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(54) Title: POLYMERIC DRUG DELIVERY SYSTEMS FOR TREATMENT OF DISEASE

(57) Abstract: Provided are compositions and nanoparticle formulations that may be used, e.g., to deliver a therapeutic compound to a subject. In some embodiments, the nanoparticles may be used to deliver one or more chemotherapeutic agents to treat a cancer such, e.g., as a pancreatic ductal adenocarcinoma.



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DESCRIPTION

POLYMERIC DRUG DELIVERY SYSTEMS FOR TREATMENT OF DISEASE

BACKGROUND OF THE INVENTION

[0001] This application claims the benefit of United States Provisional Patent
5 Application No. 62/265,167, filed December 9, 2015, the entirety of which is incorporated
herein by reference.

1. Field of the Invention

[0002] The present invention relates generally to the field of drug delivery and
nanoparticles. More particularly, it concerns polymers and polymeric micelles thereof for the
10 delivery of chemotherapeutic compounds.

2. Description of Related Art

[0003] Pancreatic cancer remains one of the most lethal types of cancer despite
vigorous research on this disease during the past several decades. The overall 5-year survival
rate of patients with pancreatic cancer is below 5%; therefore, there is a dire need to develop
15 an effective therapy for this disease (Tempero *et al.*, 2011). Pancreatic tumors are characterized
by excessive desmoplastic stroma (Feig *et al.*, 2012), a fibrotic deposition produced by
pancreatic stellate cells, also called fibroblasts. The desmoplastic stroma protects tumor cells
against radiation, restricts the delivery of chemotherapy drugs into tumors, and promotes tumor
progression and metastasis (Rucki and Zheng, 2014). Thus, stroma depletion has been
20 investigated as a potential treatment for pancreatic cancer. However, stroma depletion alone
has not been successful thus far.

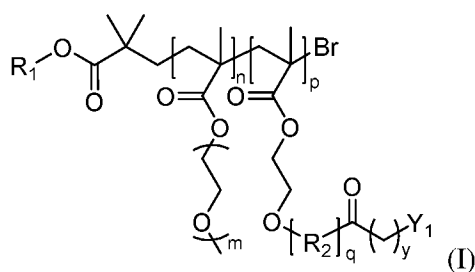
The sonic hedgehog (SHH) signaling pathway plays a critical role in the initiation and
progression of pancreatic cancer, and sustained activation of this pathway contributes to
excessive deposition of tumor stroma and maintenance of tumor-initiating cells (Thayer *et al.*,
25 2003; Geng *et al.*, 2007; Watkins and Peacock, 2004; Onishi and Katano, 2014). Many
preclinical studies have shown that inhibitors of SHH pathway, such as cyclopamine (CPA),
saridegib, and vismodegib, are effective against pancreatic cancer (Chen *et al.*, 2002; Kim *et al.*,
2014). CPA is a natural plant product with potent antagonist activity against the
smoothened (SMO) receptor in the SHH pathway. CPA can deplete cancer stem cells (CSCs),
30 disrupt tumor stroma, and enhance the tumor response to ionizing radiation (Shafaei *et al.*,
2006; Kelleher, 2011). CPA also increases the cytotoxic effects of paclitaxel (PTX) in SHH-

expressing pancreatic cancer cells (Shafae *et al.*, 2006). However, in many cases, CPA monotherapy only delayed pancreatic tumor growth (Chitkara *et al.*, 2012; You *et al.*, 2015). Moreover, both CPA and PTX are water insoluble and are highly toxic which presents potential co-administration problems. Several polymer-based nanoformulations have been prepared by
5 covalent conjugation or physical encapsulation of CPA (Chitkara *et al.*, 2012; You *et al.*, 2015; Cho *et al.*, 2013; Zhou *et al.*, 2012), and such formulations have shown promising antitumor efficacy against different tumor types. However, these formulations displayed undesirable burst release effects or fast release profiles over a 24-h incubation period under physiological conditions. It was also difficult to achieve CPA payload greater than 5% by weight. These
10 shortcomings could limit further development of these CPA nanoparticle formulations. Therefore, there remains a need for improved methods of delivery CPA and other chemotherapeutics for administration to cancers such as pancreatic cancer.

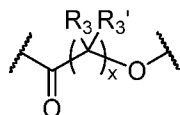
SUMMARY OF THE INVENTION

[0004] In some aspects, the present disclosure provides polymers and polymeric micelles thereof for use in the delivery of therapeutic agents. In some embodiments, these drug delivery systems may be used in the treatment of cancers such as pancreatic cancer.

5 [0005] An aspect of the present invention relates to a polymer, wherein the polymer is a block copolymer comprising a polyacrylic acid backbone with a plurality of PEG sidechains and a plurality of ϵ -caprolactone sidechains. The PEG sidechain component may comprises from 5 to 50, from 3 to 40, from 3 to 20, or 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, or 40, or any range derivable therein of ethylene glycol monomers. In some
10 embodiments, the PEG sidechain is capped with a methyl group. In some embodiments, the ϵ -caprolactone sidechain comprises from 2 to 20, from 2 to 15, or 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or any range derivable therein of ϵ -caprolactone repeating units. In some embodiments, the ϵ -caprolactone sidechain is esterified with a succinate group. The ϵ -caprolactone sidechain may be linked to the polyacrylic acid backbone by an ethylene glycol
15 linker. In some embodiments, the polyacrylic acid backbone is capped with an benzyl N-(2-hydroxyethyl)carbamate or ethanolamine group. In some embodiments, the polyacrylic acid backbone is terminated with a halogen group. In some embodiments, the polymer is further defined by the formula:

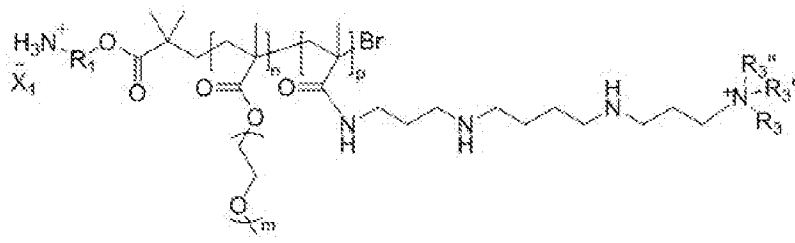
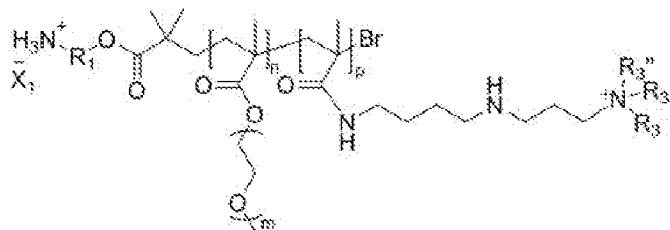
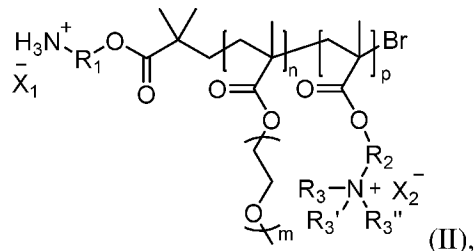


20 wherein: R_1 is alkyl_(C1-18), cycloalkyl_(C1-18), aryl_(C1-18), or a substituted version of any of these groups; or an alkyl_(C1-18) substituted with a protected amine group; R_2 is a group of the formula:



wherein: R_3 and R_3' are each independently hydrogen, alkyl_(C1-6), or substituted alkyl_(C1-6); and x is 1-10; m is 5-40 or 10-40; n is 10-40; p is 5-40 or 5-20; q is 1-20 or 1-10; y is 1-10; and Y_1 is carboxy, phosphonate, or hydroxysulfonyl; or a pharmaceutically acceptable salt thereof.

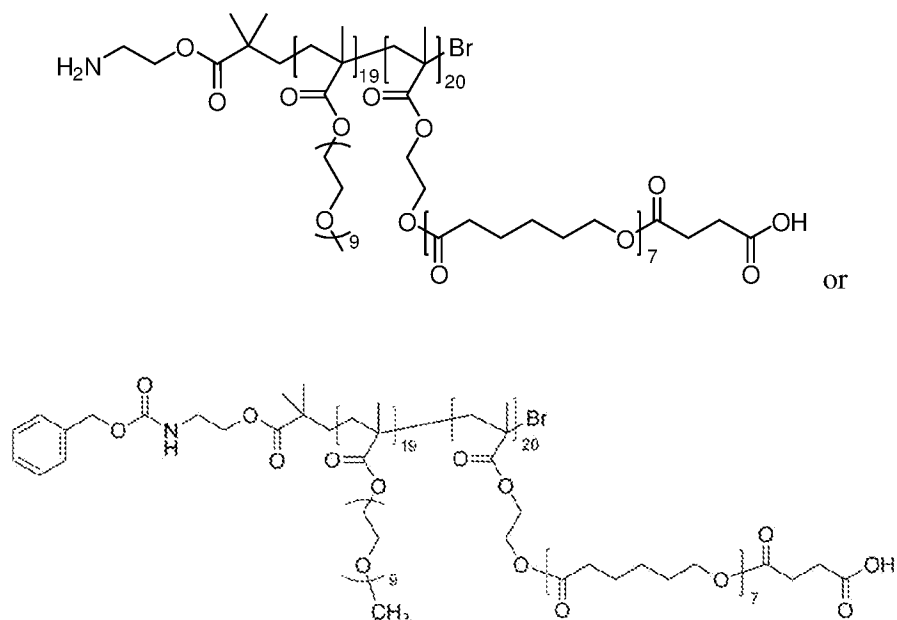
[0006] In some embodiments, the polymer further comprises a second polymer of the
5 formula:



wherein: m is 5-40 or 10-40; n is 10-40; p is 5-30; R_1 and R_2 are each independently
10 alkanediyl_(C1-8), arenediyl_(C6-12), or a substituted version of either of these groups; R_3 , R_3' , and
 R_3'' are each independently alkyl_(C1-8), aryl_(C3-12), or a substituted version of any of these groups;
and X_1 and X_2 are each independently a monovalent anion; or a pharmaceutically salt thereof.
In some embodiments, m is 5-25, 15-25, 8-10, or any range derivable therein. In some
embodiments, n is 15-25, 18-20, or any range derivable therein. In some embodiments, p is 5-
15 25, 18-22, 5-15, or any range derivable therein. In some embodiments, q is 5-10. In some
embodiments, x is 5-10, 5, 6, 7, 8, 9, or 10. In some embodiments, R_3 and R_3' are hydrogen.
In some embodiments, R_3 and R_3' are hydrogen and x is 5. R_1 may be ethylene. R_2 may be

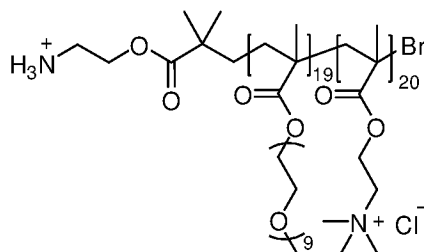
ethylene. In some embodiments, R_3 , R_3' , and R_3'' are each methyl. X_1 may be a halide (*e.g.*, chloride). X_2 may be a halide (*e.g.*, chloride).

[0007] In some embodiments, the polymer has the structure:

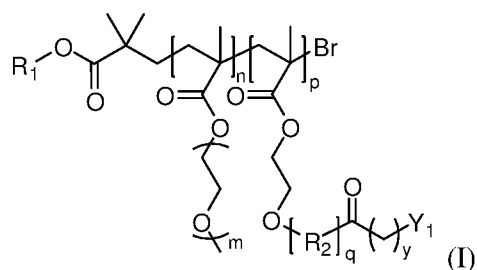


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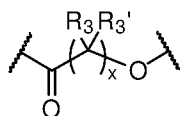
In some embodiments, the second polymer has the structure:



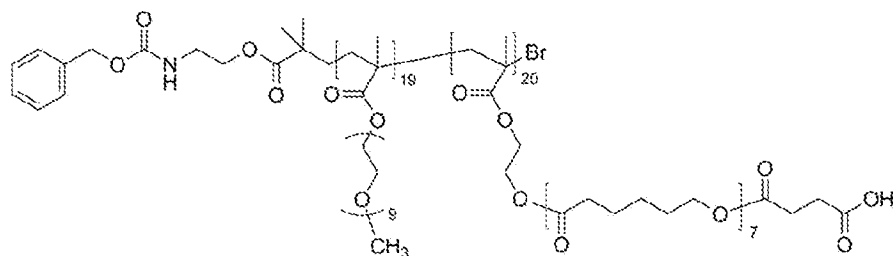
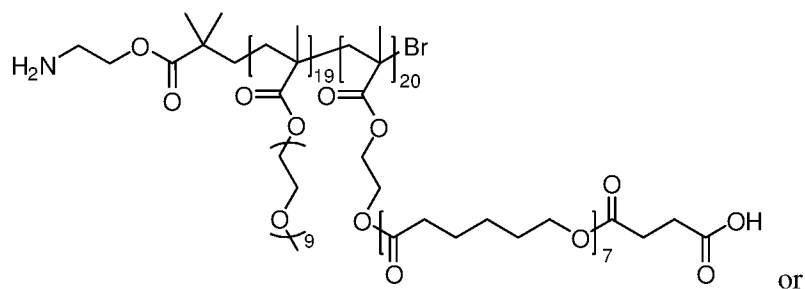
[0008] Another aspect of the present invention relates to a pharmaceutical composition comprising: (a) a micelle, liposome, or nanoparticle; and (b) a therapeutic agent (*e.g.*, one or two therapeutic agents); wherein the micelle, liposome, or nanoparticle comprises a first polymer of the present invention or as defined above, and the micelle, liposome, or nanoparticle encapsulates the therapeutic agent. In some embodiments, the pharmaceutical composition further comprises an excipient such as, for example, a monosaccharide or a disaccharide (*e.g.*, sucrose). The micelle, liposome, or nanoparticle further comprises a second polymer. In some embodiments, the first polymer comprises a net anionic charge. In some embodiments, the first polymer is further defined by the formula:



wherein: R_1 is alkyl_(C1-18), cycloalkyl_(C1-18), aryl_(C1-18), or a substituted version of any of these groups; or an alkyl_(C1-18) substituted with a protected amine group; R_2 is a group of the formula:



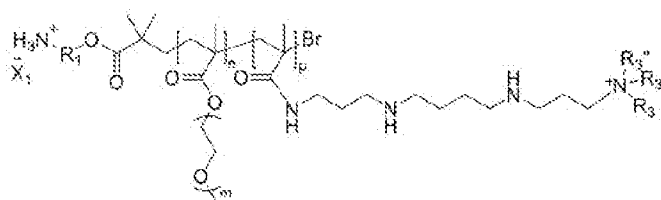
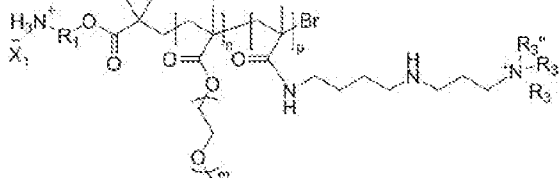
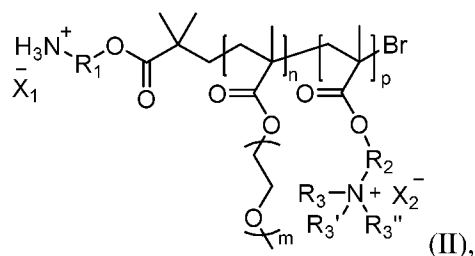
- 5 wherein: R_3 and R_3' are each independently hydrogen, alkyl_(C1-6), or substituted alkyl_(C1-6); and x is 1-10; m is 5-40 or 10-40; n is 10-40; p is 5-40 or 5-20; q is 1-20 or 1-10; y is 1-10; and Y_1 is carboxy, phosphonate, or hydroxysulfonyl; or a pharmaceutically acceptable salt thereof. In some embodiments, the first polymer is:



