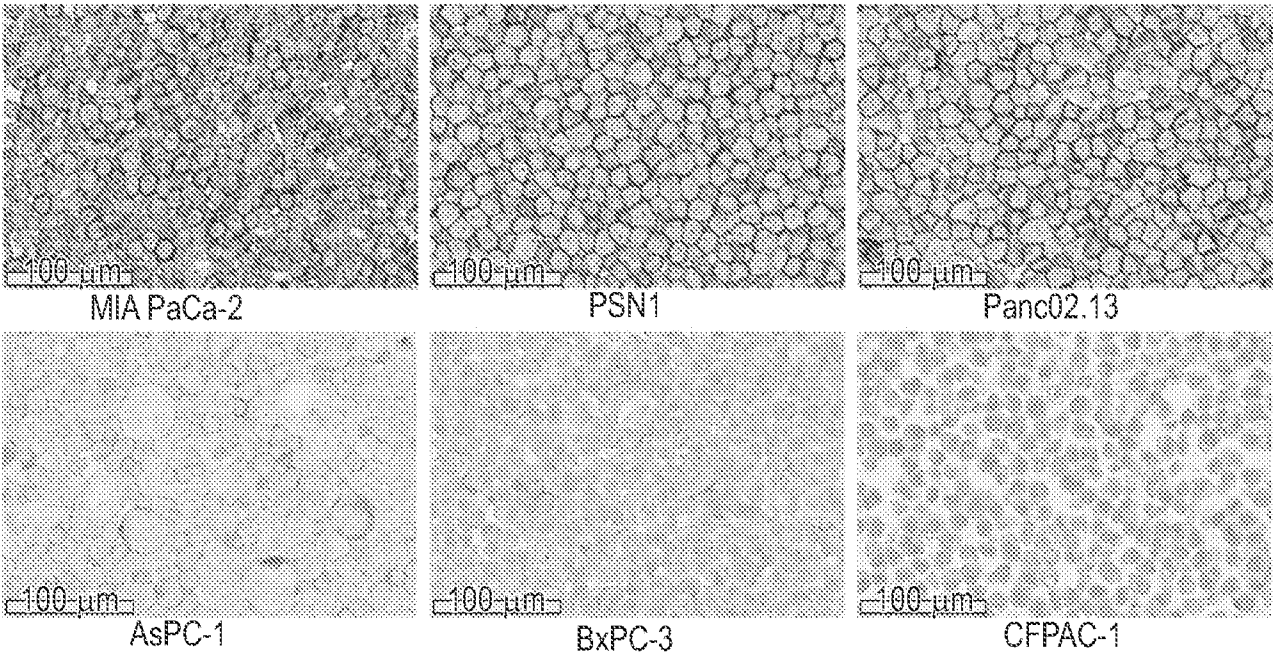


FIG. 2A

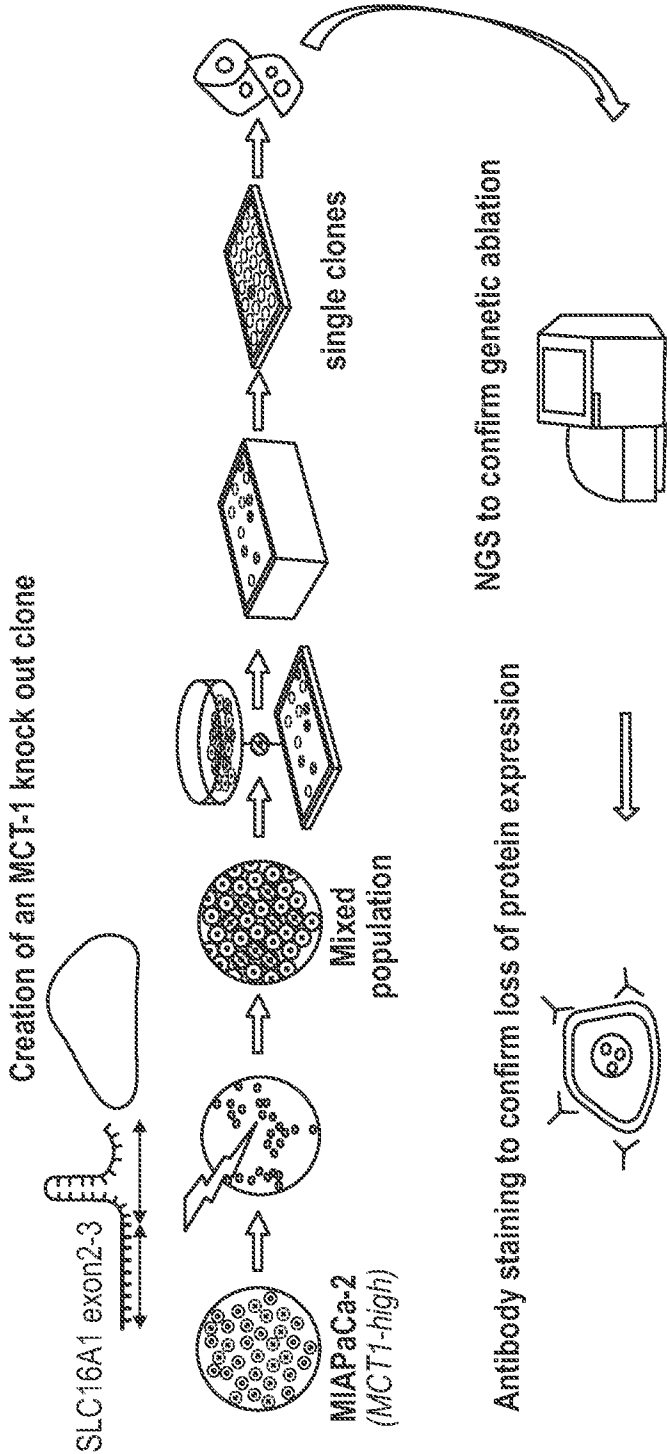


FIG. 2B

Clone No	Clone ID	Chromosome	Position	Mutation
1	A6	chr1	113471751	Indel
2	A4	chr1	113471757	Indel
3	B4	chr1	113471751	Indel
4	D1	chr1	113471762	Indel
5	C1	chr1	113471762	Indel
6	A2	chr1	1134717657/ 113471760	Indel