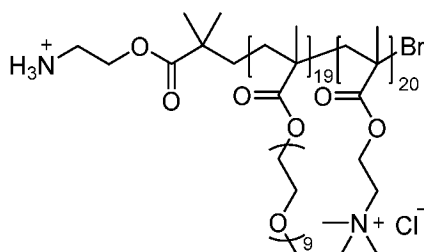
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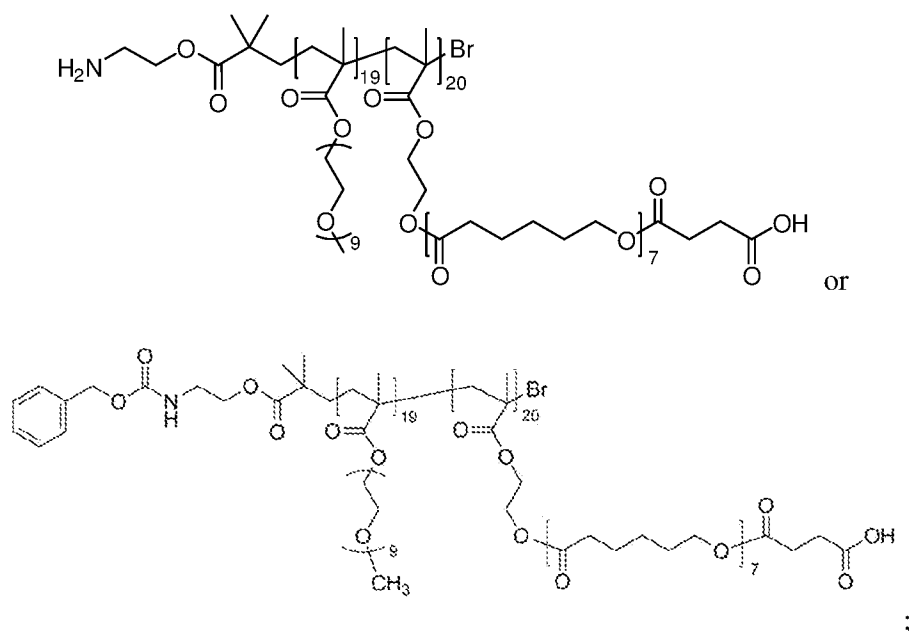
or a pharmaceutically acceptable salt thereof. In some embodiments, the second polymer comprises a net cationic charge. In some embodiments, the second polymer is further defined by the structure:



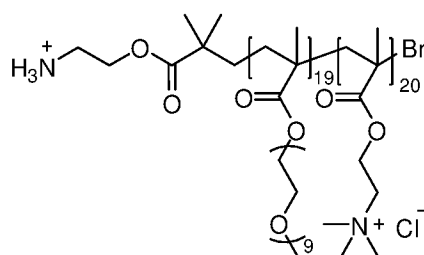
wherein: m is 5-40 or 10-40; n is 10-40; p is 5-30; R₁ and R₂ are each independently
 5 alkanediyl_(C1-8), arenediyl_(C6-12), or a substituted version of either of these groups; R₃, R_{3'}, and
 R_{3''} are each independently alkyl_(C1-8), aryl_(C3-12), or a substituted version of any of these groups;
 and X₁ and X₂ are each independently a monovalent anion; or a pharmaceutically salt thereof.
 In some embodiments, the second polymer is further defined by the formula:



10 or a pharmaceutically acceptable salt thereof. In some embodiments, the first polymer has the
 structure:



and wherein the second polymer has the structure:



- 5 or a pharmaceutically acceptable salt thereof. In some embodiments, the ratio of the first polymer to the second polymer is from about 100:1 w/w to about 1:10 w/w, from about 100:1 w/w to about 1:1, from about 100:1 w/w to about 10:1, from about 10:1 w/w to about 1:1 w/w, or about 100:1 w/w, 50:1 w/w, 10:1 w/w, 5:1 w/w, or any range derivable therein. In some
- 10 embodiments, the pharmaceutical composition comprises two therapeutic agents (*e.g.*, two chemotherapeutic agents) that each independently treat or selectively target or bind tumors cells and/or tumor stroma cells. The therapeutic agent may be a chemotherapeutic such as, *e.g.*, paclitaxel or cyclophosphamide. In some embodiments, the pharmaceutical composition comprises paclitaxel and cyclophosphamide. In some embodiments, the chemotherapeutic is GANT58, GANT61, vitamin D, or 17-N-allylamino-17-demethoxygeldanamycin (17-AAG).
- 15 In some embodiments, the pharmaceutical composition comprises: (i) GANT58 and paclitaxel, (ii) GANT61 and paclitaxel, (iii) vitamin D and paclitaxel, (iv) GANT58 and 17-AAG, (v) GANT61 and 17-AAG, or (vi) vitamin D and 17-AAG. In some embodiments, the

pharmaceutical composition is formulated for intravenous, intra-arterial, intraperitoneal, parenteral, subcutaneous, or intra-tumoral administration. In some embodiments, the pharmaceutical composition further comprises an excipient. The excipient may be a monosaccharide or a disaccharide (*e.g.*, sucrose). In some embodiments, the concentration of the monosaccharide or disaccharide is from about 1% to about 10% (w/w). In some
5 embodiments, the concentration of the excipient is about 5% (w/w).

[0009] Another aspect of the present invention relates to a method of treating a disease in a mammalian subject comprising administering a therapeutically effective amount of a pharmaceutical composition of the present invention or as defined above to the subject. In
10 some embodiments, the disease is a cancer. The cancer may be a pancreatic cancer such as, *e.g.*, pancreatic ductal adenocarcinoma (PDAC). In some embodiments, the pancreatic cancer is a pancreatic cancer in which one or more proteins in the sonic hedgehog signaling pathway is defective (*e.g.*, the sonic hedgehog signaling pathway is upregulated). In some
15 embodiments, the pancreatic cancer is a pancreatic cancer which the smoothened receptor (SMO) is misregulated (*e.g.*, upregulated). In some embodiments, the cancer is liver cancer (*e.g.*, hepatocellular carcinoma). In some embodiments, the cancer is ovarian cancer. The ovarian cancer may be a high grade serous carcinoma. In some embodiments, the cancer is basal-cell carcinoma. In some embodiments, the cancer is medulloblastoma. In some
20 embodiments, the pharmaceutical composition comprises paclitaxel or cyclopamine. In some embodiments, the pharmaceutical composition comprises paclitaxel and cyclopamine. In some embodiments, the mammalian subject is a human. In some embodiments, the pharmaceutical composition may be administered to the subject in combination with a second therapeutic or anticancer therapy such as, *e.g.*, a checkpoint inhibitor, an immunotherapy (*e.g.*, a therapeutic antibody, an antibody fragment, or an immunotoxin), or a radiotherapy. In some embodiments,
25 the method further comprises administering a checkpoint inhibitor, an immunotherapy, or a radiotherapy to the subject.

[0010] In some embodiments, a pharmaceutical composition may be administered to a patient in combination with one or more additional therapeutic agents. For example, the additional therapeutic agent may be an immunotherapy (*e.g.*, to treat a cancer). For example,
30 one or more therapeutic antibodies, antibody fragments (*e.g.*, scFv, *etc.*), or immunotoxins may be administered to a subject, such as a human patient, in combination with a nanoparticle or micelle as described herein.

[0011] As used herein, “substantially free,” in terms of a specified component, is used herein to mean that none of the specified component has been purposefully formulated into a composition and/or is present only as a contaminant or in trace amounts. The total amount of the specified component resulting from any unintended contamination of a composition is therefore below 0.05%. When used herein, the term “more substantially free” means below 0.01%. In some embodiments, when the term “essentially free” is used to describe a composition in which no amount of the specified component can be detected using standard analytical methods.

[0012] As used herein the specification, “a” or “an” may mean one or more. As used herein in the claim(s), when used in conjunction with the word “comprising,” the words “a” or “an” may mean one or more than one.

[0013] The use of the term “or” in the claims is used to mean “and/or” unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and “and/or.” As used herein “another” may mean at least a second or more.

[0014] Throughout this application, the term “about” is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

[0015] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0017] **FIGs. 1A-1D:** Chemical structures and ^1H -NMR spectra of the polymers used to formulate the micelles. **(A and C)** Chemical structures of the anionic polymer **(A)** and cationic polymer **(C)**. **(B and D)** ^1H -NMR spectra of the anionic polymer **(B)** and cationic polymer **(D)**. The bold lowercase letters indicate the characteristic chemical shifts of protons.

[0018] **FIGs. 2A-2G:** TEM micrographs of polymeric micelles M-CPA **(A)**, M-PTX **(B)**, and M-CPA/PTX **(C)**; and drug-release profiles at 37 °C **(D-G)** of drug-loaded micelles. TEM was done with uranyl acetate negative staining. Each release study was repeated three times. Data are presented as mean $\pm$ SD.

[0019] **FIGs. 3A-3I:** Effects of M-CPA and M-PTX on Miapaca-2 cells. **(A)** Inhibition of CPA-BODIPY binding to SMO receptors after 24-h incubation with M-CPA. **(B and C)** Representative fluorescence microscopic images of CPA-BODIPY-treated cells **(B)** not incubated with M-CPA and **(C)** incubated with 20 μM M-CPA. Green, CPA-BODIPY; blue, nuclei; scale bar = 20 μm . **(D)** Normalized Gli-1 expression after 48-h incubation with M-CPA. **(E and F)** Cell viability after 72-h incubation with **(E)** CPA or M-CPA or **(F)** PTX, M-PTX, or M-CPA/PTX. **(G)** Normalized number of colonies formed after 10-day incubation with CPA or M-CPA. **(H and I)** Representative images of colonies after incubation with **(H)** 3 μM CPA or **(I)** 3 μM M-CPA.

[0020] **FIGs. 4A-4J:** Effect of M-CPA and/or M-PTX treatment of Miapaca-2 cells on CD133 expression and tumorsphere formation. **(A-D)** Representative flow cytometry plots of CD133 expression after 48-h exposure to **(A)** CTL, **(B)** 10 μM M-CPA, **(C)** 10 nM M-PTX, or **(D)** 10 μM M-CPA + 10 nM M-PTX. CD133⁺ cells are those within polygons. **(E)** Percentages of CD133⁺ cells after the indicated treatments (N = 3). M-PTX treatment significantly enriched the CD133⁺ population compared to control ($p < 0.001$); M-CPA and M-CPA/PTX significantly depleted the CD133⁺ population compared to control ($p < 0.001$). **(F)** Numbers of formed tumorspheres (> 50 μm) per 1000 cells after the indicated treatments (N = 6). The control and M-PTX groups had significantly more tumorspheres than the M-CPA or M-

CPA/PTX group ($p < 0.001$). **(G-J)** Representative micrographs of cells treated with **(G)** control (CTL), **(H)** 10 μ M M-CPA, **(I)** 10 nM M-PTX, or **(J)** 10 μ M M-CPA + 10 nM M-PTX.

[0021] FIGs. 5A-5E: Effects of drug-loaded micelles on HPSC proliferation and SHH pathway activities. **(A)** Cell viability after 72-h incubation with M-CPA or free CPA (concentration in CPA equivalent) ($N = 6$). **(B)** Cell viability after 72-h incubation with M-PTX or M-CPA/PTX (concentration in PTX equivalent) ($N = 6$). M-CPA/PTX had equal loading of CPA and PTX. **(C)** Normalized Gli-1 expression of HPSCs after 48-h incubation with M-CPA. Results are means from two independent western blot analyses and are normalized against β -actin. **(D)** Cell viability after 72-h incubation in the presence of SHH ligand, with or without 10 μ M M-CPA ($N = 6$). **(E)** Normalized Gli-1 expression of HPSCs after 48-h incubation in the presence of M-CPA, with or without 1.5 μ g/mL SHH. Results are means from two independent western blot analyses and are normalized against β -actin.

[0022] FIGs. 6A-6D: Antitumor efficacy of M-CPA, M-PTX, and M-CPA/PTX in an orthotopic Miapaca-2/HPSC xenograft model in mice. **(A)** Relative tumor growth measured by bioluminescence ($N = 9$). Dosages were as follows: M-CPA and M-PTX, 10 mg/kg/injection, 3 injections in 1 week; M-CPA/PTX, 10 mg/kg/drug/injection, 3 injections in 1 week. Time of injections is marked with arrows. Tumor growth was slower in the M-CPA/PTX group than in any other group ($p < 0.01$). **(B and C)** Representative micrographs of hematoxylin-eosin-stained tumor sections from control and M-CPA/PTA-treated mice. **(B)** Tumor section from nontreated control mouse was packed with viable tumor cells and tumor stroma (boundary outlined by white arrows). **(C)** Portions of tumor section from M-CPA/PTX-treated mouse showed loose distribution of tumor cells. **(D)** Representative micrograph of hematoxylin-eosin-stained section of pancreas from a tumor-free mouse treated with M-CPA/PTX showed no residual tumor cells.

[0023] FIGs. 7A-7O: Immunohistochemical staining of MiaPaca tumor sections after various treatments. **(A-E)** Ki67 staining for proliferating tumor cells in the **(A)** control, **(B)** M-CPA, **(C)** M-PTX, and **(D)** M-CPA/PTX groups and **(E)** comparison of the number of Ki67⁺ nuclei per 20 $\times$ microscope field among treatment groups. All three treated groups had fewer Ki67⁺ nuclei than the control group ($p < 0.001$); the M-CPA/PTX group had fewer Ki67⁺ nuclei than the M-CPA group ($p < 0.05$). **(F-J)** CD31 staining for tumor vasculature in the **(F)** control, **(G)** M-CPA, **(H)** M-PTX, and **(I)** M-CPA/PTX groups and **(J)** comparison of vasculature density among treatment groups. Compared to control, M-CPA/PTX increased the tumor

vasculature over 10-fold ($p < 0.001$), whereas M-CPA increased the tumor vasculature 3.6-fold ($p < 0.05$). **(K-O)** α -SMA staining for activated stroma in the **(K)** control, **(L)** M-CPA, **(M)** M-PTX, and **(N)** M-CPA/PTX groups and **(O)** comparison of activated stroma composition among treatment groups. M-CPA and CPA/M-PTX significantly reduced α -SMA staining compared to the control ($p < 0.001$), whereas M-PTX did not cause a significant change compared to the control ($p > 0.05$). At least 15 randomly chosen 20 $\times$ fields of view were analyzed for each group.

[0024] FIG. 8: Schematic illustration for the preparation of anionic polymer poly(PEGMA)₁₉-poly[HEMA-g-(CL₇)-mono-succinate ester]₂₀

[0025] FIG. 9: ¹H-NMR spectrum of 2-(benzyloxycarbonyl amino) ethyl 2-bromo-isobutyrate (initiator **1**). Hydrogen atoms and their corresponding peak integrations are marked by lowercase letters.

[0026] FIG. 10: ¹H-NMR spectrum of P(PEGMA)₁₉-b-P(HEMA)₂₀ (polymer **2**). Hydrogen atoms and their corresponding peak integrations are marked by lowercase letters.

[0027] FIG. 11: ¹H-NMR spectrum of poly(PEGMA)₁₉-b-poly[HEMA-g-(ϵ -caprolactone)₇]₂₀ (polymer **3**). Hydrogen atoms and their corresponding peak integrations are marked by lowercase letters.

[0028] FIG. 12: ¹H-NMR spectrum of poly(PEGMA)₁₉-poly[HEMA-g-(ϵ -caprolactone)₇-mono-succinate ester]₂₀ (polymer **4**). Hydrogen atoms and their corresponding peak integrations are marked by lowercase letters.

[0029] FIG. 13: Schematic illustration for the preparation of cationic polymer poly(PEGMA)₁₉-poly(MeDMA)₂₀ (polymer **5**).

[0030] FIG. 14: ¹H-NMR spectrum of the reaction mixture of cationic polymer poly(PEGMA)₁₉-poly(MeDMA)₂₀ (polymer **5**) at the end of the reaction.

[0031] FIG. 15: Relative Gli-1 expression in Miapaca-2, L3.6pl, and Panc-1 cells after 48-hr treatment with M-CPA.

[0032] FIGs. 16A-16B: Colony formation of L3.6pl cells **(A)** and Panc-1 cells **(B)** during incubation with M-CPA at various concentrations.

[0033] **FIGs. 17A-17D:** Immunofluorescence staining of SMO (green) in Miapaca-2 cells (A), L3.6pl cells (B), HPSCs (C), and Panc-1 cells (D). Cell nuclei were counterstained with Hoechst (blue).

[0034] **FIG. 18:** Survival of orthotopic murine PDAC tumors with Kras mutation (Kras*). The median survivals were: (1) CTL 16.5 days, (2) M-CPA/PTX 16.5 days, (3) Anti-PD1 23 days, (4) M-CPA/PTX + Anti-PD1 31 days, and (5) M-CPA/PTX + Anti-PD1 + Anti-CD8 α 14.5 days. The survival of group 4 was significantly longer than group 3 ($p = 0.0013$).

[0035] **FIG. 19:** Tumor weight of Kras* tumors. Differences were noted by * ($p < 0.05$), ** ($p < 0.01$), or ns (not significant). At 10 days after the start of treatment, the tumors treated with M-CPA/PTX + Anti-PD1 were significantly smaller than the other three groups.

[0036] **FIGs. 20A-B:** ^{64}Cu -anti-PD1 in tumor-draining lymph nodes and Kras* tumor. **FIG. 20A**, Quantification of ^{64}Cu -anti-PD1 in tumor-draining lymph nodes and Kras* tumor, showing a trend of increased distribution of the antibody, which recognizes PD1+ T cells, after M-CPA/PTX treatment. **FIG. 20B**, Autoradiographs of Kras* tumors obtained at 24 h after intravenous injection of ^{64}Cu -anti-PD1, demonstrating more homogenous distribution of the antibody, which recognizes PD1+ T cells, after M-CPA/PTX treatment.

[0037] **FIG. 21:** Effect of M-CPA/PTX on T cells infiltration of tumors *in vivo*. The treated tumors had significantly more T cells infiltration than untreated CTL tumors. Data was presented as mean $\pm$ standard error of mean. Differences between the two groups were compared using unpaired t-test. ** indicates a p -value < 0.01 .

[0038] **FIG. 22:** Antitumor efficacy of M-CPA/PTX in a transgenic mice model with spontaneous PDAC in the pancreas of KPC-Luc mice. Mice with positive bioluminescence signals were enrolled at 95 to 100 days of age. M-CPA/PTX was given at 5 mg/kg/dose per injection. The treatment schedule was: 3 intravenous injections per week for the first 2 weeks, followed by 2 intraperitoneal injections per week until death or 90 days from the initiation of treatment. Untreated mice were kept as control (CTL). Gemcitabine and abraxane were used as the standard therapies. Gemcitabine was intraperitoneally injected at 100 mg/kg every other day until death. Abraxane was given at 5 mg/kg/injection following the same schedule as M-CPA/PTX.

[0039] **FIG. 23:** Histological analysis of tumors removed from untreated control mice and KPC-Luc mice treated with M-CPA/PTX. Mice of about 125 days old when tumors became palpable were enrolled into the study. Each mouse in the M-CPA/PTX treatment group was given six intravenous injections of M-CPA/PTX (5 mg/kg/dose/injection) over 2 weeks.

5 At 24 hours after the last injection, mice were sacrificed to collect tumors and processed for histological evaluation.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0040] In some aspects, the present disclosure provides comb-like polymer structures which are used to form micelles for the delivery of drugs. These micelles may be used to treat disease such as cancer by improving the delivery of one or more therapeutic agent *in vivo*. In some embodiments, these polymers also slow the release of one or more of the therapeutic agents within the body.

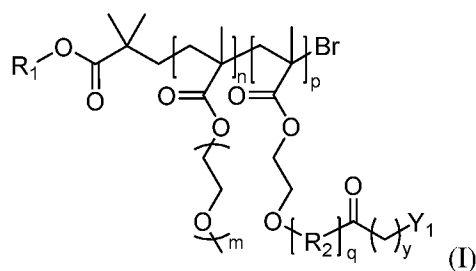
I. Polymer Compositions

[0041] In some aspects, the present disclosure presents polymers comprising a polyacrylate backbone which contains blocks of a plurality of PEG sidechains and a plurality of sidechains resulting from a ring opening polymerization. In some embodiments, the polyacrylate backbone is terminated with a group traditionally characteristic of a radical polymerization technique such as atom-transfer radical polymerization (ATRP). Some non-limiting examples of such chemical groups include hydrogen, hydroxy, or halides. In some embodiments, the polyacrylate backbone may be capped with a C1-C8 aminoalkyl such as ethanolamine or amine protected aminoalkyl such as, *e.g.*, benzyl N-(2-hydroxyethyl)carbamate (N-Cbz-ethanolamine). The polymers described herein may contain either a polyacrylic acid backbone or a polyacrylamide backbone. In some embodiments, the backbone of the polymer is a polyacrylic acid backbone. In some embodiments, the carboxylic acid of the polyacrylic acid backbone is esterified with a polyethylene glycol group to obtain a PEG sidechain. Polyethylene glycol is a polymer of ethylene glycol repeating units which the terminus is a hydroxyl group or is capped with a methyl group. The polymers described herein comprise a polyacrylate backbone with from 10 monomers to 40 monomers with PEG sidechains. In some embodiments, the number of monomers with PEG sidechains is from 16 to 22 monomers. The number of monomers in the polymer of the present disclosure is preferably from 10, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 26, 28, 30, 32, 34, 36, 38, to 40, or any range derivable therein. In some embodiments, the number of monomers with PEG sidechains is 19.

[0042] In some embodiments, the polyacrylate backbone further comprises monomers wherein the carboxylic acid has been esterified with a polymer derived from the ring opening of a lactone. In some embodiments, the polymer resulting from a ring opening is a polycaprolactone. The polycaprolactone may be esterified to the carboxylic acid through an

ethylene glycol linker or cyclic anhydride. In some embodiments, the number of monomers with polycaprolactone sidechains is from 10 to 40 monomers, more preferably from 16 to 22 monomers. The number of monomers in the polymer of the present disclosure is from 10, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 26, 28, 30, 35, to 40, or any range derivable therein.

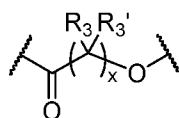
- 5 In some embodiments, the number of monomers with polycaprolactone sidechains is 20. In some embodiments, the terminus of the polycaprolactone is esterified with a carboxylic acid linked to another group which has a net negative charge at physiological pH. In some embodiments, the group is a phosphonate, a sulfonate, or a second carboxylic acid. In some
10 embodiments, the terminus of the polycaprolactone is esterified with butanedioic acid (succinic acid), pentanedioic acid, or hexanedioic acid. In some embodiments, the terminus of the polycaprolactone is esterified with succinic acid. In some aspects, the polymer has a net negative charge. In some embodiments, the present disclosure provides a polymer of the formula:



- 15 wherein:

R_1 is alkyl_(C1-18), cycloalkyl_(C1-18), aryl_(C1-18), or a substituted version of any of these groups; or an alkyl_(C1-18) substituted with a protected amine group;

R_2 is a group of the formula:



- 20 wherein:

R_3 and R_3' are each independently hydrogen, alkyl_(C1-6), or substituted alkyl_(C1-6); and

x is 1-10;

m is 5-40 or 10-40;

n is 10-40;

p is 5-40 or 5-20;

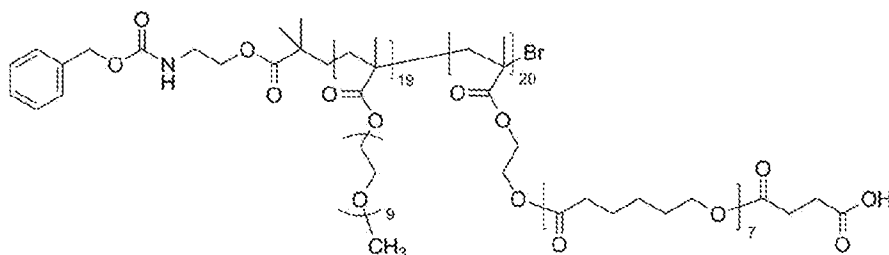
q is 1-20 or 1-10;

5 y is 1-10; and

Y_1 is carboxy, phosphonate, or hydroxysulfonyl;

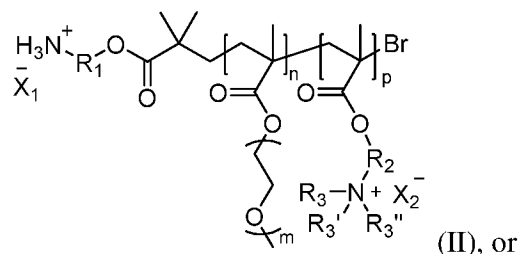
or a pharmaceutically salt thereof.

[0043] In some non-limiting examples, formula I is poly[oligo(ethylene glycol) methyl ether methacrylate]-*b*-poly[2-(methacryloyloxy)ethoxy-oligo(ϵ -caprolactone)-4-oxobutanoic acid] or

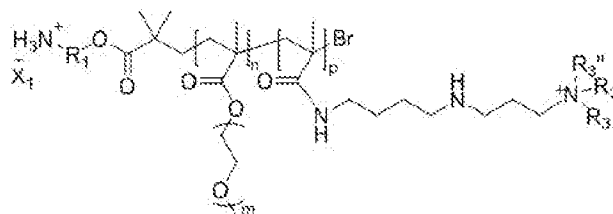


[0044] In some aspects, the present disclosure provides a second polymer containing one or more polyacrylate with a plurality of PEG sidechains and a plurality sidechains terminated with quaternary amine. In some embodiments, the carboxylic acid of the polyacrylic acid backbone in the second polymer is esterified with a polyethylene glycol group to obtain a PEG sidechain. Polyethylene glycol is a polymer of ethylene glycol repeating units which the terminus is a hydroxyl group or is capped with a methyl group. The polymers described herein comprise a polyacrylate backbone with from 10 monomers to 40 monomers with PEG sidechains. In some embodiments, the number of monomers with PEG sidechains is from 16 to 22 monomers. The number of monomers in the polymer of the present disclosure is from 10, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 26, 28, 30, 35, to 40, or any range derivable therein. In some embodiments, the number of monomers with PEG sidechains is 19.

[0045] In some embodiments, the carboxylic acid of the polyacrylic acid backbone in the second polymer is esterified with a quaternary amine groups. The polymers described herein comprise a polyacrylate backbone with from 10 monomers to 40 monomers with sidechains terminated with quaternary amines. The number of monomers in the polymer of the present disclosure is from 10, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 26, 28, 30, 35, to 40, or any range derivable therein. In some embodiments, the number of monomers with choline sidechains is 20. In some embodiments, the polymers described herein comprise a polyacrylate backbone with from 10 monomers to 40 monomers with choline side chains. In some embodiments, the second polymer comprises a net positive charge. In some embodiments, the present disclosure provides a second polymer of the formula:

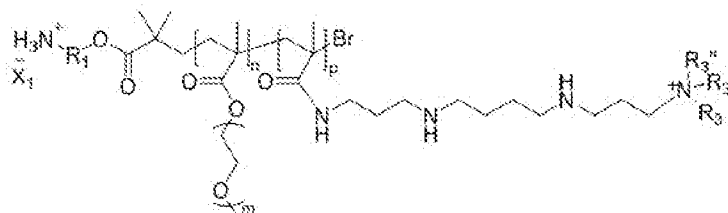


With
Spermidine
Pendent



or

With
Spermine
Pendent



wherein:

m is 5-40 or 10-40;

n is 10-40;

p is 5-30;

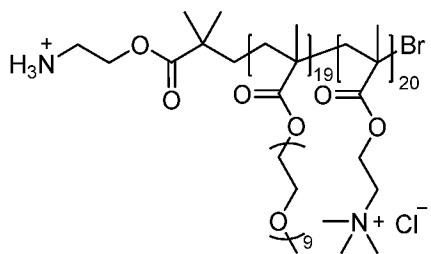
R_1 and R_2 are each independently alkanediyl_(C1-8), arenediyl_(C6-12), or a substituted version of either of these groups;

R_3 , R_3' , and R_3'' are each independently alkyl_(C1-8), aryl_(C3-12), or a substituted version of any of these groups; and

5 X_1 and X_2 are each independently a monovalent anion;

or a pharmaceutically salt thereof.

[0046] For example, Structure II can be ammonium capped poly[oligo(ethylene glycol) methyl ether methacrylate]-*b*-poly{[2-(methacryloyloxy)ethyl]trimethylammonium chloride}, as shown below:



II. Micelle Compositions

[0047] In some aspects, the present disclosure provides a nanoparticle or micelle prepared from a polymer described herein. Within the context of this disclosure, the term micelle and nanoparticle are used interchangeably. In some embodiments, the nanoparticle or micelle comprises a mixture of a first polymer and a second polymer wherein the first polymer has a net negative charge and the second polymer has a net positive charge. These nanoparticles or micelles may comprises a mixture of the first polymer to the second polymer in a ratio from about 100:1, 90:1, 80:1, 70:1, 60:1, 55:1, 50:1, 45:1, 40:1, 30:1, 20:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, to about 1:10, or any range derivable therein. In some embodiments, the ratio is from about 10:1 to about 1:1 or from about 100:1 to about 10:1.

[0048] In some aspects, the micelles are loaded with one or more therapeutic agents. In some embodiments, these therapeutic agents are chemotherapeutics. In some embodiments, the micelles are loaded with one therapeutic agent. In other embodiments, the micelles are loaded with two or more therapeutic agents. In some embodiments, the two therapeutic agents

may act synergistically to obtain the desired therapeutic results. As such, the two therapeutic agents may target different molecular mechanisms associate with the disease or disorder.

[0049] In some aspects, the micelles prepared with different therapeutic agents may show a number average size from about 25 nm to about 250 nm. In some embodiments, the size is from about 40 nm to about 150 nm. In some embodiments, the size of the micelles may be from about 30 nm, 40 nm, 50 nm, 55 nm, 60 nm, 65 nm, 70 nm, 75 nm, 80 nm, 85 nm, 90 nm, 95 nm, 100 nm, 105 nm, 110 nm, 115 nm, 120 nm, 125 nm, 130 nm, 135 nm, 140 nm, 145 nm, to about 150 nm, or any range derivable therein. Furthermore, the micelles may have a polydispersity index (PDI) of less than 0.20. In some embodiment, the micelles may have a PDI of 0.05 to 0.15. In some embodiments, the micelles have a PDI of less than 0.2. Both micelle size and PDI may be determined by dynamic light scattering at a 90° scatter angle using a particle electrophoresis system using methods known to a person of skill in the art.

[0050] In some embodiments, the micelle have high relative loading of the therapeutic agents. The relative loading yield of the therapeutic agent may be greater than 85%. In some embodiments, it may be greater than 95%. In some embodiments, it may be greater than 98%. Furthermore, the amount of each therapeutic agent loaded in the micelle may comprise from about 1% w/w to about 15% w/w. In some embodiments, the amount of therapeutic agent is from 1%, 2%, 2.5%, 3%, 4%, 4.5%, 5%, 5.5%, 6%, 7%, 8%, 9%, 10% w/w to 15%, or any range derivable therein. In some embodiments, the amount of each therapeutic agent is about 5%-10% w/w. In other embodiments, when two or more therapeutic agents are used each therapeutic agent comprises about 2.5%-5% w/w of one of the polymers.

[0051] In some embodiments, the micelles described in the present disclosure may have increased stability. The micelles may show less than a 10% loss of therapeutic agent after storage for 90 days. In some embodiments, the micelles show a loss of less than 5% after a 90 day storage period. In some embodiments, the micelles show a loss of less than 5%, 4.5%, 4%, 3.5%, 3%, 2.5%, 2%, 1.5%, or 1%. Furthermore, when stored for a period of about 90 days, the particles show a change in size and PDI of less than 30%. In some embodiments, the change in size and PDI is less than 25%.