FIG. 2C

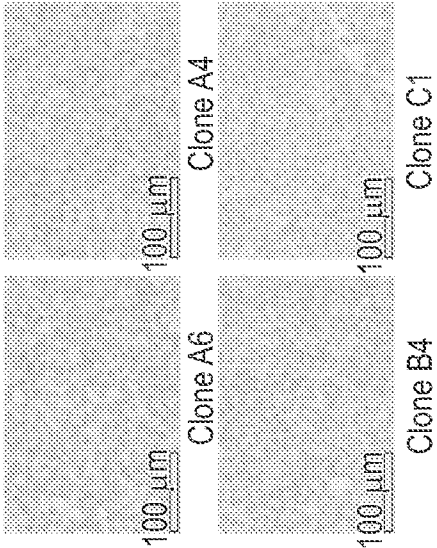


FIG. 2D

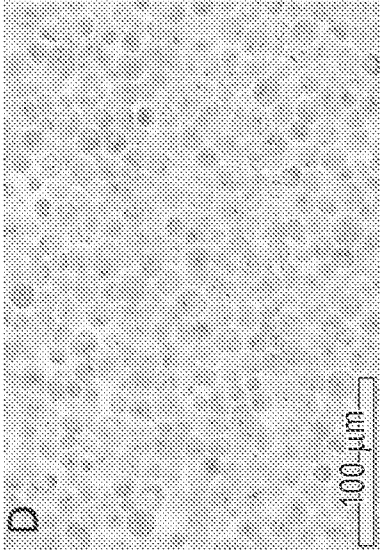


FIG. 2E

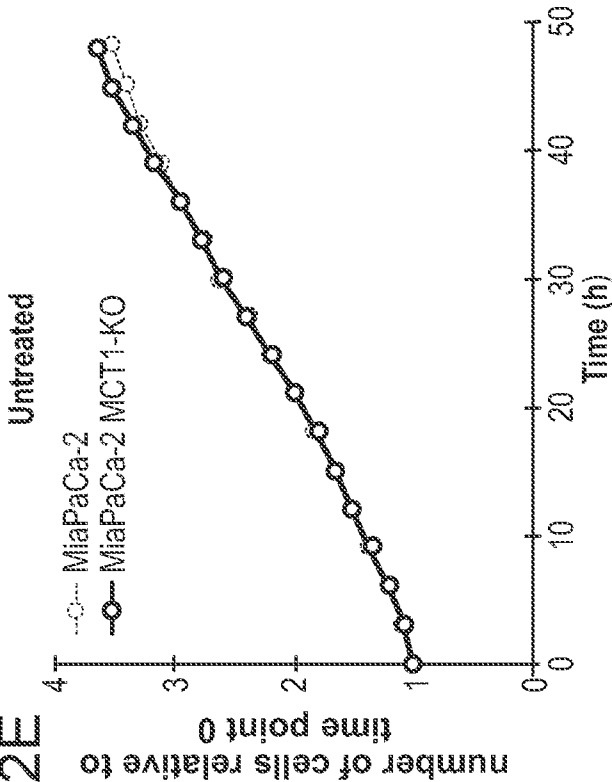


FIG. 2F

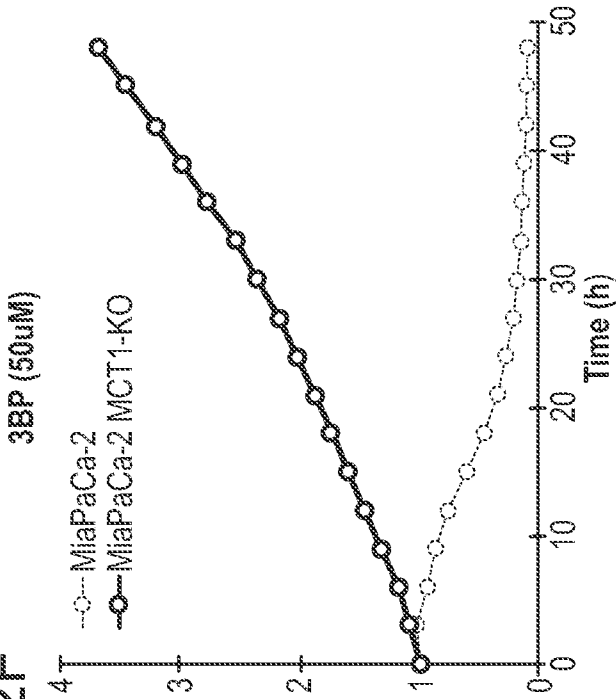


FIG. 2G

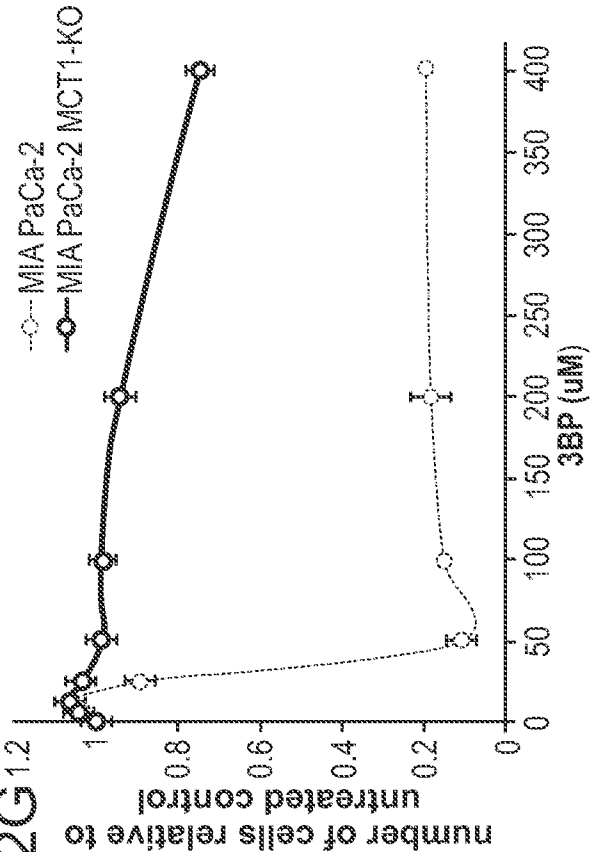
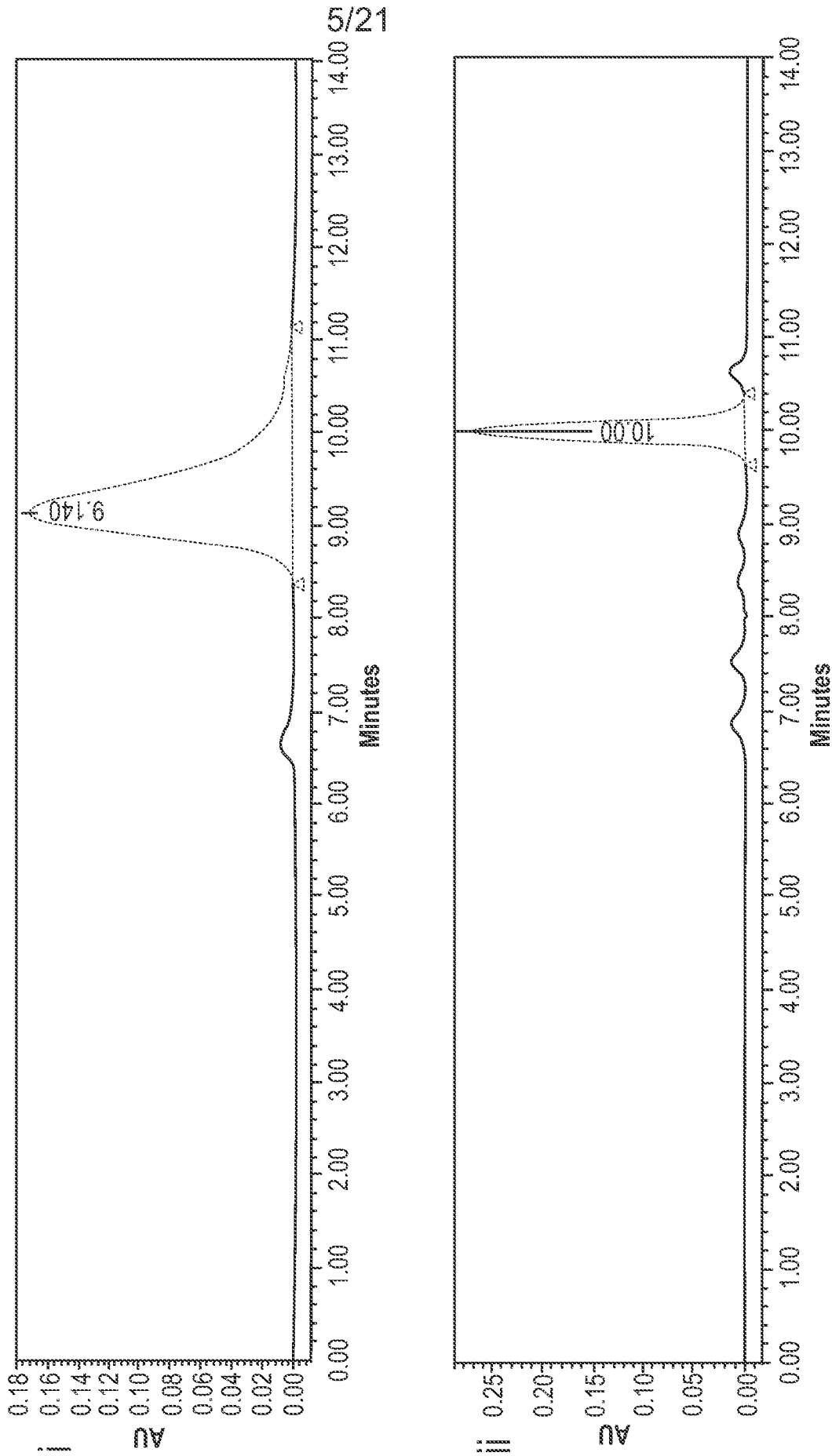
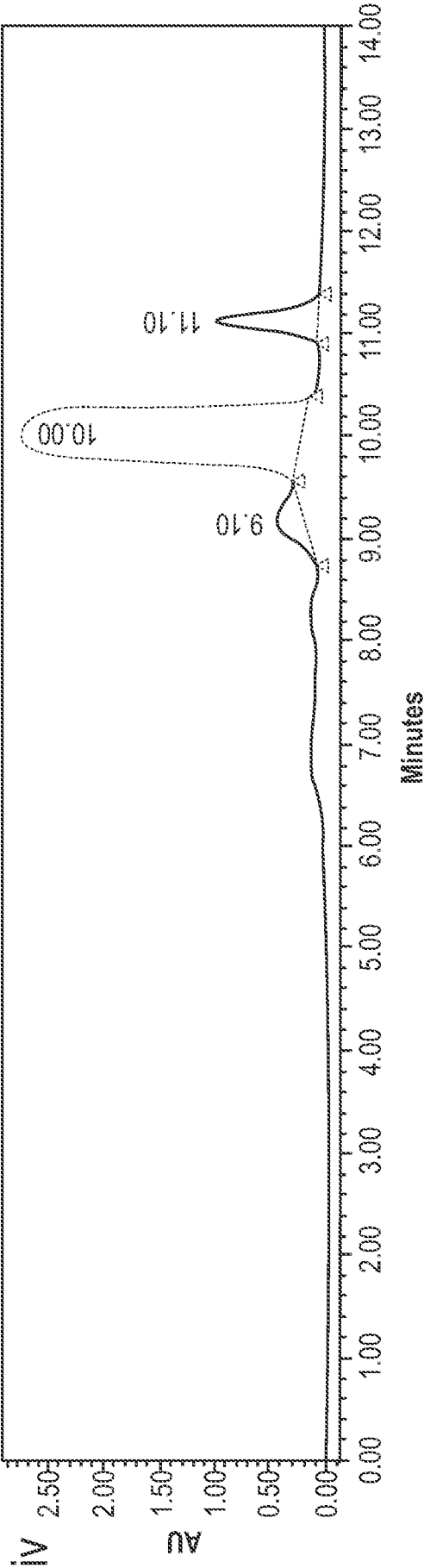
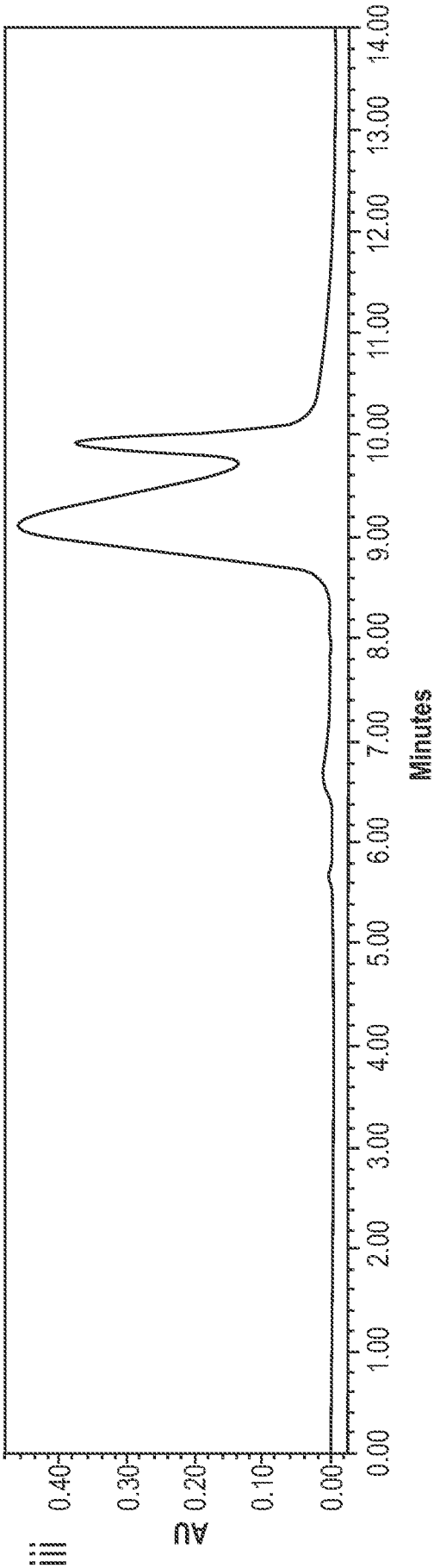