III. Pharmaceutical Preparations

[0052] In some embodiments, the micelles may be formulated as a pharmaceutical or therapeutic composition appropriate for the intended application. The therapeutic

compositions of the present embodiments are administered in the form of injectable compositions either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. These preparations also may be emulsified.

5 **[0053]** The phrases “pharmaceutical or pharmacologically acceptable” refers to molecular entities and compositions that do not produce an adverse, allergic, or other untoward reaction when administered to an animal, such as a human, as appropriate. The preparation of a pharmaceutical composition comprising additional active ingredients will be known to those of skill in the art in light of the present disclosure. In some non-limiting examples, the
10 pharmaceutical composition may further comprise a peptide or an antibody on the surface of the micelle, nanoparticle, or liposome or may be administered as a co-therapy. Moreover, for animal (*e.g.*, human) administration, it will be understood that preparations should meet sterility, pyrogenicity, general safety, and purity standards as required by FDA Office of Biological Standards.

15 **[0054]** As used herein, “pharmaceutically acceptable carrier” includes any and all aqueous solvents (*e.g.*, water, alcoholic/aqueous solutions, saline solutions, parenteral vehicles, such as sodium chloride, Ringer's dextrose, *etc.*), non-aqueous solvents (*e.g.*, propylene glycol, polyethylene glycol, vegetable oil, and injectable organic esters, such as ethyloleate), dispersion media, coatings, surfactants, monosaccharide and/or disaccharides, antioxidants,
20 preservatives (*e.g.*, antibacterial or antifungal agents, anti-oxidants, chelating agents, and inert gases), isotonic agents, absorption delaying agents, salts, drugs, drug stabilizers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, fluid and nutrient replenishers, such like materials and combinations thereof, as would be known to one of ordinary skill in the art. The pH and exact concentration of the various components in
25 a pharmaceutical composition are adjusted according to well-known parameters.

[0055] The term “unit dose” or “dosage” refers to physically discrete units suitable for use in a subject, each unit containing a predetermined quantity of the therapeutic composition calculated to produce the desired responses discussed above in association with its administration, *i.e.*, the appropriate route and treatment regimen. The quantity to be
30 administered, both according to number of treatments and unit dose, depends on the effect desired. The actual dosage amount of a composition of the present embodiments administered to a patient or subject can be determined by physical and physiological factors, such as body

weight, the age, health, and sex of the subject, the type of disease being treated, the extent of disease penetration, previous or concurrent therapeutic interventions, idiopathy of the patient, the route of administration, and the potency, stability, and toxicity of the particular therapeutic substance. For example, a dose may also comprise from about 1 µg/kg/body weight to about 1000 mg/kg/body weight (such range includes intervening doses) or more per administration, and any range derivable therein. In non-limiting examples of a derivable range from the numbers listed herein, a range of about 5 µg/kg/body weight to about 100 mg/kg/body weight, about 5 µg/kg/body weight to about 500 mg/kg/body weight, *etc.*, can be administered. The practitioner responsible for administration will, in any event, determine the concentration of active ingredient(s) in a composition and appropriate dose(s) for the individual subject.

[0056] Single or multiple doses of the agents are contemplated. Desired time intervals for delivery of multiple doses can be determined by one of ordinary skill in the art employing no more than routine experimentation. As an example, subjects may be administered two doses daily at approximately 12 hour intervals. In some embodiments, the agent is administered once a day.

[0057] The agent(s) may be administered on a routine schedule. As used herein a routine schedule refers to a predetermined designated period of time. The routine schedule may encompass periods of time which are identical or which differ in length, as long as the schedule is predetermined. For instance, the routine schedule may involve administration twice a day, every day, every two days, every three days, every four days, every five days, every six days, a weekly basis, a monthly basis or any set number of days or weeks there-between. Alternatively, the predetermined routine schedule may involve administration on a twice daily basis for the first week, followed by a daily basis for several months, *etc.* In other embodiments, the invention provides that the agent(s) may be taken orally and that the timing of which is or is not dependent upon food intake. Thus, for example, the agent may be taken every morning and/or every evening, regardless of when the subject has eaten or will eat.

[0058] The active compounds can be formulated for parenteral administration, *e.g.*, formulated for injection via the intravenous, intraarterial, intramuscular, sub-cutaneous, or even intraperitoneal routes. Typically, such compositions can be prepared as either liquid solutions or suspensions; solid forms suitable for use to prepare solutions or suspensions upon the addition of a liquid prior to injection can also be prepared; and, the preparations can also be emulsified.

[0059] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil, iodinated oil, or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that it may be easily injected. It also should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

[0060] The therapeutically compositions may be formulated into a neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts (such as those formed with the free amino groups of the polymer) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, trifluoroacetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Additionally, the pharmaceutical or therapeutic compositions may comprises one or more polycationic peptides or proteins such as protamine, polylysine, or polyarginine such that the therapeutic agent is formulated as a neutral salt or as a complex which contains significantly reduced charge.

[0061] A pharmaceutical composition can include a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion, and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. In some embodiments, the sugar used is 5% sucrose. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0062] The therapeutic compound may also be administered topically to the skin, eye, ear, or mucosal membranes. Administration of the therapeutic compound topically may include formulations of the compounds as a topical solution, lotion, cream, ointment, gel, foam,

transdermal patch, or tincture. When the therapeutic compound is formulated for topical administration, the compound may be combined with one or more agents that increase the permeability of the compound through the tissue to which it is administered.

IV. Cancers

5 [0063] In some embodiments, one or more nanoparticles or micelles as described herein may be used to treat a cancer. The cancer may be a solid tumor, metastatic cancer, or non-metastatic cancer. In some embodiments, the cancer is a pancreatic cancer such as, *e.g.*, a pancreatic ductal adenocarcinoma. For example, in some embodiments, a nanoparticle or micelle of the present invention may be used to deliver a combination of chemotherapeutic
10 agents (*e.g.*, paclitaxel and cyclopamine) to treat a pancreatic cancer, ovarian cancer, or liver cancer.

 [0064] It is anticipated that the nanoparticles may be used to treat a wide variety of cancers in a mammalian subject, such as a human patient. For example, cancer cells that may be treated with cell targeting constructs according to the embodiments include but are not
15 limited to cells from the bladder, blood, bone, bone marrow, brain, breast, colon, esophagus, gastrointestinal, gum, head, kidney, liver, lung, nasopharynx, neck, ovary, prostate, skin, stomach, testis, tongue, or uterus. In addition, the cancer may specifically be of the following histological type, though it is not limited to these: neoplasm, malignant; carcinoma; carcinoma, undifferentiated; giant and spindle cell carcinoma; small cell carcinoma; papillary carcinoma;
20 squamous cell carcinoma; lymphoepithelial carcinoma; basal cell carcinoma; pilomatrix carcinoma; transitional cell carcinoma; papillary transitional cell carcinoma; adenocarcinoma; gastrinoma, malignant; cholangiocarcinoma; hepatocellular carcinoma; combined hepatocellular carcinoma and cholangiocarcinoma; trabecular adenocarcinoma; adenoid cystic carcinoma; adenocarcinoma in adenomatous polyp; adenocarcinoma, familial polyposis coli;
25 solid carcinoma; carcinoid tumor, malignant; branchiolo-alveolar adenocarcinoma; papillary adenocarcinoma; chromophobe carcinoma; acidophil carcinoma; oxyphilic adenocarcinoma; basophil carcinoma; clear cell adenocarcinoma; granular cell carcinoma; follicular adenocarcinoma; papillary and follicular adenocarcinoma; nonencapsulating sclerosing carcinoma; adrenal cortical carcinoma; endometroid carcinoma; skin appendage carcinoma;
30 apocrine adenocarcinoma; sebaceous adenocarcinoma; ceruminous adenocarcinoma; mucoepidermoid carcinoma; 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, malignant; ovarian stromal tumor, malignant; thecoma, malignant; granulosa cell tumor, malignant; androblastoma, 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 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; 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; 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; hodgkin's disease; hodgkin's; paraganuloma; 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 cell leukemia; myeloid leukemia; basophilic leukemia; eosinophilic leukemia; monocytic leukemia; mast cell leukemia; megakaryoblastic leukemia; myeloid sarcoma; hairy cell leukemia, and medulloblastoma.

[0065] In addition to cancers, nanoparticles or micelles of the present invention may be used to deliver a therapeutic to treat other diseases. The present invention can find application in the treatment of any disease for which delivery of a drug, small molecule therapeutic, therapeutic protein, therapeutic nucleic acid, small interfering RNA (siRNA), or microRNA (mRNA) to a cell or tissue of a subject is believed to be of therapeutic benefit. Examples of such diseases include hyperproliferative diseases, inflammatory diseases, infectious diseases, degenerative diseases, and autoimmune diseases.

V. Therapeutic Agents

[0066] In some embodiments, a nanoparticle or micelle as described herein may be used to deliver a therapeutic agent such as a small molecule therapeutic, or a gene therapy (*e.g.*, a siRNA, a miRNA, an antisense, a therapeutic gene, *etc.*), or other therapeutic agent to a subject, such as a mammalian subject or a human.

a. Chemotherapeutic Agents

[0067] In some embodiments, one or more anticancer therapeutic agent(s), anticancer drug(s), or chemotherapeutic(s) may be included in a nanoparticle or micelle as described herein. For example, in some aspects the chemotherapeutic agent is a protein kinase inhibitor such as a EGFR, VEGFR, AKT, Erb1, Erb2, ErbB, Syk, Bcr-Abl, JAK, Src, GSK-3, PI3K, Ras, Raf, MAPK, MAPKK, mTOR, c-Kit, eph receptor or BRAF inhibitors. Nonlimiting examples of protein kinase inhibitors include Afatinib, Axitinib, Bevacizumab, Bosutinib, Cetuximab, Crizotinib, Dasatinib, Erlotinib, Fostamatinib, Gefitinib, Imatinib, Lapatinib, Lenvatinib, Mubritinib, Nilotinib, Panitumumab, Pazopanib, Pegaptanib, Ranibizumab, Ruxolitinib, Saracatinib, Sorafenib, Sunitinib, Trastuzumab, Vandetanib, AP23451, Vemurafenib, MK-2206, GSK690693, A-443654, VQD-002, Miltefosine, Perifosine, CAL101, PX-866, LY294002, rapamycin, temsirolimus, everolimus, ridaforolimus, Alvocidib, Genistein, Selumetinib, AZD-6244, Vatalanib, P1446A-05, AG-024322, ZD1839, P276-00, GW572016 or a mixture thereof.

[0068] Yet further combination chemotherapies include, for example, 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 bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callistatin; 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);
 5 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
 10 enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gammaII and calicheamicin omegaII; dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores, aclacinomysins, actinomycin, authrarnycin, azaserine, bleomycins, cactinomycin, carabycin, carminomycin, carzinophilin, chromomycins, dactinomycin,
 15 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,
 20 zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, 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, epitostanol, mepitostane,
 25 testolactone; anti-adrenals such as mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone;
 30 mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK polysaccharide complex; razoxane; rhizoxin; sizofiran; 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; taxoids, *e.g.*, paclitaxel and docetaxel gemcitabine; 6-thioguanine; mercaptopurine; platinum coordination complexes such as cisplatin, oxaliplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (*e.g.*, CPT-11); topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine; carboplatin, procarbazine, plicomycin, gemcitabine, navelbine, farnesyl-protein transferase inhibitors, transplatin, and pharmaceutically acceptable salts, acids or derivatives of any of the above. In certain embodiments, the compositions provided herein may be used in combination with gefitinib. In other embodiments, the present embodiments may be practiced in combination with Gleevac. In certain embodiments, one or more chemotherapeutic may be used in combination with the compositions provided herein.

[0069] In some embodiments, the therapeutic agent is a transcriptional factor inhibitor such as a hedgehog pathway inhibitor or a NF- κ B inhibitor. Nonlimiting examples of hedgehog pathway inhibitors include GANT61, GANT58, LDE225 (Sonidegib), LEQ 506, PF-04449913 (Glasdegib), TAK-441, IPI-926 (Saridegib), BMS 833923, LY2940680 (Taladegib), and GDC-0449 (Vismodegib). In some embodiments, the therapeutic agent is a vitamin. Nonlimiting examples of vitamins include vitamin D and vitamin D analogues. The therapeutic agent may be a naturally occurring anticancer agents. For example, nonlimiting examples of such naturally occurring agents include geldanamycin and its derivative 17-*N*-allylamino-17-demethoxygeldanamycin (17-AAG), triptolide, and related analogues.

b. Gene Therapies

[0070] In yet another embodiment, a gene therapy (*e.g.*, a siRNA, a miRNA, an antisense, a therapeutic gene, a nucleic acid encoding a therapeutic protein, *etc.*) may be included in a nanoparticle or micelle as described herein.

c. Anti-inflammatory Agents

[0071] In some embodiments, one or more anti-inflammatory agents may be included in a nanoparticle or micelle as described herein. The anti-inflammatory agent may be a steroid or a non-steroidal anti-inflammatory agent. Non-steroidal anti-inflammatory agents include a class of drugs used in the treatment of inflammation and pain. The exact mode of action of this class of drugs is unknown. Examples of members of this class of agents include, but are not

limited to, ibuprofen, ketoprofen, flurbiprofen, nabumetone, piroxicam, naproxen, diclofenac, indomethacin, sulindac, tolmetin, etodolac, flufenamic acid, diflunisal, oxaprozin, rofecoxib, and celecoxib. One of ordinary skill in the art would be familiar with these agents. Included in this category are salicylates and derivatives of salicylates, such as acetyl salicylic acid, sodium salicylate, choline salicylate, choline magnesium salicylate and diflunisal.

[0072] Other anti-inflammatory agents include anti-rheumatic agents, such as gold salts (*e.g.*, gold sodium thiomalate, aurothioglucose, and auranofin), anti-rheumatic agents (*e.g.*, chloroquine, hydroxychloroquine, and penicillamine), antihistamines (*e.g.*, diphenhydramine, chlorpheniramine, clemastine, hydroxyzine, and triprolidine), and immunosuppressive agents (*e.g.*, methotrexate, mechlorethamine, cyclophosphamide, chlorambucil, cyclosporine, and azathioprine). Other immunosuppressive agent contemplated include tacrolimus and everolimus. Tacrolimus suppresses interleukin-2 production associated with T-cell activation, inhibits differentiation and proliferation of cytotoxic T cells. One of ordinary skill in the art would be familiar with these agents, and other members of this class of agents, as well as the mechanism of actions of these agents and indications for use of these agents.

VI. Chemical Definitions

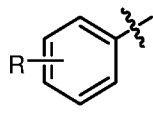
[0073] When used in the context of a chemical group: “hydrogen” means $-H$; “hydroxy” means $-OH$; “oxo” means $=O$; “carbonyl” means $-C(=O)-$; “carboxy” means $-C(=O)OH$ (also written as $-COOH$ or $-CO_2H$); “halo” means independently $-F$, $-Cl$, $-Br$ or $-I$; “amino” means $-NH_2$; “hydroxyamino” means $-NHOH$; “nitro” means $-NO_2$; imino means $=NH$; “cyano” means $-CN$; “isocyanate” means $-N=C=O$; “azido” means $-N_3$; in a monovalent context “phosphate” means $-OP(O)(OH)_2$ or a deprotonated form thereof; “phosphonate” means $-P(O)(OH)_2$ or a deprotonated form thereof; in a divalent context “phosphate” means $-OP(O)(OH)O-$ or a deprotonated form thereof; “mercapto” means $-SH$; and “thio” means $=S$; “sulfonyl” means $-S(O)_2-$; “hydroxysulfonyl” means $-S(O)_2OH$ or a deprotonated form thereof; “sulfonamide” means $-S(O)_2NH_2$; and “sulfinyl” means $-S(O)-$.

[0074] In the context of chemical formulas, the symbol “—” means a single bond, “=” means a double bond, and “≡” means triple bond. The symbol “----” represents an optional bond, which if present is either single or double. The symbol “” represents a single bond

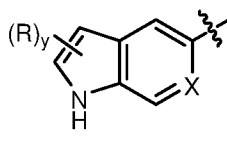
or a double bond. Thus, for example, the formula  includes , , , 

and . And it is understood that no one such ring atom forms part of more than one double bond. Furthermore, it is noted that the covalent bond symbol “—”, when connecting one or two stereogenic atoms, does not indicate any preferred stereochemistry. Instead, it covers all stereoisomers as well as mixtures thereof. The symbol “”, when drawn perpendicularly across a bond (*e.g.*, —CH₃ for methyl) indicates a point of attachment of the group. It is noted that the point of attachment is typically only identified in this manner for larger groups in order to assist the reader in unambiguously identifying a point of attachment. The symbol “” means a single bond where the group attached to the thick end of the wedge is “out of the page.” The symbol “” means a single bond where the group attached to the thick end of the wedge is “into the page.” The symbol “” means a single bond where the geometry around a double bond (*e.g.*, either *E* or *Z*) is undefined. Both options, as well as combinations thereof are therefore intended. Any undefined valency on an atom of a structure shown in this application implicitly represents a hydrogen atom bonded to that atom. A bold dot on a carbon atom indicates that the hydrogen attached to that carbon is oriented out of the plane of the paper.

[0075] When a group “R” is depicted as a “floating group” on a ring system, for example, in the formula:



then R may replace any hydrogen atom attached to any of the ring atoms, including a depicted, implied, or expressly defined hydrogen, so long as a stable structure is formed. When a group “R” is depicted as a “floating group” on a fused ring system, as for example in the formula:



then R may replace any hydrogen attached to any of the ring atoms of either of the fused rings unless specified otherwise. Replaceable hydrogens include depicted hydrogens (*e.g.*, the hydrogen attached to the nitrogen in the formula above), implied hydrogens (*e.g.*, a hydrogen of the formula above that is not shown but understood to be present), expressly defined

hydrogens, and optional hydrogens whose presence depends on the identity of a ring atom (*e.g.*, a hydrogen attached to group X, when X equals $-\text{CH}-$), so long as a stable structure is formed. In the example depicted, R may reside on either the 5-membered or the 6-membered ring of the fused ring system. In the formula above, the subscript letter “y” immediately following the group “R” enclosed in parentheses, represents a numeric variable. Unless specified otherwise, this variable can be 0, 1, 2, or any integer greater than 2, only limited by the maximum number of replaceable hydrogen atoms of the ring or ring system.

[0076] For the chemical groups and compound classes, the number of carbon atoms in the group or class is as indicated as follows: “C_n” defines the exact number (n) of carbon atoms in the group/class. “C_{≤n}” defines the maximum number (n) of carbon atoms that can be in the group/class, with the minimum number as small as possible for the group/class in question, *e.g.*, it is understood that the minimum number of carbon atoms in the group “alkenyl_(C≤8)” or the class “alkene_(C≤8)” is two. Compare with “alkoxy_(C≤10)”, which designates alkoxy groups having from 1 to 10 carbon atoms. “C_{n-n'}” defines both the minimum (n) and maximum number (n') of carbon atoms in the group. Thus, “alkyl_(C2-10)” designates those alkyl groups having from 2 to 10 carbon atoms. These carbon number indicators may precede or follow the chemical groups or class it modifies and it may or may not be enclosed in parenthesis, without signifying any change in meaning. Thus, the terms “C5 olefin”, “C5-olefin”, “olefin_(C5)”, and “olefin_{C5}” are all synonymous.

[0077] The term “saturated” when used to modify a compound or chemical group means the compound or chemical group has no carbon-carbon double and no carbon-carbon triple bonds, except as noted below. When the term is used to modify an atom, it means that the atom is not part of any double or triple bond. In the case of substituted versions of saturated groups, one or more carbon oxygen double bond or a carbon nitrogen double bond may be present. And when such a bond is present, then carbon-carbon double bonds that may occur as part of keto-enol tautomerism or imine/enamine tautomerism are not precluded. When the term “saturated” is used to modify a solution of a substance, it means that no more of that substance can dissolve in that solution.

[0078] The term “aliphatic” when used without the “substituted” modifier signifies that the compound or chemical group so modified is an acyclic or cyclic, but non-aromatic hydrocarbon compound or group. In aliphatic compounds/groups, the carbon atoms can be joined together in straight chains, branched chains, or non-aromatic rings (alicyclic). Aliphatic

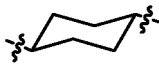
compounds/groups can be saturated, that is joined by single carbon-carbon bonds (alkanes/alkyl), or unsaturated, with one or more carbon-carbon double bonds (alkenes/alkenyl) or with one or more carbon-carbon triple bonds (alkynes/alkynyl).

[0079] The term “aromatic” when used to modify a compound or a chemical group atom means the compound or chemical group contains a planar unsaturated ring of atoms that is stabilized by an interaction of the bonds forming the ring.

[0080] The term “alkyl” when used without the “substituted” modifier refers to a monovalent saturated aliphatic group with a carbon atom as the point of attachment, a linear or branched acyclic structure, and no atoms other than carbon and hydrogen. The groups –CH₃ (Me), –CH₂CH₃ (Et), –CH₂CH₂CH₃ (*n*-Pr or propyl), –CH(CH₃)₂ (*i*-Pr, *i*Pr or isopropyl), –CH₂CH₂CH₂CH₃ (*n*-Bu), –CH(CH₃)CH₂CH₃ (*sec*-butyl), –CH₂CH(CH₃)₂ (isobutyl), –C(CH₃)₃ (*tert*-butyl, *t*-butyl, *t*-Bu or *t*Bu), and –CH₂C(CH₃)₃ (*neo*-pentyl) are non-limiting examples of alkyl groups. The term “alkanediyl” when used without the “substituted” modifier refers to a divalent saturated aliphatic group, with one or two saturated carbon atom(s) as the point(s) of attachment, a linear or branched acyclic structure, no carbon-carbon double or triple bonds, and no atoms other than carbon and hydrogen. The groups –CH₂– (methylene), –CH₂CH₂–, –CH₂C(CH₃)₂CH₂–, and –CH₂CH₂CH₂– are non-limiting examples of alkanediyl groups. The term “alkylidene” when used without the “substituted” modifier refers to the divalent group =CRR' in which R and R' are independently hydrogen or alkyl. Non-limiting examples of alkylidene groups include: =CH₂, =CH(CH₂CH₃), and =C(CH₃)₂. An “alkane” refers to the class of compounds having the formula H–R, wherein R is alkyl as this term is defined above. When any of these terms is used with the “substituted” modifier one or more hydrogen atom has been independently replaced by –OH, –F, –Cl, –Br, –I, –NH₂, –NO₂, –CO₂H, –CO₂CH₃, –CN, –SH, –OCH₃, –OCH₂CH₃, –C(O)CH₃, –NHCH₃, –NHCH₂CH₃, –N(CH₃)₂, –C(O)NH₂, –C(O)NHCH₃, –C(O)N(CH₃)₂, –OC(O)CH₃, –NHC(O)CH₃, –S(O)₂OH, or –S(O)₂NH₂. The following groups are non-limiting examples of substituted alkyl groups: –CH₂OH, –CH₂Cl, –CF₃, –CH₂CN, –CH₂C(O)OH, –CH₂C(O)OCH₃, –CH₂C(O)NH₂, –CH₂C(O)CH₃, –CH₂OCH₃, –CH₂OC(O)CH₃, –CH₂NH₂, –CH₂N(CH₃)₂, and –CH₂CH₂Cl. The term “haloalkyl” is a subset of substituted alkyl, in which the hydrogen atom replacement is limited to halo (*i.e.* –F, –Cl, –Br, or –I) such that no other atoms aside from carbon, hydrogen and halogen are present. The group, –CH₂Cl is a non-limiting example of a haloalkyl. The term “fluoroalkyl” is a subset of substituted alkyl, in which the hydrogen atom

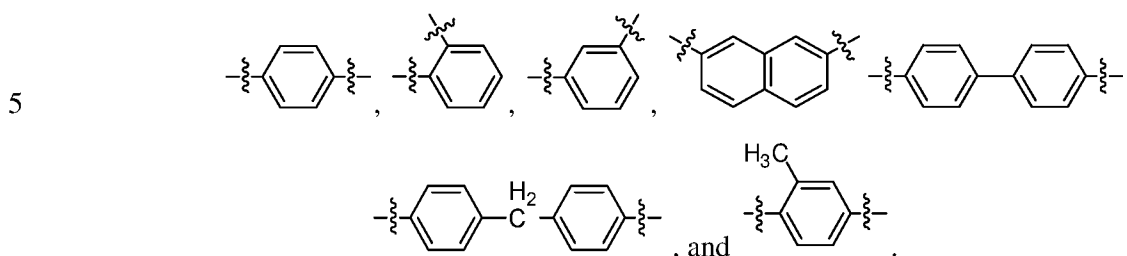
replacement is limited to fluoro such that no other atoms aside from carbon, hydrogen and fluorine are present. The groups $-\text{CH}_2\text{F}$, $-\text{CF}_3$, and $-\text{CH}_2\text{CF}_3$ are non-limiting examples of fluoroalkyl groups.

[0081] The term “cycloalkyl” when used without the “substituted” modifier refers to a monovalent saturated aliphatic group with a carbon atom as the point of attachment, said carbon atom forming part of one or more non-aromatic ring structures, no carbon-carbon double or triple bonds, and no atoms other than carbon and hydrogen. Non-limiting examples include: $-\text{CH}(\text{CH}_2)_2$ (cyclopropyl), cyclobutyl, cyclopentyl, or cyclohexyl (Cy). The term “cycloalkanediyl” when used without the “substituted” modifier refers to a divalent saturated aliphatic group with two carbon atoms as points of attachment, no carbon-carbon double or

triple bonds, and no atoms other than carbon and hydrogen. The group  is a non-limiting example of cycloalkanediyl group. A “cycloalkane” refers to the class of compounds having the formula $\text{H}-\text{R}$, wherein R is cycloalkyl as this term is defined above. When any of these terms is used with the “substituted” modifier one or more hydrogen atom has been independently replaced by $-\text{OH}$, $-\text{F}$, $-\text{Cl}$, $-\text{Br}$, $-\text{I}$, $-\text{NH}_2$, $-\text{NO}_2$, $-\text{CO}_2\text{H}$, $-\text{CO}_2\text{CH}_3$, $-\text{CN}$, $-\text{SH}$, $-\text{OCH}_3$, $-\text{OCH}_2\text{CH}_3$, $-\text{C}(\text{O})\text{CH}_3$, $-\text{NHCH}_3$, $-\text{NHCH}_2\text{CH}_3$, $-\text{N}(\text{CH}_3)_2$, $-\text{C}(\text{O})\text{NH}_2$, $-\text{C}(\text{O})\text{NHCH}_3$, $-\text{C}(\text{O})\text{N}(\text{CH}_3)_2$, $-\text{OC}(\text{O})\text{CH}_3$, $-\text{NHC}(\text{O})\text{CH}_3$, $-\text{S}(\text{O})_2\text{OH}$, or $-\text{S}(\text{O})_2\text{NH}_2$.

[0082] The term “aryl” when used without the “substituted” modifier refers to a monovalent unsaturated aromatic group with an aromatic carbon atom as the point of attachment, said carbon atom forming part of a one or more six-membered aromatic ring structure, wherein the ring atoms are all carbon, and wherein the group consists of no atoms other than carbon and hydrogen. If more than one ring is present, the rings may be fused or unfused. As used herein, the term does not preclude the presence of one or more alkyl or aralkyl groups (carbon number limitation permitting) attached to the first aromatic ring or any additional aromatic ring present. Non-limiting examples of aryl groups include phenyl (Ph), methylphenyl, (dimethyl)phenyl, $-\text{C}_6\text{H}_4\text{CH}_2\text{CH}_3$ (ethylphenyl), naphthyl, and a monovalent group derived from biphenyl. The term “arenediyl” when used without the “substituted” modifier refers to a divalent aromatic group with two aromatic carbon atoms as points of attachment, said carbon atoms forming part of one or more six-membered aromatic ring structure(s) wherein the ring atoms are all carbon, and wherein the monovalent group consists of no atoms other than carbon and hydrogen. As used herein, the term does not preclude the presence of one or more alkyl, aryl or aralkyl groups (carbon number limitation permitting)

attached to the first aromatic ring or any additional aromatic ring present. If more than one ring is present, the rings may be fused or unfused. Unfused rings may be connected via one or more of the following: a covalent bond, alkanediyl, or alkenediyl groups (carbon number limitation permitting). Non-limiting examples of arenediyl groups include:



An “arene” refers to the class of compounds having the formula H–R, wherein R is aryl as that term is defined above. Benzene and toluene are non-limiting examples of arenes. When any of these terms are used with the “substituted” modifier one or more hydrogen atom has been
 10 independently replaced by –OH, –F, –Cl, –Br, –I, –NH₂, –NO₂, –CO₂H, –CO₂CH₃, –CN, –SH, –OCH₃, –OCH₂CH₃, –C(O)CH₃, –NHCH₃, –NHCH₂CH₃, –N(CH₃)₂, –C(O)NH₂, –C(O)NHCH₃, –C(O)N(CH₃)₂, –OC(O)CH₃, –NHC(O)CH₃, –S(O)₂OH, or –S(O)₂NH₂.

[0083] An “amine protecting group” is well understood in the art. An amine protecting group is a group which prevents the reactivity of the amine group during a reaction which
 15 modifies some other portion of the molecule and can be easily removed to generate the desired amine. Amine protecting groups can be found at least in Greene and Wuts, 1999, which is incorporated herein by reference. Some non-limiting examples of amino protecting groups include formyl, acetyl, propionyl, pivaloyl, t-butylacetyl, 2-chloroacetyl, 2-bromoacetyl, trifluoroacetyl, trichloroacetyl, o-nitrophenoxyacetyl, α-chlorobutyryl, benzoyl, 4-
 20 chlorobenzoyl, 4-bromobenzoyl, 4-nitrobenzoyl, and the like; sulfonyl groups such as benzenesulfonyl, p-toluenesulfonyl and the like; alkoxy- or aryloxycarbonyl groups (which form urethanes with the protected amine) such as benzyloxycarbonyl (Cbz), p-chlorobenzyloxycarbonyl, p-methoxybenzyloxycarbonyl, p-nitrobenzyloxycarbonyl, 2-nitrobenzyloxycarbonyl, p-bromobenzyloxycarbonyl, 3,4-dimethoxybenzyloxycarbonyl, 3,5-
 25 dimethoxybenzyloxycarbonyl, 2,4-dimethoxybenzyloxycarbonyl, 4-methoxybenzyloxycarbonyl, 2-nitro-4,5-dimethoxybenzyloxycarbonyl, 3,4,5-trimethoxybenzyloxycarbonyl, 1-(p-biphenyl)-1-methylethoxycarbonyl, α,α--dimethyl-3,5-dimethoxybenzyloxycarbonyl, benzhydryloxycarbonyl, t-butyloxycarbonyl (Boc), diisopropylmethoxycarbonyl, isopropylloxycarbonyl, ethoxycarbonyl, methoxycarbonyl,

allyloxycarbonyl (Alloc), 2,2,2-trichloroethoxycarbonyl, 2-trimethylsilylethoxycarbonyl (Teoc), phenoxycarbonyl, 4-nitrophenoxycarbonyl, fluorenyl-9-methoxycarbonyl (Fmoc), cyclopentyloxycarbonyl, adamantyloxycarbonyl, cyclohexyloxycarbonyl, phenylthiocarbonyl and the like; aralkyl groups such as benzyl, triphenylmethyl, benzyloxymethyl and the like; and silyl groups such as trimethylsilyl and the like. Additionally, the “amine protecting group” can be a divalent protecting group such that both hydrogen atoms on a primary amine are replaced with a single protecting group. In such a situation the amine protecting group can be phthalimide (phth) or a substituted derivative thereof wherein the term “substituted” is as defined above. In some embodiments, the halogenated phthalimide derivative may be tetrachlorophthalimide (TCphth). The term “protected amine group” references to an amine radical which is substituted on another groups comprising one or more monovalent amine protecting groups or a divalent amine protected group.