FIG. 3A



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FIG. 3A continued



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FIG. 3B

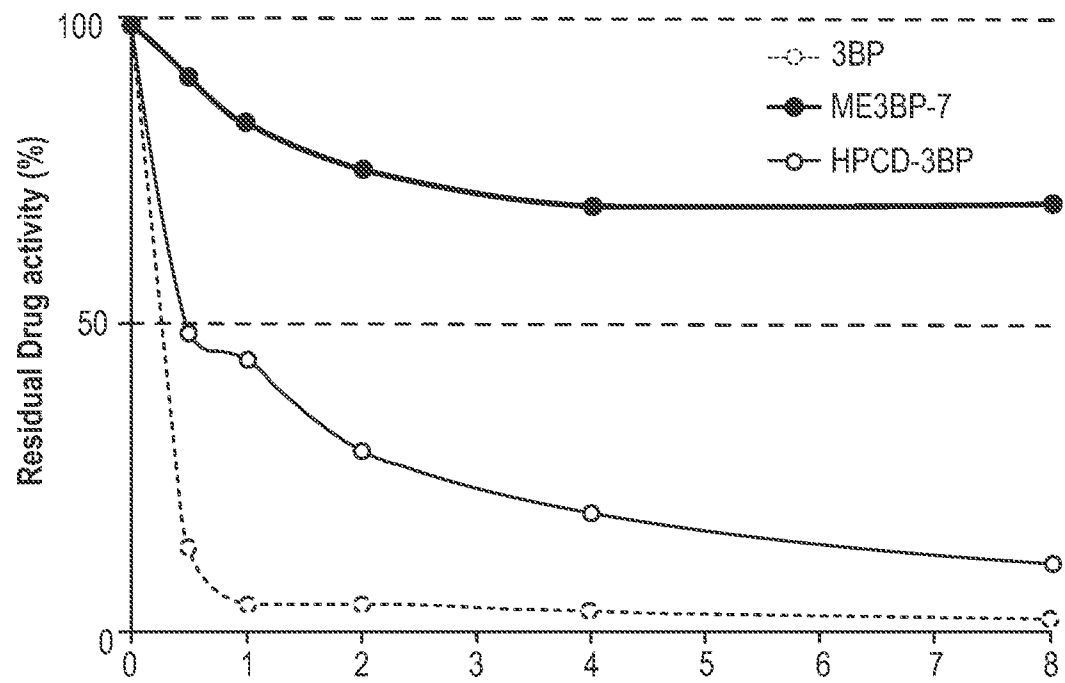
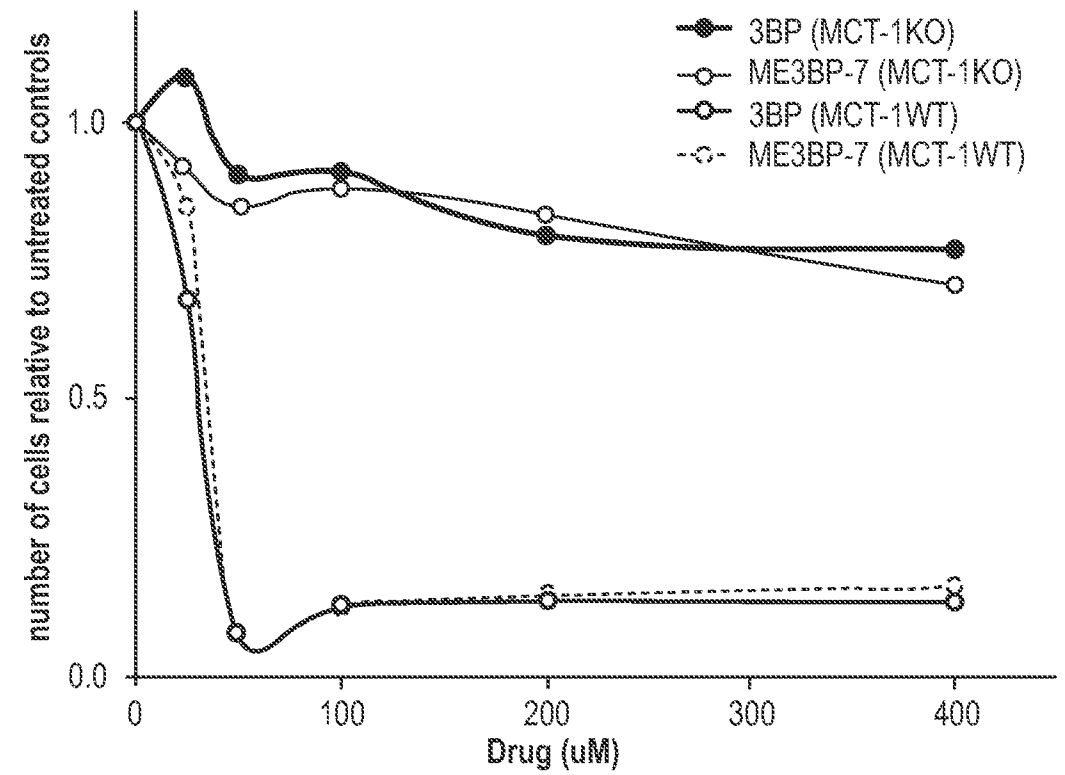
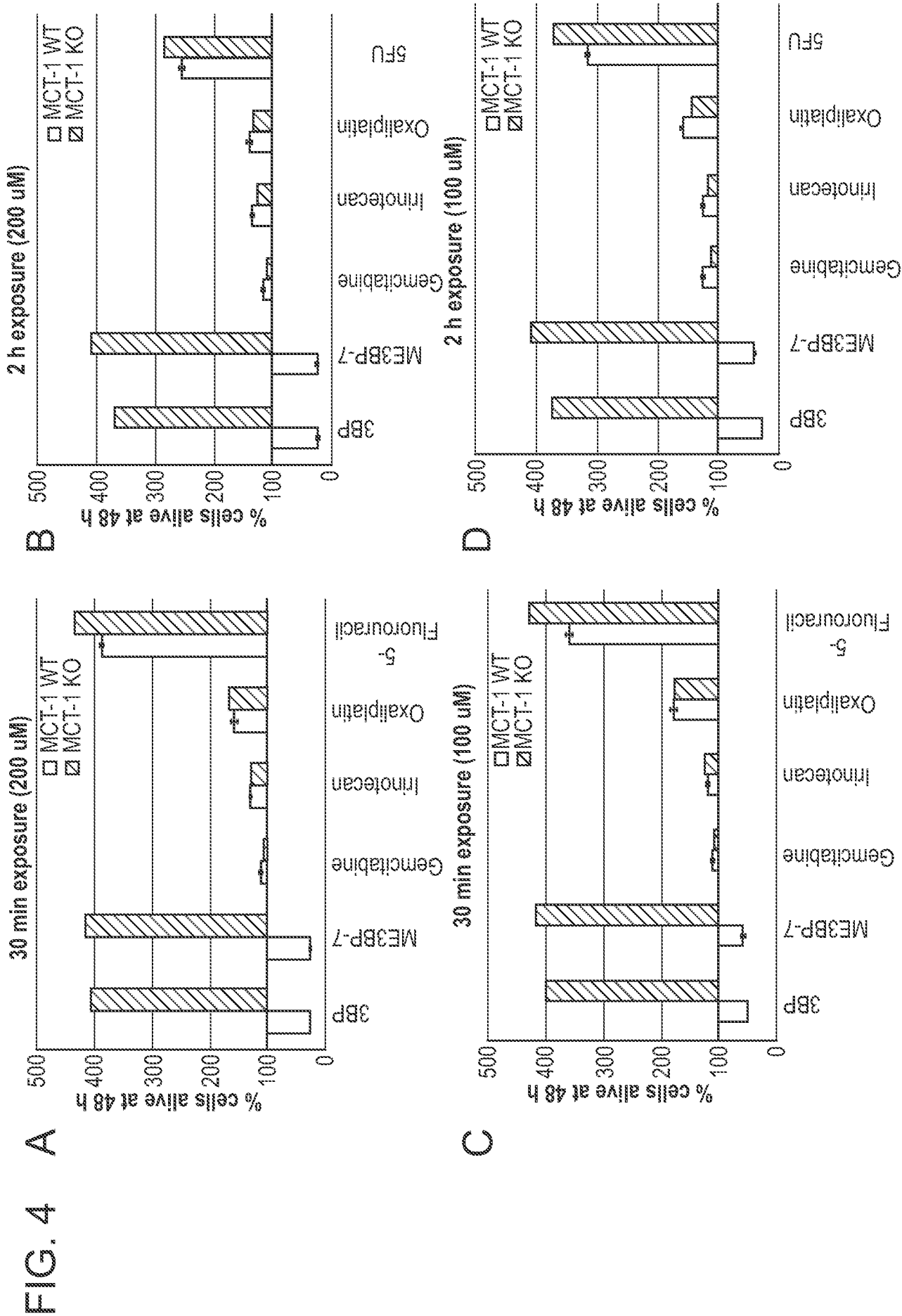


FIG. 3C



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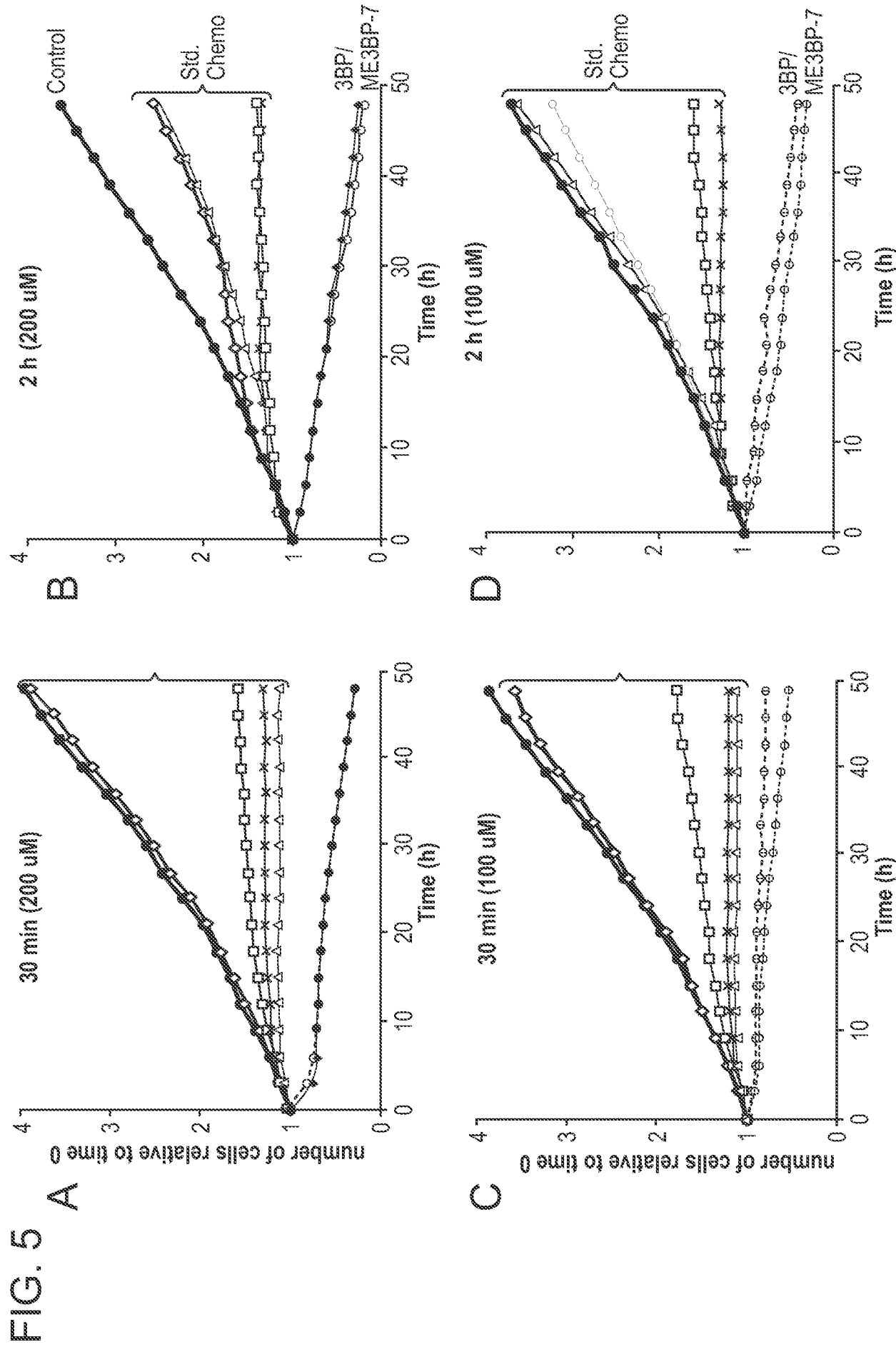


FIG. 6A

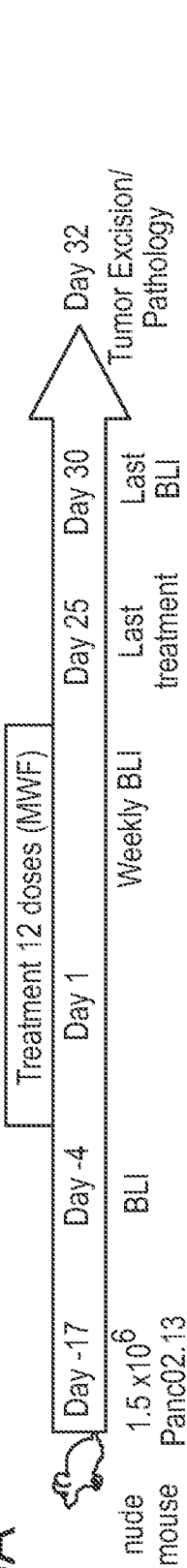


FIG. 6B

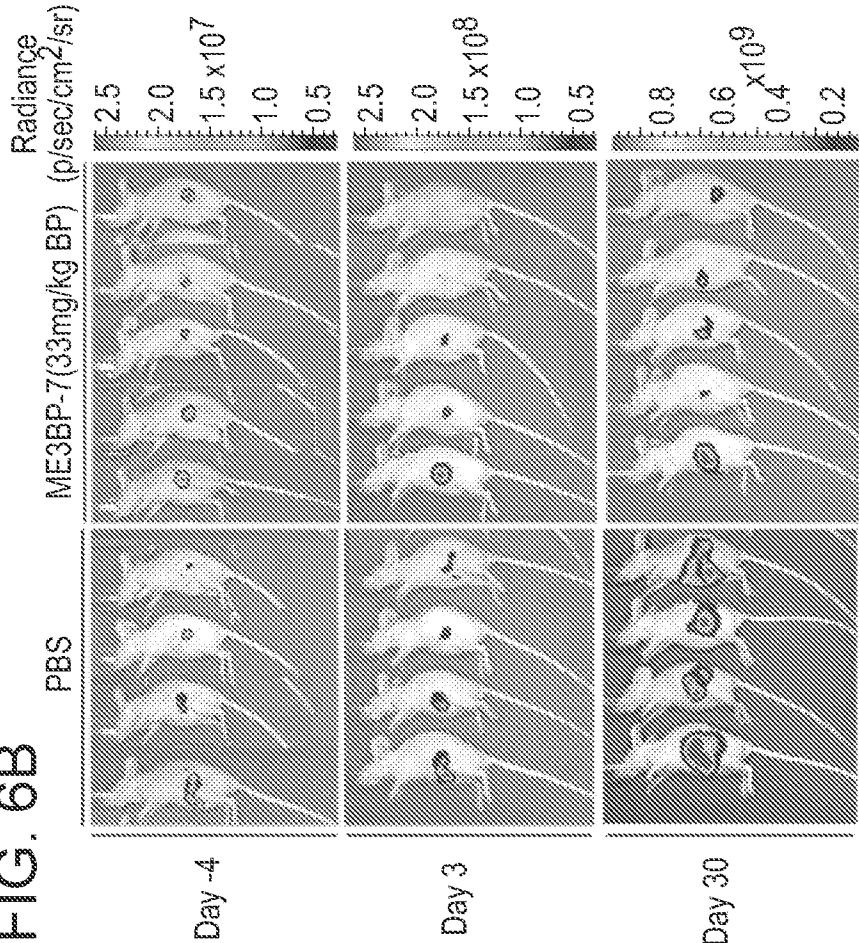


FIG. 6C

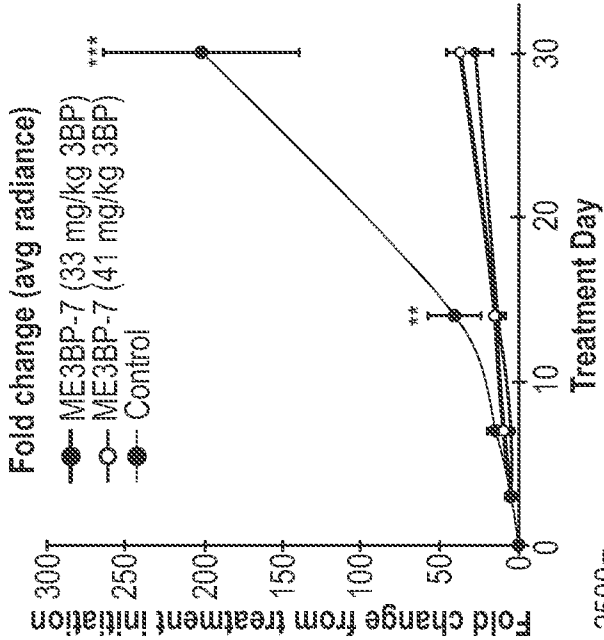


FIG. 6D

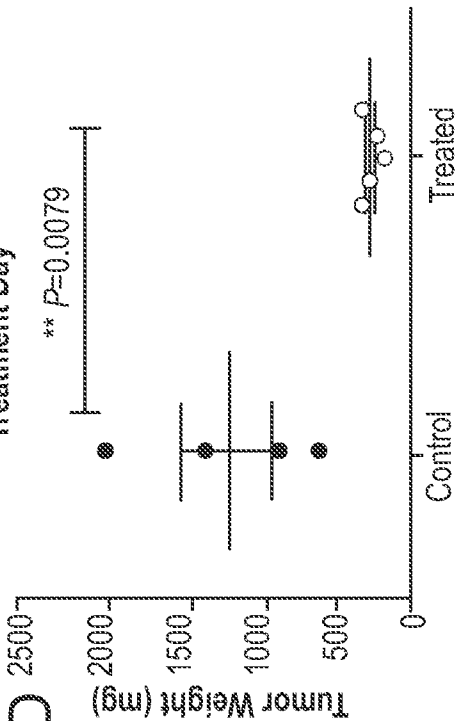


FIG. 7A

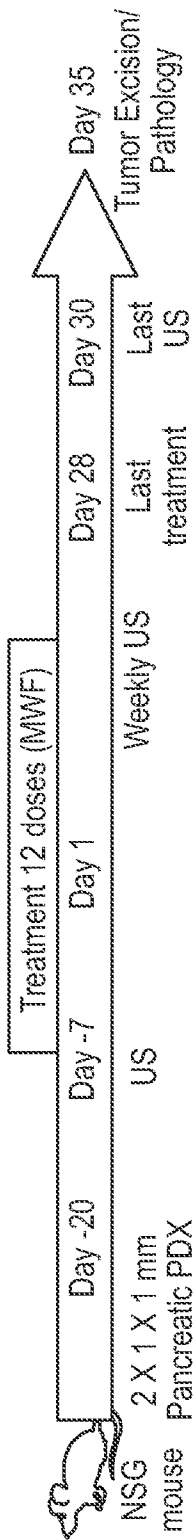


FIG. 7B

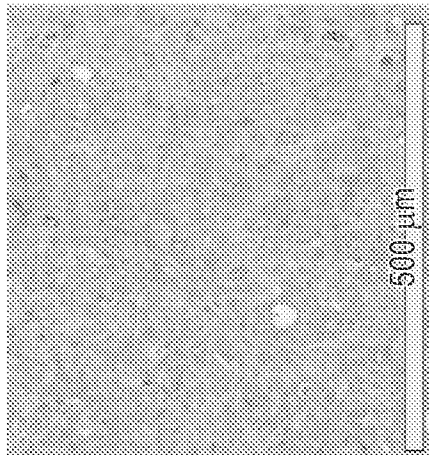


FIG. 7C

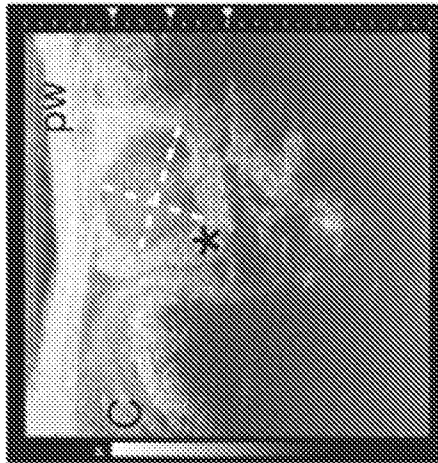
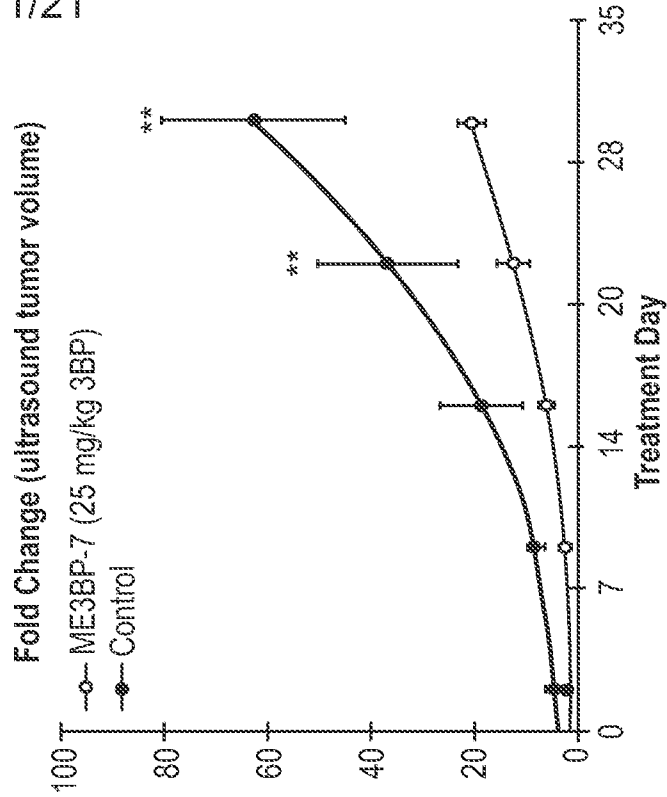