VII. Examples

[0084] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1 – Materials and Methods

[0085] **Materials.** Common chemical reagents were purchased from Sigma-Aldrich (St. Louis, MO) or VWR (West Chester, PA) and used as received. CPA-BODIPY was purchased from Toronto Research Chemicals Inc. (Toronto, Canada). ¹H and ¹³C NMR spectra were recorded on a Bruker Avance 600 spectrometer (Billerica, MA) with tetramethyl silane as internal standard.

[0086] **Preparation of micelles loaded with CPA and/or PTX.** The anionic and cationic polymers used to formulate the micelles (FIG. 1) were prepared using atom transfer radical polymerization and ring-opening polymerization. The detailed procedures for polymerization and polymer characterization are described in Example 2. For preparation of

CPA-loaded micelles (M-CPA), 5 mg of CPA was added to 200 μ L of ethanol, and the solution was warmed to 50 $^{\circ}$ C to completely dissolve the CPA. Anionic polymer (100 mg) was dissolved in 0.8 mL of tetrahydrofuran, this solution was added to the CPA-ethanol solution, and the resulting solution was incubated at room temperature for 10 min with agitation.

5 Cationic polymer (20 mg) was dissolved in 2 mL of deionized water, and this solution was pipetted into the CPA-anionic polymer solution under violent vortexing at room temperature. The mixture was vortexed for another 1 min after addition of cationic polymer was complete. Tetrahydrofuran and ethanol were removed on a rotary evaporator under 130 mBar at 45 $^{\circ}$ C for 10 min, after which the polymer was purified by dialysis against deionized water at 4 $^{\circ}$ C.

10 Un-encapsulated drug was removed by centrifugation at 5000 RPM for 5 min. The supernatant was collected and filtered through a sterile 0.22- μ m nylon filter before use. For long-term storage, the micelles were mixed with sucrose solution (50% by weight in water at a volume ratio of 9:1) and then frozen in a -80 $^{\circ}$ C freezer. Micelles loaded with PTX or both CPA and PTX were prepared similarly.

15 **[0087] Characterization of drug-loaded micelles.** Drug-loaded micelles were characterized with respect to size, surface charge, drug loading, drug release, and morphology. Particle size and surface charge were measured using dynamic light scattering at a 90 $^{\circ}$ scatter angle on a ZetaPlus particle electrophoresis system (Brookhaven Instruments Corp., Holtsville, NY). To determine drug loading, micelle suspensions were dissociated in ethanol and sonicated

20 for 5 min to release the encapsulated drugs. After centrifugation at 13,000 RPM for 15 min, the supernatant was analyzed using an Agilent 1100 series high-performance liquid chromatography (HPLC) (Santa Clara, CA). The mobile phase was a mixture of 0.1% trifluoroacetic acid (solvent A) and acetonitrile (solvent B), using a gradient (solvent B from 5% to 95% v/v in 30 min). The column was an Agilent C18 column (4.6 $\times$ 250 mm) with 5-

25 μ m particles. The flow rate was 1.0 mL/min, and the detection wavelength was 210 nm for CPA and 227 nm for PTX. To determine drug release, micelles were added to a microdialyzer (molecular weight cut-off $\sim$ 3000) and incubated in PBS (pH 7.4) or sodium acetate buffer (pH 6.0 and pH 5.2) at 37 $^{\circ}$ C with agitation. At predetermined time points, 20 μ L of the solution inside the dialyzer was retrieved and centrifuged at 5000 RPM for 5 min. Aliquots of the

30 supernatant (10 μ L) were analyzed using HPLC to determine the concentrations of CPA and PTX. The retention times of CPA and PTX were 14.7 min and 19.6 min, respectively.

[0088] The morphology of drug-loaded micelles was examined by transmission electron microscopy (TEM). Samples were placed on 100 mesh formvar-coated copper grids treated with poly-L-lysine and left for 1 h. The grids were blotted with filter paper to remove excess samples and then stained with filtered 1% uranyl acetate for 1 min. Stain was blotted from the grids with filter paper, and the samples were allowed to dry. TEM was performed on a JEM 1010 transmission electron microscope (JEOL USA, Inc., Peabody, MA) at an accelerating voltage of 80 kV. Digital images were collected using the AMT Imaging System (Advanced Microscopy Techniques Corp., Danvers, MA).

[0089] **Cell lines.** Three pancreatic cancer cell lines were obtained from American Type Culture Collection: Miapaca-2 (high CPA sensitive), L3.6pl (moderately CPA sensitive) (Feldmann *et al.*, 2007), and Panc-1 (CPA resistant) (Feldmann *et al.*, 2007).

[0090] **Assays of cell proliferation and clonogenicity.** Cells were seeded at 2000 cells/well in 96-well plates and treated at 37 °C for 72 h before MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] assay. The proportion of viable cells was normalized to that of untreated cells and expressed as mean $\pm$ standard error of the mean (n = 6). Clonogenicity assay was performed as previously reported (Shoemaker *et al.*, 1985). Cells were seeded at 1000 cells per 10-cm plate in growth medium overnight and treated at 37 °C for 10 days. At the end of this 10-day period, colonies were fixed with formalin and stained with crystal violet. Colonies with more than 20 cells were counted.

[0091] **SMO competition assay.** SMO competition assay was performed using a commercial fluorescent SMO ligand, CPA-BODIPY (Bee *et al.*, 2012). Cells in 96-well plates were first treated with M-CPA for 24 h at 37 °C. CPA-BODIPY (10 nM) was then added to the cells, and cells were incubated for another 4 h. Then the treatment solutions were removed, and cells were washed with PBS and lysed with DMSO. The residual fluorescence of each well was recorded (λ_{ex} = 480 nm, λ_{em} = 525 nm). For fluorescence images, cells were fixed with methanol, counterstained with Hoechst, and observed using a Zeiss Axio Observer.Z1 fluorescence microscope (Carl Zeiss MicroImaging GmbH, Thornwood, NY).

[0092] **Western blot analysis.** Cell lysate was fractioned and transferred to a polyvinylidene fluoride membrane (Millipore, Billerica, MA). Membranes were blotted with rabbit anti-human monoclonal Gli-1 antibody (Abcam Inc., Cambridge, MA, 1:1000 dilution) and visualized using fluorescent IRDye 680RD goat anti-rabbit IgG (H+L) (LI-COR, Lincoln,

NE, 1:5000 dilution). Beta-actin (Sigma-Aldrich) was used as an internal control to ensure equal protein loading.

[0093] Flow cytometry analysis of CD133 stem cell marker and tumorsphere formation. Miapaca-2 cells were treated with M-CPA and/or M-PTX at concentrations close to their respective IC₅₀ values: 10 μ M for M-CPA and 10 nM for M-PTX. Treated cells were detached, and stained with mouse anti-human CD133 antibody–phycoerythrin conjugate (Miltenyi Biotec Inc., San Diego, CA). Mouse IgG-phycoerythrin was used as a negative control. Cell suspensions were analyzed using flow cytometry (BD FACSCalibur, San Jose, CA). Tumorspheres were cultured in 24-well ultralow attachment plates as previously reported (Hermann *et al.*, 2007). Miapaca-2 cells in monolayer were detached and re-suspended at 1000 cells/well in sphere-forming medium consisting of DMEM/F-12 with B-27, N-2, 20 ng/mL epidermal growth factor, 20 ng/mL basic fibroblast growth factor, and 4 μ g/mL heparin. M-CPA and/or M-PTX was added at the beginning of incubation. After 10 days of incubation, tumorspheres larger than 50 μ m in each well were counted under a microscope.

[0094] Assays of antitumor efficacy in mice with orthotopic xenografts. All animal studies were approved by the Institutional Animal Care and Use Committee of The University of Texas MD Anderson Cancer Center and were carried out in accordance with institutional guidelines. Orthotopic Miapaca-2-luc pancreatic tumor xenografts were established in female NCR nude mice (Taconic, Hudson, NY). A small incision was made in the left abdomen of each mouse. Directly into the pancreas was injected 50 μ L of cell suspension (1×10^6 Miapaca-2-luc cells and 3×10^6 HPSCs) in a 1:1 volume mixture of Hank's balanced salt solution and Matrigel. The incision was closed with absorbable sutures, and the tumors were allowed to grow for 4 weeks before treatment. Mice were randomly assigned to four different groups consisting of 9 mice per group and treated with (1) saline (control); (2) M-CPA, 10 mg/kg/injection, 3 injections in 1 week; (3) M-PTX, 10 mg/kg/injection, 3 injections in 1 week; or (4) M-CPA/PTX, 10 mg/kg/drug/injection, 3 injections in 1 week. Luciferase activity was monitored weekly by intraperitoneal injection of D-luciferin (150 mg/kg) according to the manufacturer's instructions. The luminescence signals were recorded up to 20 min after D-luciferase injection and the peak value was recorded using a Xenogen IVIS-200 optical system (Perkin Elmer, Waltham, MA).

[0095] Immunohistochemical staining. Formalin-fixed, paraffin-embedded tumor sections were deparaffinized and rehydrated. After antigen retrieval, slides were blocked in

PBS with 10% goat serum and incubated with primary antibodies overnight at 4 °C. The antibody dilutions were as follows: α -smooth muscle actin, 1:100; CD31, 1:50; Ki67, 1:400; and Gli-1, 1:100 (Abcam Inc.). Slides were washed and incubated with biotinylated goat-anti-rabbit IgG (1:200) (Vector Labs, Burlingame, CA) and streptavidin-conjugated horseradish peroxidase (DAKO, Carpinteria, CA) for 30 min each. A positive reaction was detected by exposure to 3,3'-diaminobenzidine. Slides were counterstained with hematoxylin and visualized under a microscope. Staining was quantified using at least 10 randomly selected 20 $\times$ fields of view. For Ki67 staining, the positively stained nuclei were counted and recorded in each field of view. For CD31 and α -SMA staining, the percentage of positively stained pixels in the total pixels within each field of view was recorded using Image J software.

[0096] Statistics. Values are expressed as mean $\pm$ standard error of the mean. Data were evaluated using Student's *t* test or one-way analysis of variance. A *p* value of less than 0.05 was considered statistically significant.

Example 2 – Preparation of Polymers

[0097] 2-(benzyloxycarbonyl amino) ethyl 2-bromo-isobutyrate (initiator 1).

Cbz-N-ethanolamine 2.0 g (10.2 mmol) and triethylamine (1.6 mL, 11.3 mmol) were dissolved in 25 mL of ethyl acetate and cooled with an ice-water bath. 2-Bromoisobutyryl bromide (1.3 mL, 10.7 mmol) in 5 mL of ethyl acetate was slowly dropped in under vigorous stirring. The mixture was stirred at room temperature for another 4 hr and then filtered to remove the precipitate. The ethyl acetate solution was subsequently washed with saturated NaHCO₃, 5% HCl, and double-distilled water; dried over anhydrous MgSO₄; and condensed *in vacuo* at 35 °C. The crude product was purified via flash silica column using ethyl acetate/hexane as eluent to give colorless viscous oil 3.1 g (88%). ¹H-NMR (ppm): 7.3 ~ 7.5 (5H), 5.05 (2H), 4.15 (2H), 3.30 (2H), 1.87 (6H) (FIG. 9).

[0098] Poly[oligo(ethylene glycol) monomethyl ether methacrylate]₁₉-b-poly(2-hydroxyethyl methacrylate)₂₀ (polymer 2). Into a round-bottom flask was added the initiator **1** (24.2 mg, 70.2 μ mol), oligo ethylene glycol methyl methacrylate (PEGMA, MW ~ 50, 1.0 g, 2.1 mmol), 2,2'-bipyridyl (21.9 mg, 140.4 μ mol), and 0.5 mL of anhydrous methanol. The flask was flushed with anhydrous argon for 20 min and immersed in a 50 °C oil bath. CuBr (10.0 mg, 70.2 μ mol) was quickly added under argon protection, and the reaction was allowed to proceed for 30 min. A separate mixture of 2-hydroxyethyl methacrylate (0.25 mL, 2.1 mmol)

in 0.5 mL of methanol was de-oxygenized and added into the reaction mixture. The polymerization was allowed to continue for 18 hr at 50 °C and then stopped by exposure to open air. The reaction mixture was diluted with tetrahydrofuran, passed through a basic aluminum column to remove the catalyst, and condensed *in vacuo* at 50 °C to give block copolymer **2** as viscous oil 1.2 g (94%). ¹H-NMR showed that polymer **2** contained 19 units of PEGMA and 20 units of 2-hydroxyethyl methacrylate (HEMA) (FIG. 10). Polymer **2** was abbreviated as P(PEGMA)₁₉-*b*-P(HEMA)₂₀.

[0099] Poly(PEGMA)₁₉-*b*-poly[HEMA-*g*-(ϵ -caprolactone)₇]₂₀ (polymer 3). Polymer **2** (2.0 g, 2.8 mmol -OH) was dried by azeotropic distillation with toluene at 110 °C. Excess toluene was removed under vacuo. Compound **2** was then dissolved in 4 mL of anhydrous toluene and transferred into a flame-dried flask. Anhydrous ϵ -caprolactone (CL, 1.6 mL, 14.1 mmol) and tin(II) 2-ethylhexanoate (Sn(Oct)₂, 9 μ L, 28.1 μ mol) were added. The flask was flushed with anhydrous argon and heated at 110 °C overnight. The polymerization mixture was precipitated in hexane twice to give 3.1 g (yield = 85%) of the block copolymer **3** as white wax. ¹H-NMR showed that each oligo(ϵ -caprolactone) side chain polymer **2** contained 7 units of ϵ -caprolactone (FIG. 11). Polymer **3** was abbreviated as P(PEGMA)₁₉-*b*-P(HEMA-CL₇)₂₀.

[00100] Poly(PEGMA)₁₉-poly[HEMA-*g*-(CL₇)-mono-succinate ester]₂₀ (polymer 4). Polymer **3** (1.0 g, 0.78 mmol -OH) was dissolved in 5 mL of anhydrous toluene. Then 3-isocyanatopropyl triethoxysilane (0.2 mL, 0.78 mmol) and dibutyltin dilaurate (, 5 μ L, 7.8 μ mol) were added. The flask was heated at 40 °C overnight under argon protection. The reaction mixture was precipitated in diethyl ether/hexane and dried in vacuo at room temperature to give the anionic polymer **4**, abbreviated as P(PEGMA)₁₉-*b*-P(HEMA-CL₇-SAn)₂₀. ¹H-NMR showed that each polymer contained about 20 units of succinates (FIG. 12).

[00101] Preparation of Cationic Polymer Poly(PEGMA)₁₉-poly(MeDMA)₂₀. The initiator, trifluoroacetic acid salt of 2-aminoethyl 2-bromoisobutyrate, was synthesized via published protocol (Tempero *et al.*, 2011). Briefly, to a 50-mL flask were added the initiator (22.7 mg, 70.2 μ mol), 2,2'-Bipyridyl (21.9 mg, 140.4 μ mol), methoxy-PEG-methacrylate (PEGMA, MW 500) (1.00 g, 2.10 mmol) and 0.5 mL of anhydrous isopropanol. The flask was deoxygenized via three cycles of freeze-pump-thaw and refilled with N₂, after which CuBr (10.0 mg, 70.2 μ mol) was quickly added. Polymerization was started by heating the flask in an oil bath at 50 °C. Thirty minutes later, a pre-deoxygenized solution of 2-(methacryloyloxy) ethyl trimethyl ammonium chloride (MeDMA) (1.77 g, 4.21 mmol) at 40% by weight in water

was quickly added into the reaction mixture. The polymerization was allowed to continue for 2 hr at 50 °C. ¹H-NMR showed conversion > 98% (FIG. 14). The reaction mixture was dialyzed against deionized water for 3 days to remove un-reacted monomers and catalysts. A solid powder was obtained after lyophilization.