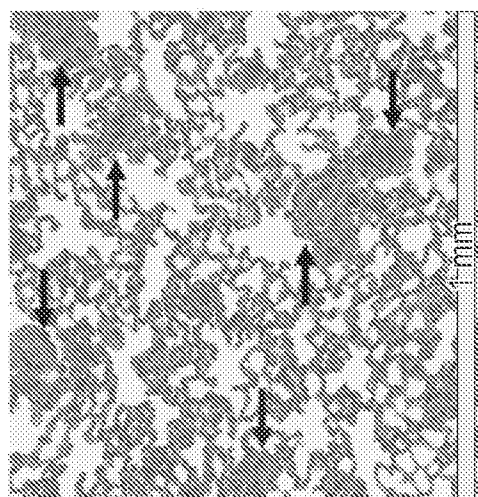
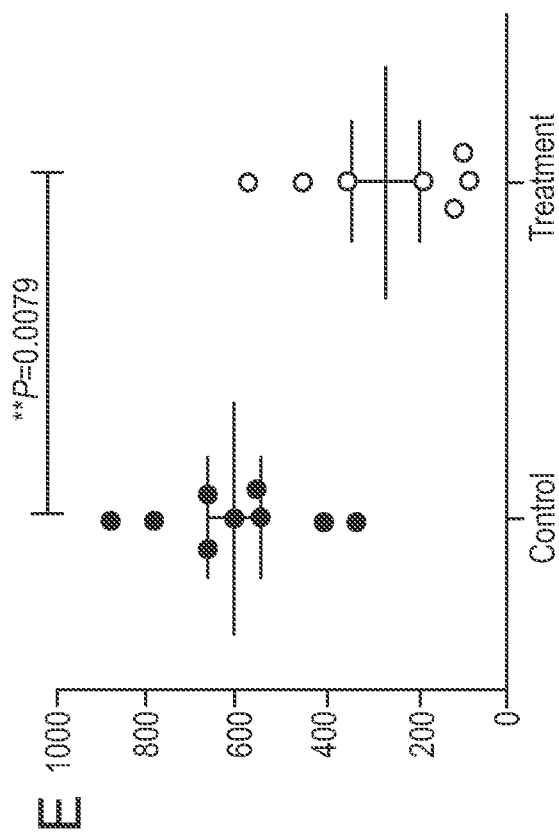
FIG. 7D

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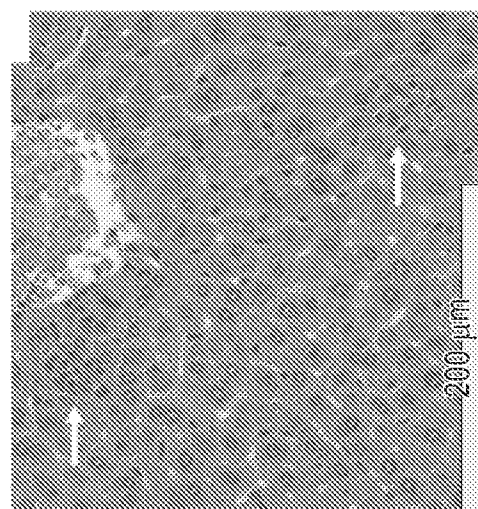


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WZ^xGL



GF²



HELIX

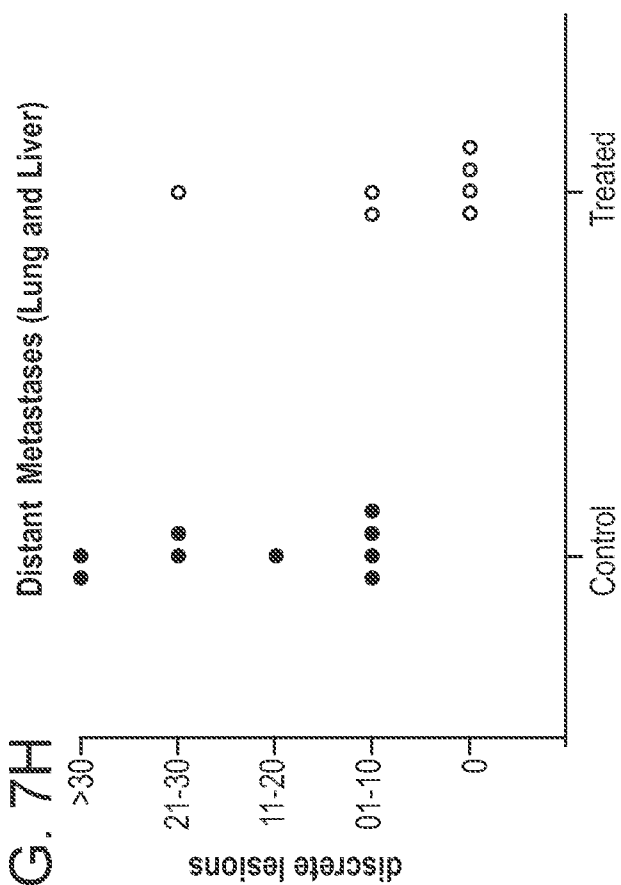


FIG. 8A

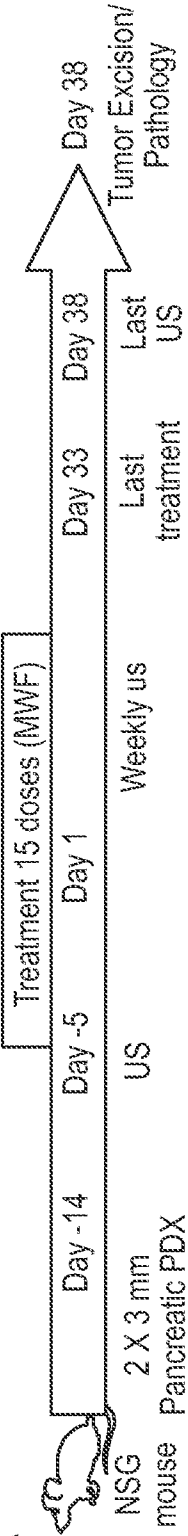


FIG. 8B

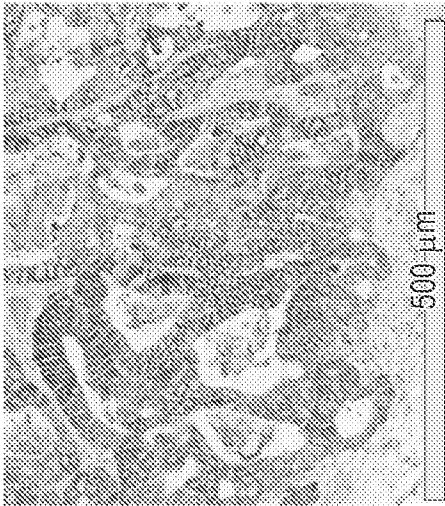


FIG. 8D

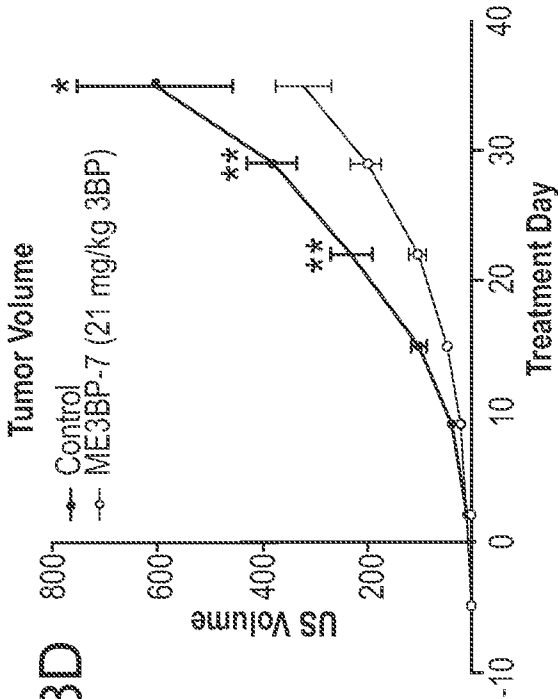


FIG. 8C

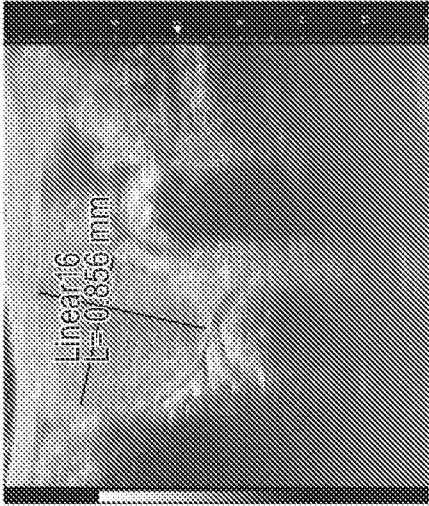


FIG. 8E

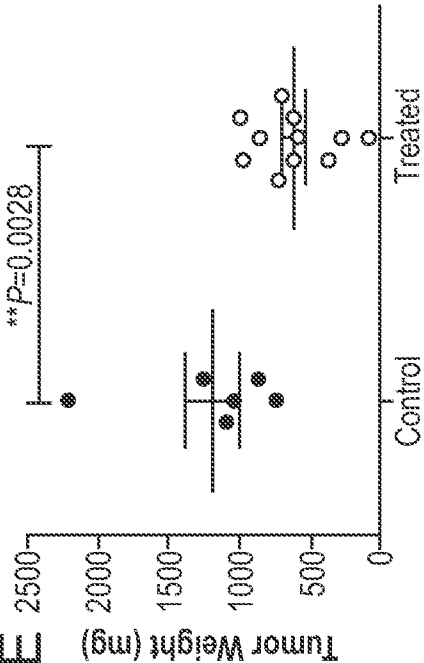


FIG. 9

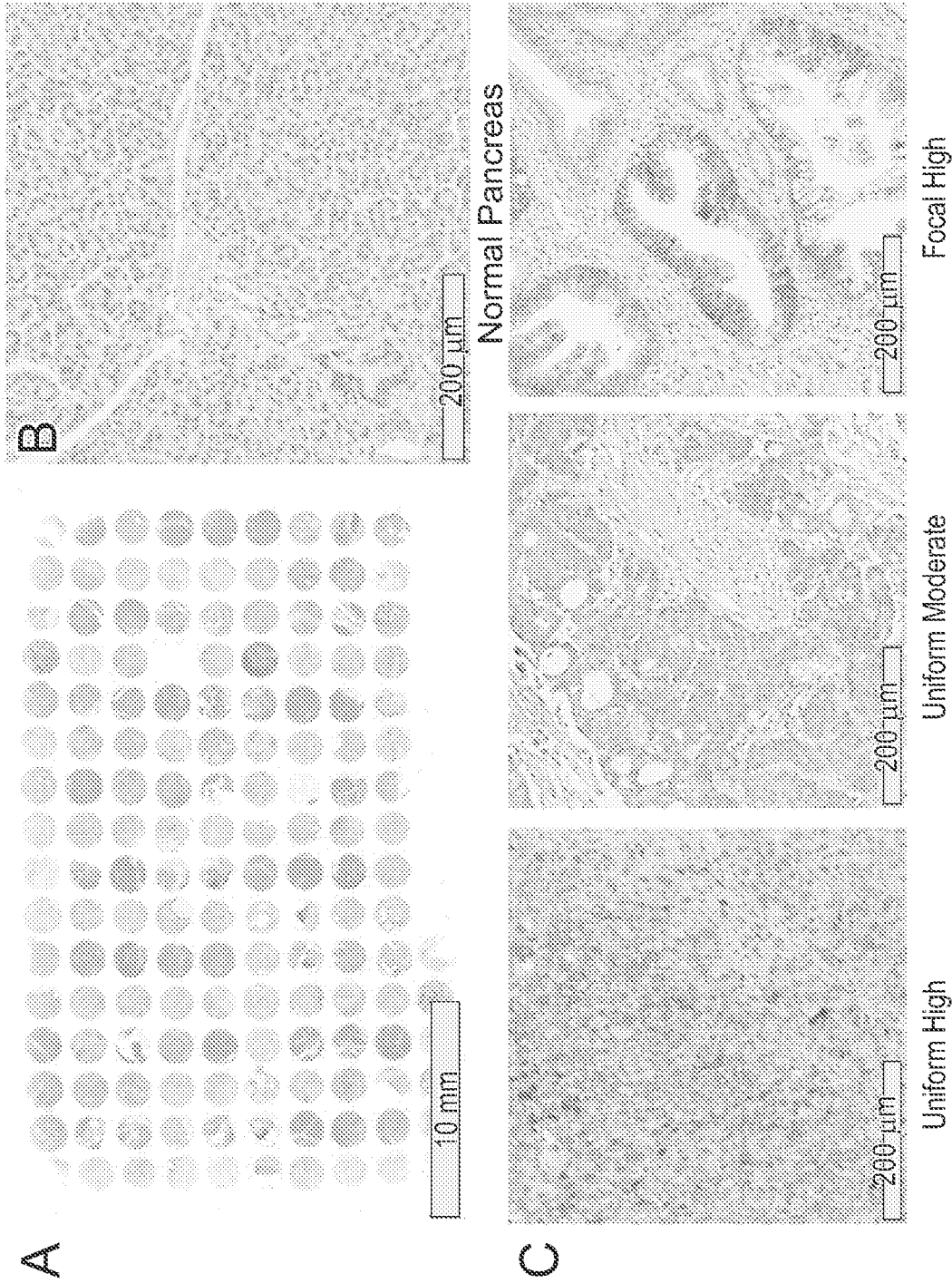
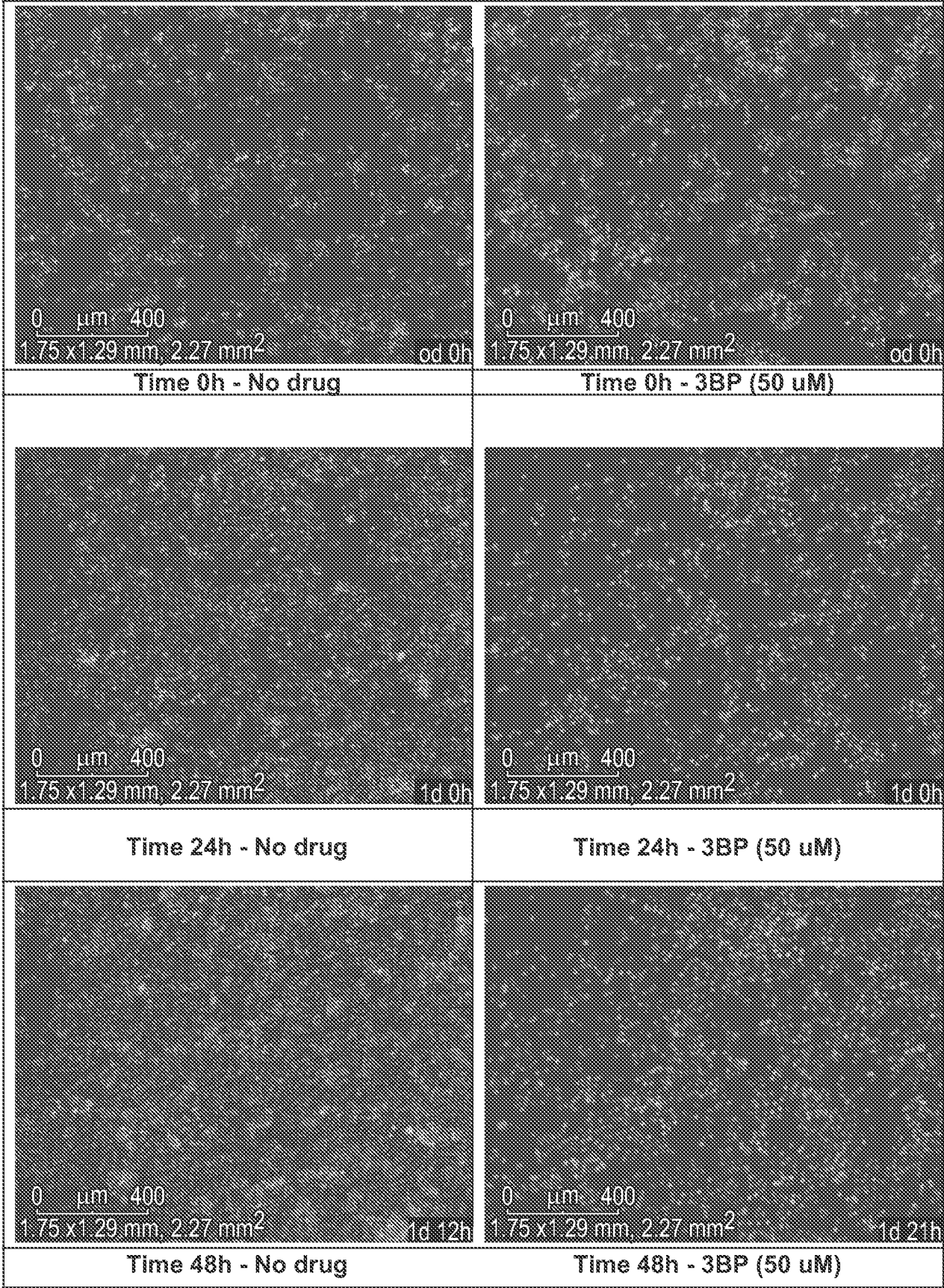
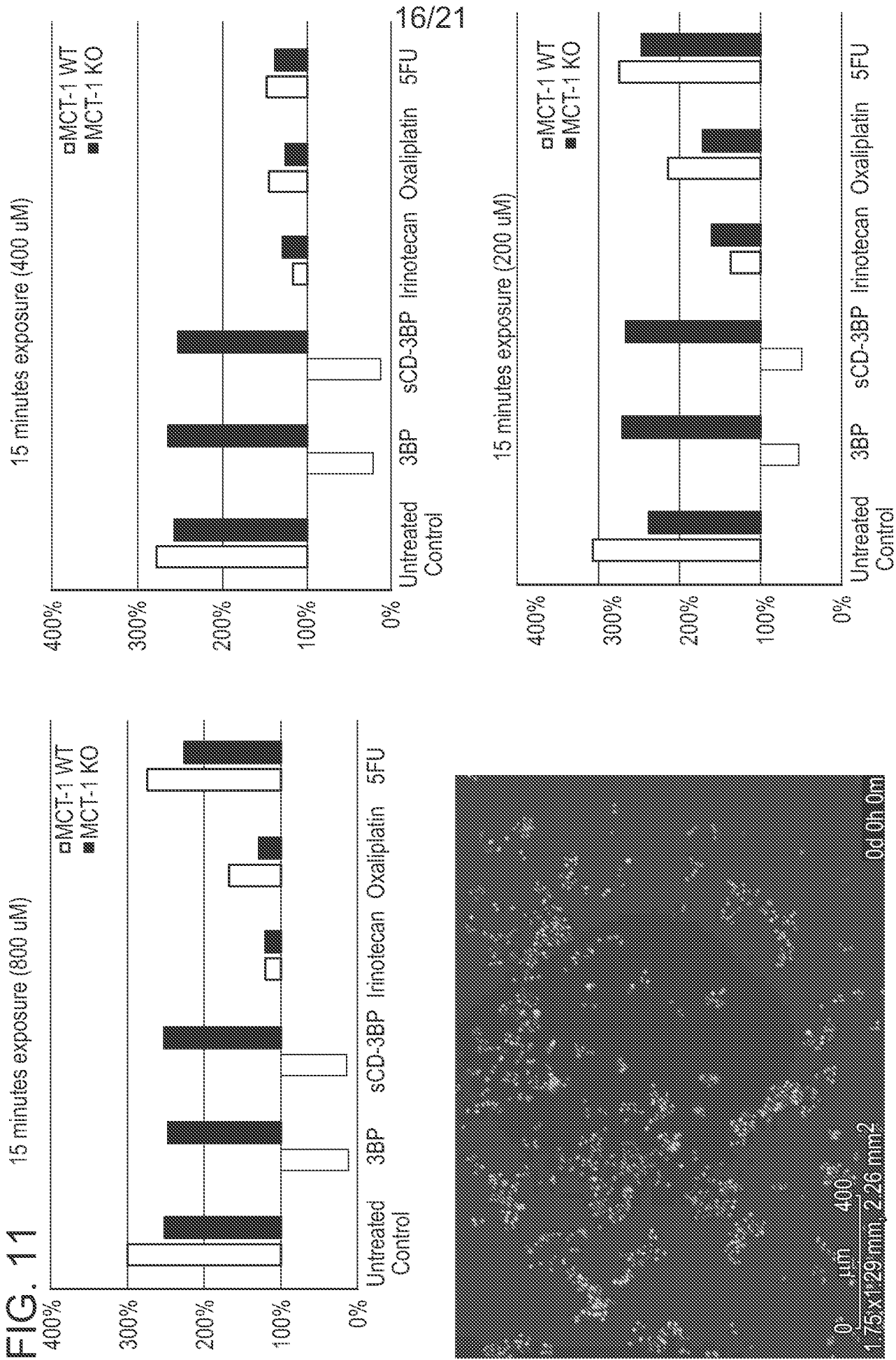