5 **Example 3 - Synthesis and characterization of polyion polymers and drug-loaded micelles**

[00102] The chemical structures of the anionic and cationic polymers are shown in FIG. 1. The anionic polymer (FIG. 1A) consisted of a hydrophilic brush-like polyethylene glycol (PEG) block and a hydrophobic block with biodegradable oligo(ϵ -caprolactone) side chains. Succinates were conjugated to the termini of the caprolactone side chains. The ¹H-NMR spectrum of the anionic polymer was as follows (FIG. 1B): 1.6 ppm (β and δ -CH₂- of the caprolactone units), 2.6 ppm (-CH₂- of succinates), and 3.4 ppm (terminal -OCH₃ of PEG brushes). Integration of ¹H-NMR spectra (FIGs. 9-12) indicated that each anionic macromolecule had 19 PEG brushes and 20 caprolactone side chains; each side chain in the hydrophobic block contained seven caprolactone monomers and one succinate terminal group. The cationic polymer (FIG. 1C) consisted of a hydrophilic brush-like PEG block and a cationic block of poly(2-[(methacryloyloxy) ethyl] trimethylammonium chloride).

[00103] The ¹H-NMR spectrum of the cationic polymer was as follows (FIG. 1D): 3.3 ppm (terminal -OCH₃ of PEG brushes) and 3.5 ppm (N⁺-CH₃ of ammonium). Each polymer had approximately 19 PEG brushes and 20 units of 2-[(methacryloyloxy) ethyl] trimethylammonium chloride. The complete polymerization schemes and ¹H-NMR spectra are presented in FIGs. 8-14. As shown in Table 1, all of the drug-loaded micelles had size in the range of 60-125 nm, had slightly negative surface charges, and had drug-loading efficiency above 95%. Representative TEM micrographs of each type of micelle are shown in FIGs. 2A-C. The dark color at the micellar core in M-PTX and M-CPA/PTX (FIG. 2C) may arise from the uranyl ions chelated by the carboxylates of anionic polymers. The drug release (FIGs. 2D-G) profiles showed that at pH 7.4, micelles retained more than 80% of the encapsulated CPA or PTX for up to 3 days. As the pH value of incubating buffer decreased, the release of both CPA and PTX increased in all three formulations. When drug-loaded micelles were stored for 90 days at -80 °C, no changes were noted between day 0 and day 90 in micelle size or drug loading (Table 2).

Table 1. Physicochemical characterization of drug-loaded micelles.

Characteristic	M-CPA ¹	M-PTX ²	M-CPA/PTX ³
Size, nm ⁴	74.7 ± 1.5	121.6 ± 2.1	61.5 ± 1.9
PDI	0.148	0.124	0.131
Zeta-potential, mV	-13.5 ± 10.1	-6.9 ± 3.5	-9.7 ± 4.1
Drug loading efficiency	> 95%	> 95%	> 95%

PDI, polydispersity index.

¹CPA/anionic polymer = 5% by weight.

²PTX/anionic polymer = 5% by weight.

5 ³CPA/anionic polymer = 2.5% by weight; PTX/anionic polymer = 2.5% by weight.

⁴Number-average size. Anionic polymer/cationic polymer = 5/1 by weight in all specimens.

Table 2. Long-term Stability of Drug-loaded Polymeric Micelles¹ at -80 °C.

Micelle Type and Characteristic	Day 0	Day 90
M-CPA²		
Size, nm ⁵	74.7 ± 1.5	70.6 ± 2.7
PDI	0.148	0.184
Relative CPA loading, %	100 ± 4.3	98.5 ± 1.4
M-PTX³		
Size, nm ⁵	121.6 ± 2.1	94.9 ± 1.3
PDI	0.111	0.173
Relative PTX loading, %	100 ± 1.8	101.8 ± 3.5
M-CPA/PTX⁴		
Size, nm ⁵	61.5 ± 1.9	53.2 ± 3.2
PDI	0.131	0.181
Relative CPA loading, %	100 ± 3.1	99.4 ± 1.7
Relative PTX loading, %	100 ± 2.6	100.5 ± 3.5

10 PDI, polydispersity index.

¹Micelles were snap-frozen in the presence of 5% sucrose.

²CPA/anionic polymer = 5% by weight.

³PTX/anionic polymer = 5% by weight.

⁴CPA/anionic polymer = 2.5% by weight; PTX/anionic polymer = 2.5% by weight.

⁵Number-average size. Anionic polymer/cationic polymer = 5/1 by weight in all specimens.

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Example 4 - Effects of drug-loaded micelles on SHH pathway signaling molecules, proliferation, and stem cell population in pancreatic cancer cell lines

[00104] CPA inhibits SHH activity by binding to SMO. FIG. 3A shows that M-CPA could compete with CPA-BODIPY, a fluorescent ligand for SMO. Pretreatment of Miapaca-2 cells with $1.3 \pm 0.3 \mu\text{M}$ M-CPA resulted in 50% loss of CPA-BODIPY binding ($\text{EC}_{50} = 1.3 \pm 0.3 \mu\text{M}$). The EC_{50} values of M-CPA in L3.6pl and Panc-1 cells were 0.9 ± 0.2 and $0.35 \pm 0.05 \mu\text{M}$, respectively (Table 3). Fluorescence micrographs confirmed that the fluorescence of CPA-BODIPY-treated cells was strong in the absence of M-CPA (green, FIG. 3B) but diminished in the presence of $20 \mu\text{M}$ M-CPA (FIG. 3C).

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Table 3. Effects of M-CPA on SMO Receptor Binding and Cell Proliferation.

	Miapaca-2	L3.6pl	Panc-1
EC₅₀ for inhibition of SMO binding			
M-CPA, μM	1.3 ± 0.3	0.9 ± 0.2	0.35 ± 0.05
IC₅₀ for cell proliferation			
CPA, μM	10.2 ± 0.1	16.0 ± 1.5	~ 163
M-CPA, μM	7.3 ± 1.4	6.2 ± 0.6	~ 249
PTX, nM	1.2 ± 0.1	1.4 ± 0.1	2.4 ± 0.4
M-PTX, nM	1.3 ± 0.1	1.3 ± 0.1	1.6 ± 0.2
M-CPA/PTX, ¹ nM in PTX equivalent	0.9 ± 0.1	1.1 ± 0.2	1.2 ± 0.5

¹CPA and PTX were equally loaded in micelles.

[00105] Gli-1 is a downstream target of SMO in the SHH pathway. M-CPA treatment reduced Gli-1 expression in Miapaca-2 cells in a dose-dependent manner (FIG. 3D). Compared to Gli-1 expression with no treatment (control), the relative Gli-1 expression (%) was 75.5 ± 2.5 , 52.6 ± 2.2 , 33.4 ± 7.0 , and 7.3 ± 0.7 after treatment with 5, 10, 20, and $50 \mu\text{M}$

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M-CPA, respectively. Similar M-CPA-induced downregulation of Gli-1 was observed in L3.6pl cells; M-CPA slightly downregulated Gli-1 in Panc-1 cells (FIG. 15).

[00106] Next, the cell proliferation during drug treatments was assessed. M-CPA was more cytotoxic than CPA in Miapaca-2 (FIG. 3E) and L3.6pl cells (Table 3), but neither treatment was effective against Panc-1 cells (Table 3). Both M-PTX and PTX showed excellent cytotoxicity against all three cell lines (Table 3). When CPA and PTX were equally loaded in micelles, the resultant M-CPA/PTX had slightly higher cytotoxicity against all three cell lines than M-PTX did (FIG. 3F, Table 3). Colony formation assay in Miapaca-2 cells showed that M-CPA inhibited colony formation more effectively than CPA did at higher concentrations (FIGs. 3G-I). Compared to no treatment (control), 3 μ M CPA reduced the number of colonies by $18.2 \pm 1.3\%$, and 3 μ M M-CPA reduced the number of colonies by $57.7 \pm 2.3\%$. M-CPA also inhibited colony formation against L3.6pl cells and Panc-1 cells at high equivalent drug concentration (30 μ M), although only L3.6pl showed dose-dependent response at lower drug concentrations (0- 3 μ M) (FIG. 16).

[00107] CD133 is a surface marker for the CSCs in pancreatic cancer (Simeone, 2008). The impact of treatments on the CD133-positive (CD133+) population of Miapaca-2 cells is summarized in FIGs. 4A-E. The percentage of CD133+ cells was 12.2 ± 0.2 for control, 2.1 ± 0.1 for M-CPA, 18.9 ± 0.2 for M-PTX, and 2.2 ± 0.1 for M-CPA + M-PTX. M-PTX treatment significantly enriched the CD133+ population compared to control ($p < 0.001$), whereas both M-CPA and M-CPA/PTX significantly depleted the CD133+ population compared to control ($p < 0.001$). There was no significant difference in CD133+ depletion between M-CPA and M-CPA/PTX treatment. M-CPA/PTX also reduced the formation of tumorspheres (FIG. 4F-J). The number of tumorspheres (size $> 50 \mu\text{m}$) per 1000 cells was 28 ± 5 for control, 12 ± 2 for M-CPA, 25 ± 5 for M-PTX, and 6 ± 2 for M-CPA/PTX. The control and M-PTX groups had significantly more tumorspheres than the M-CPA group or M-CPA/PTX group ($p < 0.001$). Representative micrographs of tumorspheres are shown in FIG 4G-J.

Example 5 - Effect of M-CPA on HPSCs

[00108] Pancreatic cancer is known for its abundance of stroma tissue, which is produced mostly by activated stellate cells. The efficacy of M-CPA against stroma-producing HPSCs was examined *in vitro*. FIGs. 5A and 5B show HPSC proliferation during the various

treatments. The IC_{50} values were $13.3 \pm 1.2 \mu M$ for CPA, $12.7 \pm 1.1 \mu M$ for M-CPA, 4.4 ± 0.2 nM for M-PTX, and 3.3 ± 0.1 nM for M-CPA/PTX (in PTX equivalent). M-CPA reduced Gli-1 expression by HPSCs in a dose-dependent manner: the relative Gli-1 expression (%) was 100.2 ± 2.6 , 96.9 ± 10.9 , 48.4 ± 4.3 , and 15.2 ± 3.6 after treatment with 2, 5, 10, and 20 μM M-CPA, respectively (FIG. 5C). Proliferation of HPSCs can be stimulated by exogenous SHH ligand.

[00109] However, in cell culture using serum-free medium 15, M-CPA neutralized stimulation of HPSC proliferation by exogenous SHH ligand (FIG. 5D). In the absence of M-CPA, the relative proportions of viable cells (%) for HPSCs stimulated with 0.5, 1, and 2 $\mu g/mL$ SHH ligand were 122.2 ± 9.3 , 144.5 ± 4.1 , and 149.5 ± 4.7 , respectively, compared to no treatment (control). In the presence of 10 μM M-CPA, however, no cells were viable after 72-h incubation at any of the SHH concentrations tested. These effects of M-CPA on HPSC viability were accompanied by decreased Gli-1 expression (FIG. 5E). Compared to no treatment (control), 1.5 $\mu g/mL$ SHH ligand increased Gli-1 expression (%) to 143.7 ± 14.7 . M-CPA negated the stimulatory effect of SHH ligand: relative Gli-1 expression (%) in the presence of SHH ligand was 132.6 ± 8.5 , 116.3 ± 10.4 , and 83.3 ± 6.8 after treatment with 5, 10, and 20 μM M-CPA.

Example 6 - Effect of drug-loaded micelles on tumor growth *in vivo*

[00110] FIG. 6A shows the relative tumor growth curves in the Miapaca-2-luc xenograft model for no treatment (control), M-CPA, M-PTX, and M-CPA/PTX, where the relative tumor growth was defined as the ratio of the bioluminescence flux (photons per second) at each time point to the flux before treatment started. the antitumor efficacy was in the order M-CPA/PTX > M-PTX > M-CPA. At 5 weeks after the initial injection, the relative tumor growth for the control, M-CPA, M-PTX, and M-CPA/PTX regimens was 19.1 ± 2.2 , 5.2 ± 2.3 , 1.2 ± 0.2 , and 0.4 ± 0.1 fold, respectively. The M-CPA/PTX group had slower tumor growth than any other group ($p < 0.01$). Mice were euthanized 5 weeks after the initial treatment, and tumors were retrieved for histological analysis. Hematoxylin and eosin-stained sections revealed that tumors in the control mice were packed with viable tumor cells and had a dense stroma compartment (white arrows) (FIG. 6B), whereas tumors in the M-CPA/PTX-treated mice had loosely packed tumor cells (FIG. 6C). Notably, four of the nine mice in the M-CPA/PTX group were tumor free (FIG. 6D), whereas all mice in the other groups had residual tumors.

[00111] **Immunohistochemical analysis.** The tumor sections were stained for expression of Ki67 (proliferation), CD31 (vasculature), and α -SMA (activated cancer-associated fibroblasts). Results are summarized in FIG. 7; the micrographs are representative images from at least 15 randomly selected 20 $\times$ fields of view. The Ki67 staining results are presented in FIGs. 7A-E. The frequency of Ki67⁺ nuclei was in the order control > M-CPA > M-PTX > M-CPA/PTX. All three treated groups had fewer Ki67⁺ nuclei than the control group did ($p < 0.001$). Tumors from mice treated with M-CPA/PTX had fewer Ki67⁺ nuclei than tumors from M-CPA-treated mice did ($p < 0.05$). The CD31 staining results are presented in FIGs. 7F-J. Compared to control, treatment with M-CPA/PTX increased the tumor vasculature more than 10-fold ($p < 0.001$); treatment with M-CPA increased the tumor vasculature 3.6-fold ($p < 0.05$). M-PTX did not induce a significant change in the density of micro-blood vessels ($p > 0.05$). The α -SMA staining results are presented in FIGs. 7K-O. M-CPA and M-CPA/PTX significantly reduced the α -SMA⁺ pixels to $4.8 \pm 0.7\%$ and $3.6 \pm 0.4\%$ of total pixels, respectively, whereas α -SMA⁺ pixels were $13.0 \pm 1.0\%$ in the control group ($p < 0.001$). M-PTX (α -SMA⁺ pixels $10.0 \pm 1.0\%$) did not cause a significant change from the control ($p > 0.05$).

[00112] The above data supports the idea that M-CPA inhibited the proliferation of pancreatic cancer cells and HPSCs through the SHH signaling pathway. Moreover, the data suggests that M-CPA selectively depleted the population of pancreatic CSCs and inhibited the formation of tumorspheres. The dual-action M-CPA/PTX effectively killed tumor cells and HPSCs and depleted CSCs *in vitro*. The dual-action M-CPA/PTX increased tumor vasculature density, remodeled tumor stroma, and delayed the growth of orthotopic human pancreatic cancer Miapaca-2 xenograft tumors *in vivo*.

[00113] In the disclosure reported here, a polyion complex micelle system was prepared to encapsulate CPA. The micelles presented in this disclosure may have the following unique properties. First, the major component of the micelles (FIG. 1A) was an anionic polymer that consisted of a brush-like PEG block and a hydrophobic block with pendant oligo(ϵ -caprolactone) chains. The PEG brush is expected to provide shielding for recognition by the reticuloendothelial system and prolong the blood circulation of the micelles. Second, succinic acid was conjugated at the end of the oligo(ϵ -caprolactone) side chains, whose carboxylate could interact with CPA through its secondary amine. Third, to further increase the colloidal stability of the micelles, a cationic block polymer (FIG. 1C) was added to cross-link the

micellar core through electrostatic interaction in addition to hydrophobic interaction. Fourth, it was found that the resultant drug-loaded micelles could be frozen and stored at -80 °C for up to 3 months without significant increase in micelle size or decrease in drug loading (Table 2). The long term stability of these particles is a useful property for translating the micelle into clinical application. When drug-loaded micelles were incubated at 37 °C, the release of CPA and PTX was minimal at pH 7.4, which would prevent premature drug release during blood circulation (FIG. 2). The drug release in acidic conditions was studied in pH 5.2 and pH 6.0 buffers. The tumor microenvironment tends to be acidic (~ pH 6) (Estrella *et al.*, 2013) due to abnormal metabolism; while the pH values in lysosomes and endosomes ranges from 4.5 to 6.0 (Sorkin and von Zastrow, 2002). At acidic pH, PTX was released slowly while CPA was released much faster, probably because CPA was protonated at low pH and therefore became water soluble at pH 5.2 and pH 6.0.