FIG. 10





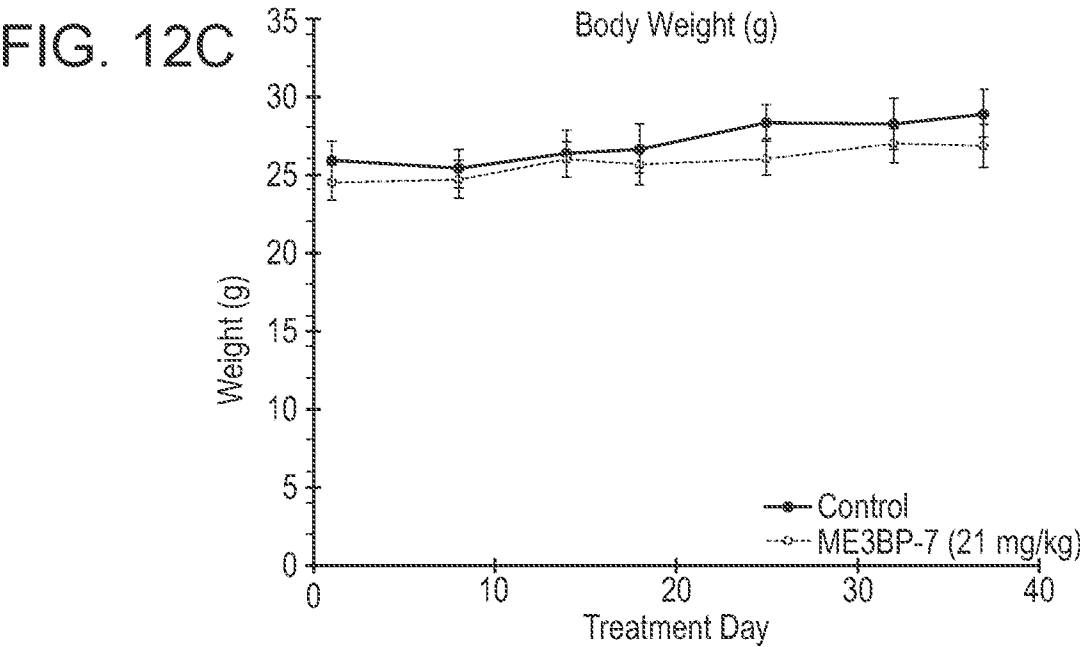
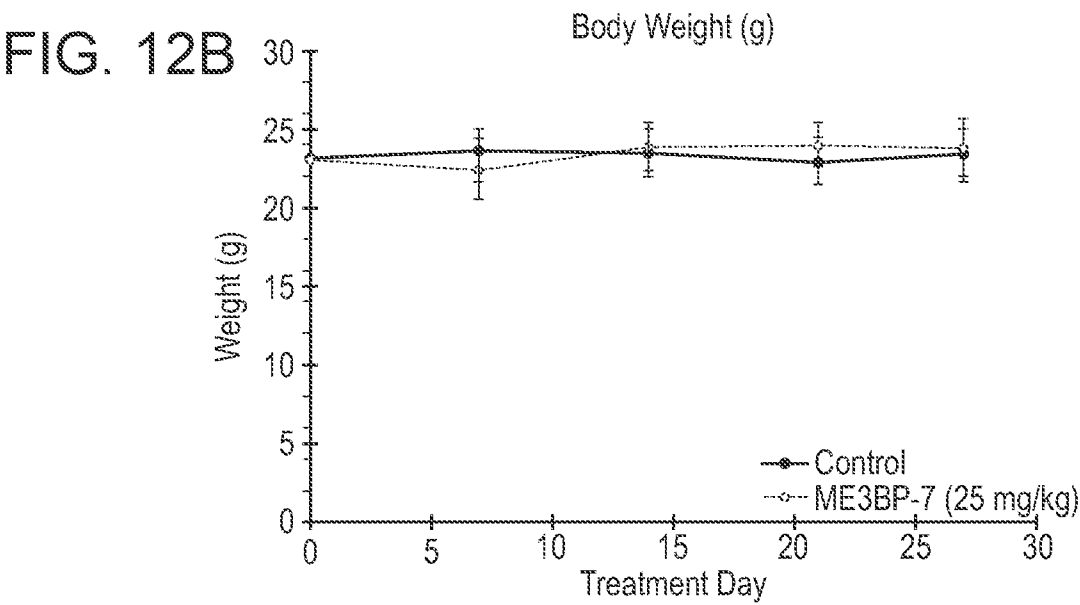
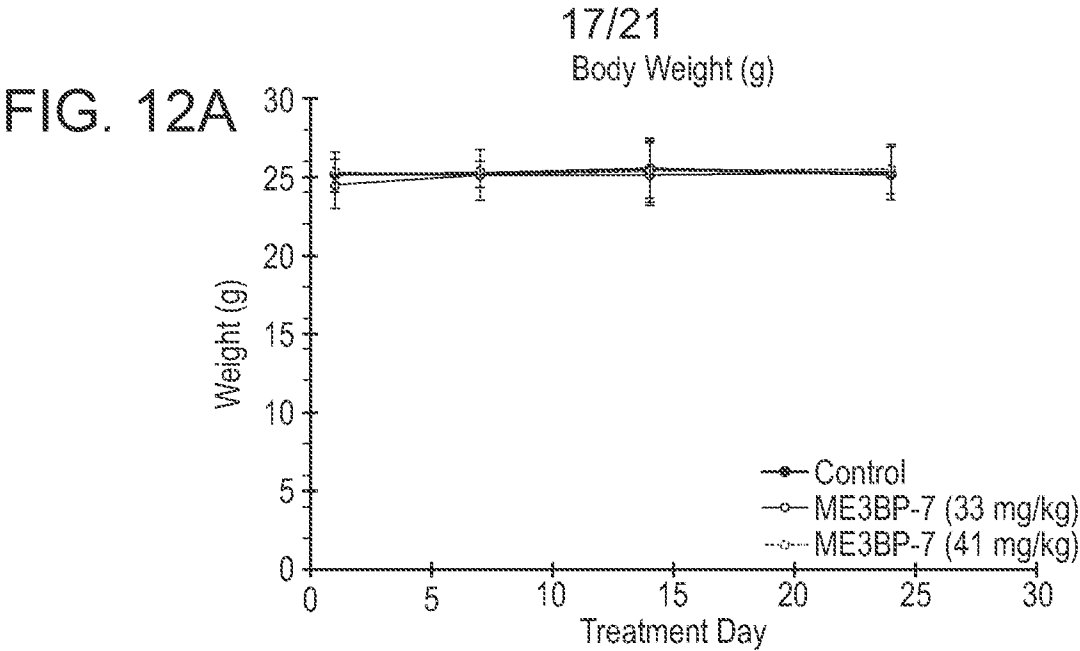


FIG. 13

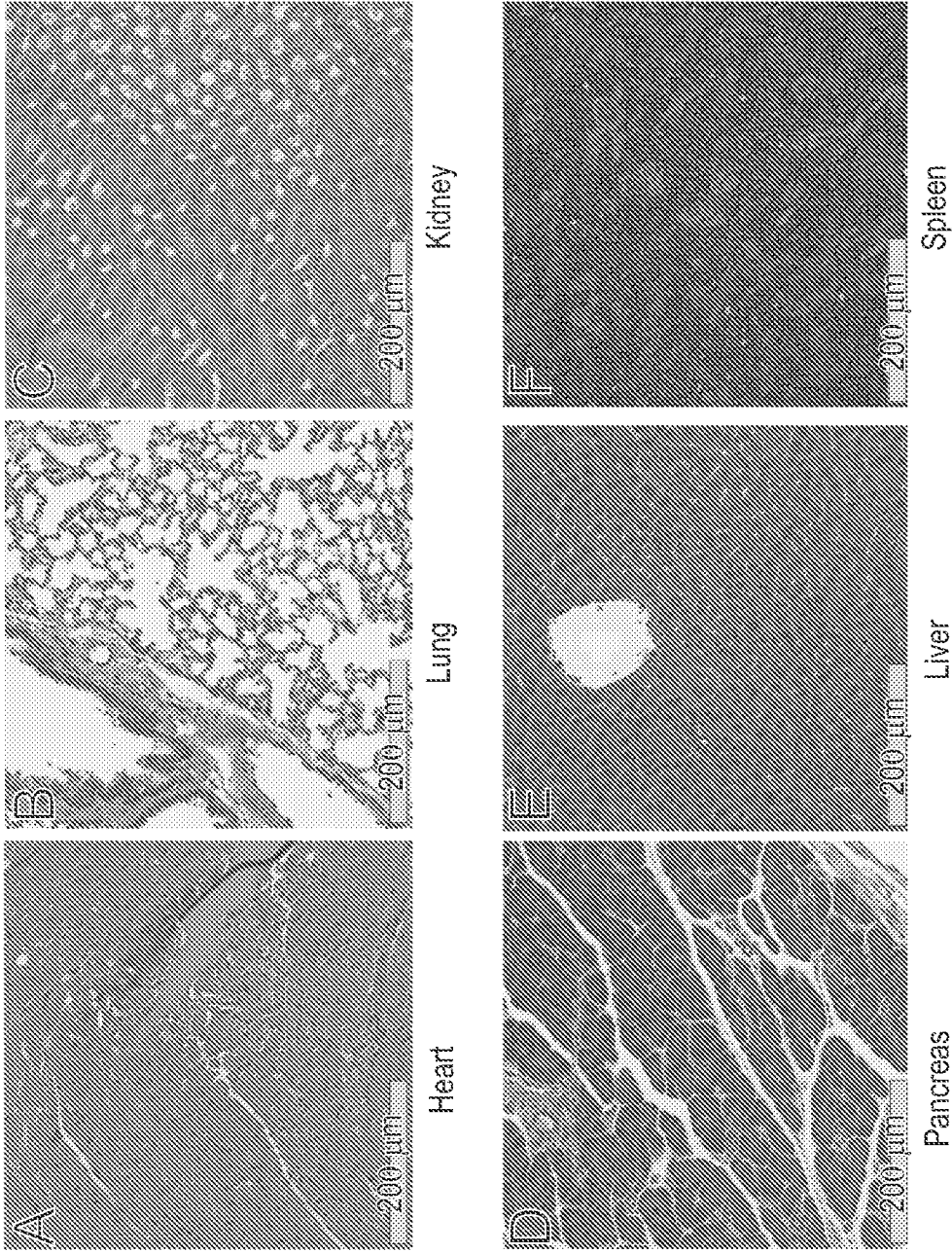


FIG. 14

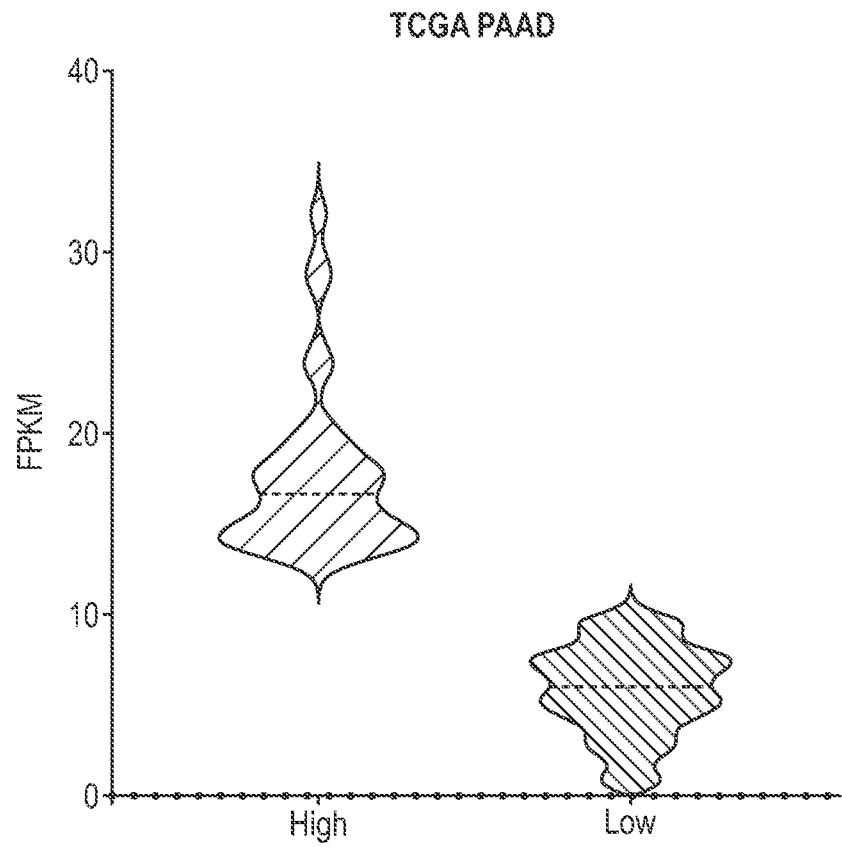


FIG. 15

SLC16A1 (TPM) expression in normal tissues
Genotype Tissue Expression (GTEx) project

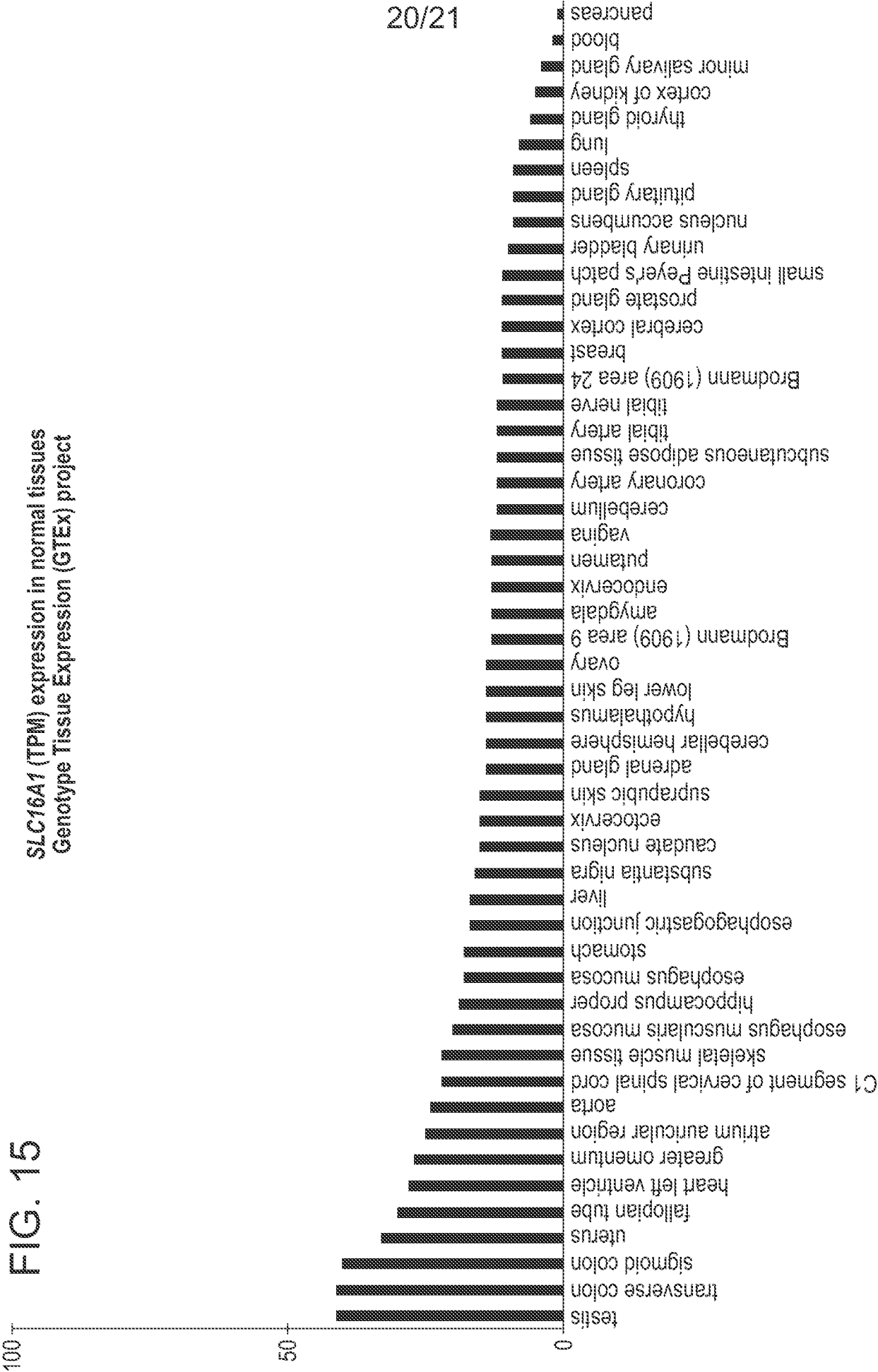
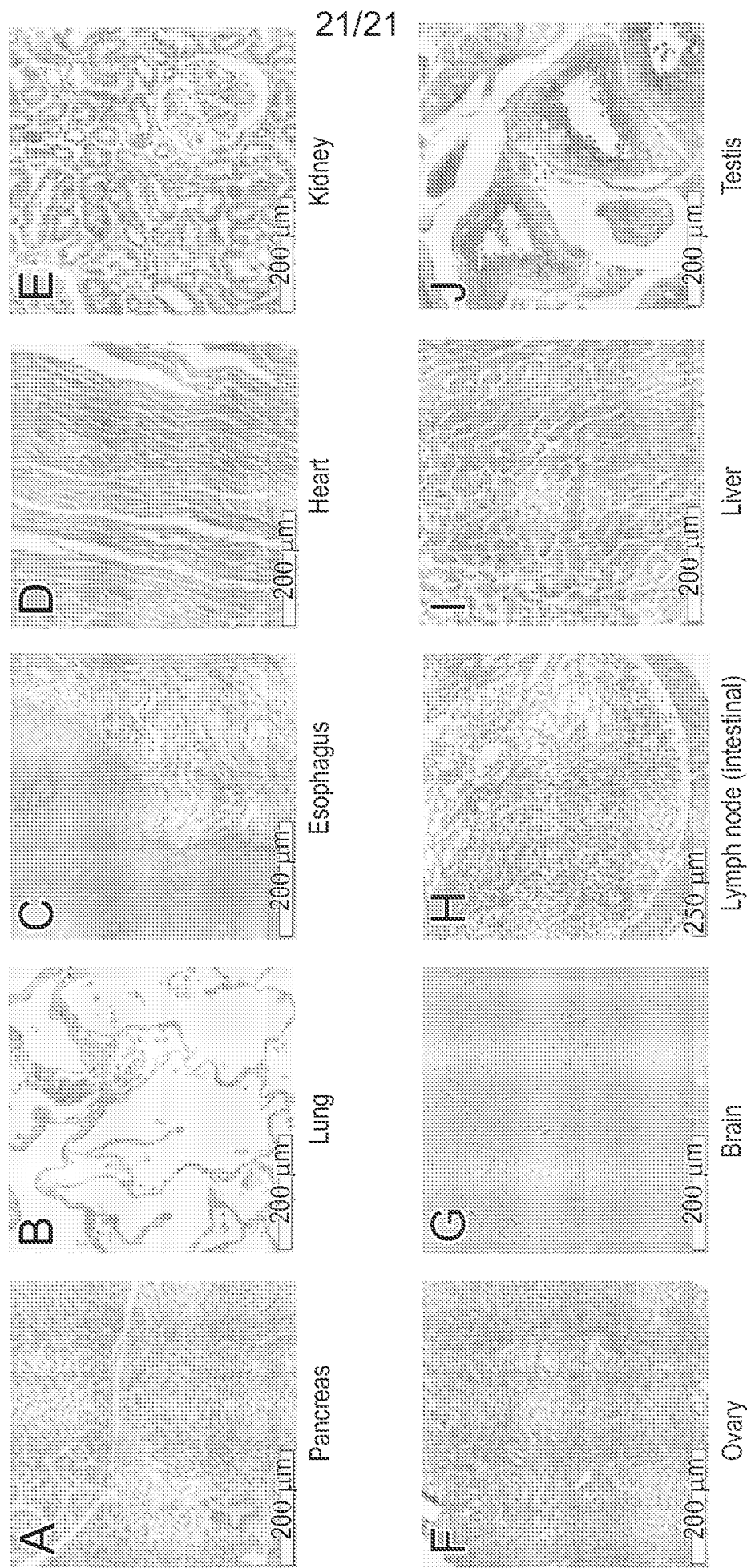


FIG. 16



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 24/30317

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. A61P 35/00, A61K 47/69, A61K 47/40 (2024.01)

ADD. A61K 9/50 (2024.01)

CPC - INV. A61P 35/00, A61K 47/6951, A61K 47/40

ADD. A61K 9/5036

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)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2016/0015639 A1 (The Johns Hopkins University) 21 January 2016 (21.01.2016); entire document, especially abstract, [0007], [0008], [0024]	1-5
A	US 5,710,268 A (Wimmer) 20 January 1998 (20.01.1998); entire document	1-5
A	US 2015/0192579 A1 (LAWRENCE LIVERMORE NATIONAL SECURITY, LLC) 09 July 2015 (09.07.2015); entire document	1-5
A	US 2003/0087961 A1 (Ko et al.) 08 May 2003 (08.05.2003); entire document	1-5

☐ 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

"D" document cited by the applicant in the international application

"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

10 July 2024

Date of mailing of the international search report

AUG 22 2024

Name and mailing address of the ISA/US

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Facsimile No. 571-273-8300

Authorized officer

Kari Rodriguez

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 24/30317

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 6-34
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

Remark on Protest

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