[00114] In these experiments, it was first assessed whether M-CPA retained the functions of CPA against the SHH pathway. SMO expression was observed in all the three pancreatic cancer cell lines as well as HPSC (FIG. 17). It was found that M-CPA could compete with CPA-BODIPY for SMO binding in all three cell lines, indicating that the SMO affinity of encapsulated CPA was preserved. Furthermore, M-CPA treatment successfully downregulated Gli-1 expression in Miapaca-2 and L3.6pl cells, although not in Panc-1 cells, probably because Panc-1 cells have mutations downstream of SMO or another signaling pathway that can activate Gli-1 and thus are resistant to CPA treatment (Steg *et al.*, 2010). Accordingly, M-CPA inhibited the proliferation of Miapaca-2 and L3.6pl cells, but not Panc-1 cells over a 72-h incubation period (Table 3). M-CPA inhibited the formation of Miapaca-2 colonies more efficiently than free CPA did (FIGs. 3G-I). Without wishing to be bound by any theory, it is believed that this increased efficiency may be due to improved solubility of CPA and drug availability.

[00115] In contrast to other chemotherapeutics such as gemcitabine, CSCs can be depleted by CPA. The effect of M-CPA and/or M-PTX on the CSC subpopulation of Miapaca-2 cells was assessed using CD133 as a CSC marker. Although M-PTX inhibited the proliferation of Miapaca-2 with high efficiency ($IC_{50} = 1.3 \pm 0.1$ nM, FIG. 3F), it did not eliminate CD133⁺ cells; rather, M-PTX enriched the CD133⁺ population by 59% compared to no treatment (control) (FIG. 4A-E). In contrast, M-CPA depleted the CD133⁺ population.

Treatment with M-CPA alone also reduced the ability of Miapaca-2 cells to form tumorspheres, a measure of the stemness of cancer cells (FIG. 4F-J).

[00116] In this disclosure, the data suggests that M-CPA reverted these stimulatory effects of SHH ligands on HPSCs. M-CPA inhibited the proliferation of HPSCs through the SHH signaling pathway (FIGs. 5A and 5B). In addition, M-CPA abolished the stimulating effects of exogenous SHH ligand on cell proliferation and Gli-1 expression (FIGs. 5C-5D). *In vivo* studies also showed that M-CPA altered the tumor stroma by increasing the intratumoral vasculature and reducing the activated stroma (FIGs. 7F-7O). Because CPA or M-CPA as a single agent is expected to primarily impact the cancer-associated fibroblasts and stroma of pancreatic cancer, without wishing to be bound by any theory, it is believed that by co-encapsulating CPA and PTX in the same polyion micelles, both the tumor cells and stroma-producing cancer-associated fibroblasts could be attacked simultaneously, leading to increased antitumor efficacy. As expected, M-CPA/PTX was cytotoxic to 2 of the 3 pancreatic cancer cell lines and HPSCs that had showed drug-induced Gli1 down-regulation *in vitro* (FIG. 3 and Table 3). Similar to M-CPA, M-CPA + M-PTX depleted CD133⁺ Miapaca-2 cells and reduced the formation of tumorspheres (FIG. 4). These data suggest that co-encapsulation of CPA with PTX could abolish the enrichment of CD133⁺ cells caused by treatment with M-PTX alone.

[00117] In the disclosure with the orthotopic human pancreatic cancer Miapaca-2 xenograft model, M-CPA/PTX exhibited better antitumor efficacy than control, M-PTX, or M-CPA (FIG. 6A). Furthermore, four of nine mice treated with M-CPA/PTX were rendered histologically tumor free (FIG. 6D). The enhanced antitumor activity observed with M-CPA/PTX was accompanied by an increase in vasculature density, remodeling of tumor stroma, and decrease in proliferation of tumor cells (FIG. 7).

[00118] Next, drug-loaded micelles were tested on the orthotopic Kras* (with Kras mutation) syngeneic mouse model of PDAC. Survival of mice with Kras* syngeneic PDAC tumors was tested (FIG. 18). C57BL/6 mice were inoculated with murine Kras* PDAC tumors in the pancreas. The mice then received treatment once the tumor was palpable (~ 5 mm in diameter). Mice were randomly divided into the following groups: (1) CTL: no treatment. (2) Anti-PD1: intraperitoneal injection at 100 µg every 48 hours, until death. (3) M-CPA/PTX: intravenous injection per day at 5 mg/kg/drug for three consecutive days, then monitored until death. (4) M-CPA/PTX + Anti-PD1: intravenous injection per day at 5 mg/kg/drug for three consecutive days. The mice rested for 1 day, and received anti-PD1

antibody at 100 µg/mouse every 48 hours until death. (5) M-CPA/PTX + Anti-PD1 + Anti-CD8α: Anti-CD8 antibody was injected intraperitoneally at 100 µg/dose along with the anti-PD1 antibody. Other treatments were the same as group (4). Each group had 9 to 10 mice. Mice were monitored daily and sacrificed if moribund or with excessive tumor burden. All mice were sacrificed at the end of the experiments, *i.e.*, 40 days after the start of treatment. Mouse survivals of different groups were fit into Kaplan-Meier curves and compared using log-rank test. A p-value less than 0.05 indicated significant difference. As shown in FIG. 18, The medium survivals were: (1) CTL 16.5 days, (2) M-CPA/PTX 16.5 days, (3) Anti-PD1 23 days, (4) M-CPA/PTX + Anti-PD1 31 days, and (5) M-CPA/PTX + Anti-PD1 + Anti-CD8α 14.5 days. The survival of group 4 was significantly longer than group 2 ($p = 0.0013$).

[00119] The effect of drug-loaded micelles on tumor weight using the Kras* syngeneic model of PDAC was tested. Inoculated mice were randomly divided into 4 groups once the tumors were palpable (~ 5 mm) (set as day 0), as follows: (1) CTL: no treatment. (2) M-CPA/PTX: mice were intravenously injected with 5 mg/kg/drug once per day on days 0, 1, and 2. (3) Anti-PD1: mice were intraperitoneally injected with 100 µg antibody per day on days 4, 6, 8, and 10. (4) M-CPA/PTX + Anti-PD1: mice were intravenously injected with 5 mg/kg/drug once per day on days 0, 1, and 2; then intraperitoneally injected with 100 µg antibody per day on days 4, 6, 8, and 10. Mice were sacrificed on day 10, 4 hrs after the last injection of antibody. Tumors were collected and weighed immediately. Each group had 10 mice. Data was presented as mean ± standard error of mean. Tumor weights were compared using one-way ANOVA with ad-hoc Tukey multiple comparison test. Differences were noted by * ($p < 0.05$), ** ($p < 0.01$), or ns (not significant). Results are shown in FIG. 19. As shown in FIG. 19, At 10 days after the start of treatment, the tumors treated with M-CPA/PTX + Anti-PD1 were 0.39 ± 0.05 g, significantly smaller than the other three groups: CTL (0.82 ± 0.16 g), M-CPA/PTX (0.97 ± 0.12 g), and Anti-PD1 (0.87 ± 0.16 g). There was no difference among control and monotherapy groups.

[00120] The inoculated Kras* tumor-bearing mice were randomly divided into two groups once the tumors were palpable (~ 5 mm) (set as day 0), as follows: (1) CTL: no treatment. (2) M-CPA/PTX: mice were intravenously injected with 5 mg/kg/drug once per day on days 0, 1, and 2. Both groups were intravenously injected with ⁶⁴Cu-labeled anti-PD1 antibody on day 4. Mice were sacrificed, tumors and lymph nodes were collected at 24 hours after antibody injection. Radioactivity associated with tumors or lymph nodes was measured

on a γ -counter. The tumor uptake in each organ was calculated as percent of injected radioactivity per gram of tissue (%ID/g), and presented as (mean $\pm$ standard deviation). Each group had 4 mice. Frozen sections of tumors were scanned for autoradiographs to show the intratumoral distribution of ^{64}Cu -labeled anti-PD1 antibody. As shown in FIG. 20A, quantification of ^{64}Cu -anti-PD1 in tumor-draining lymph nodes and Kras* tumor showed a trend of increased distribution of the antibody, which recognizes PD1+ T cells, after M-CPA/PTX treatment. As shown in FIG. 20B, autoradiographs of Kras* tumors obtained at 24 h after intravenous injection of ^{64}Cu -anti-PD1 were observed to result in a more homogenous distribution of the antibody, which recognizes PD1+ T cells, after M-CPA/PTX treatment.

[00121] A genetically engineered mouse model of PDAC (the KPC-luc mouse model) was also used to test for the effects of drug-loaded micelles on tumors. KPC-luc mice at ages of 125 to 130 days were used for the studies. The mice were assigned into two different groups: (1) untreated control mice (CTL), or (2) mice intravenously injected with M-CPA/PTX at 5 mg/kg/dose for 6 doses over two weeks. Mice were then sacrificed at 24 hrs after the last dose. Tumors were collected, sectioned, and stained for CD3⁺ T cells. Twenty random visual fields at 200x magnification were photographed, and the number of CD3⁺ cells were counted. Results are shown in FIG. 21. As shown in FIG. 21, the treated tumors had significantly more T cells infiltration than untreated CTL tumors. The treated tumor had 55 ± 5 CD3⁺ cells per 200x visual field, as compared to the CTL tumor with 32 ± 3 CD3⁺ cells per 200x visual field ($p < 0.01$).

[00122] The genetically engineered mouse model of PDAC (the KPC-luc mouse model) was also used to test for the antitumor efficacy of drug-loaded micelles on tumors in a survival study. KPC-luc mice that showed positive luminescence ($> 1 \times 10^9$ photons/second) at ages of 95 to 100 days were used for the studies. The mice were assigned into four different groups: (1) mice with untreated cytotoxic T lymphocytes (CTL) (control group), (2) mice intravenously injected gemcitabine at 100 mg/kg every other day until death, (3) mice given Abraxane at 5 mg/kg/injection, and (4) M-CPA/PTX given at 5 mg/kg/dose per injection. The treatment schedule for Abraxane and M-CPA/PTX was: 3 intravenous injections per week for the first 2 weeks, followed by 2 intraperitoneal injections per week until death or 90 days from the initiation of treatment. As shown in FIG. 22, The median survivals of CTL, gemcitabine, and abraxane groups were 139, 167, and 155 days, respectively. The M-CPA/PTX group had

its first mortality at 167 days of age. Its median survival was 236.5 days, significantly longer than the other groups ($p < 0.0001$, log-rank test).

[00123] FIG. 23 shows histological analysis of tumors removed from untreated control mice and KPC-Luc mice treated with M-CPA/PTX. In this study, mice with palpable
5 tumors (about 125 days old) either not treated (control group, CTL) or treated with M-CPA/PTX (six intravenous injections at 5 mg/kg/drug/injection over 2 weeks). At 24 hours after the last injection, mice were sacrificed to collect tumors. Analyses of tumor histopathology features showed that, compared to CTL, M-CPA/PTX reduced the poorly differentiated PDAC by 82% ($p=0.037$) and the moderately differentiated PDAC by 92%
10 ($p<0.0001$). It increased the benign pancreas by 3.7 folds ($p<0.0001$).

* * *

[00124] All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be
15 apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar
20 substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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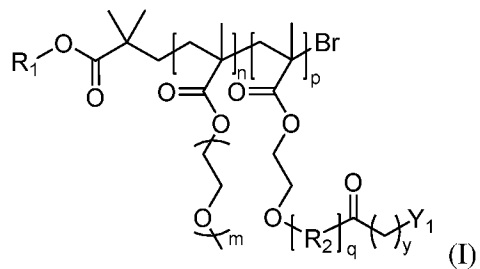
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CLAIMS

What is Claimed is:

1. A polymer, wherein the polymer is a block copolymer comprising a polyacrylic acid backbone with a plurality of PEG sidechains and a plurality of ϵ -caprolactone sidechains.
5
2. The polymer of claim 1, wherein the PEG sidechain component comprises from 5 ethylene glycol monomers to 50 ethylene glycol monomers.
3. The polymer of claim 2, wherein the polyacrylic acid backbone comprises from 3 repeating units to 40 repeating units with the PEG sidechain.
- 10 4. The polymer of claim 3, wherein the polyacrylic acid backbone comprises from 3 repeating units to 20 repeating units with the PEG sidechain.
5. The polymer of claim 4, wherein the PEG sidechain is capped with a methyl group.
6. The polymer of claim 1, wherein the ϵ -caprolactone sidechain comprises from 2 ϵ -caprolactone repeating units to 20 ϵ -caprolactone repeating units.
- 15 7. The polymer of claim 1, wherein the ϵ -caprolactone sidechain comprises from 2 ϵ -caprolactone repeating units to 15 ϵ -caprolactone repeating units.
8. The polymer of claim 7, wherein the ϵ -caprolactone sidechain is esterified with a succinate group.
9. The polymer of claim 1, wherein the ϵ -caprolactone sidechain is linked to the
20 polyacrylic acid backbone by an ethylene glycol linker.
10. The polymer of claim 1, wherein the polyacrylic acid backbone is capped with an benzyl N-(2-hydroxyethyl)carbamate or ethanolamine group.
11. The polymer of claim 1, wherein the polyacrylic acid backbone is terminated with a halogen group.

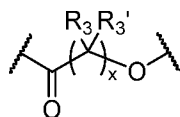
12. The polymer of claim 1, wherein the polymer is further defined by the formula:



wherein:

R₁ is alkyl_(C1-18), cycloalkyl_(C1-18), aryl_(C1-18), or a substituted version of any of these groups; or an alkyl_(C1-18) substituted with a protected amine group;

R₂ is a group of the formula:



wherein:

R₃ and R₃' are each independently hydrogen, alkyl_(C1-6), or substituted alkyl_(C1-6); and

x is 1-10;

m is 5-40;

n is 10-40;

p is 5-40;

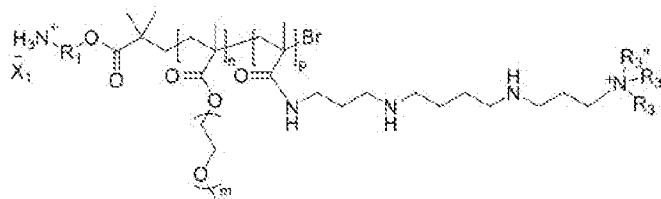
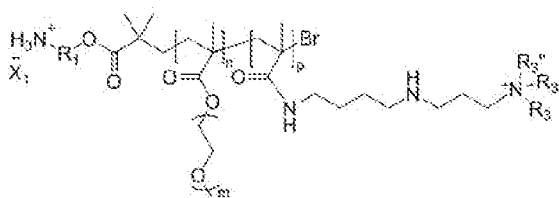
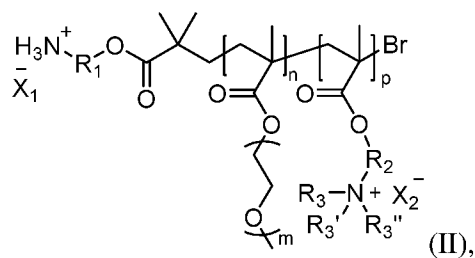
q is 1-20;

y is 1-10; and

Y₁ is carboxy, phosphonate, or hydroxysulfonyl;

or a pharmaceutically acceptable salt thereof.

13. The polymer of claim 1, wherein the polymer further comprises a second polymer of the formula:



wherein:

m is 5-40;

n is 10-40;

p is 5-30;

10 R_1 and R_2 are each independently alkanediyl_(C1-8), arenediyl_(C6-12), or a substituted version of either of these groups;

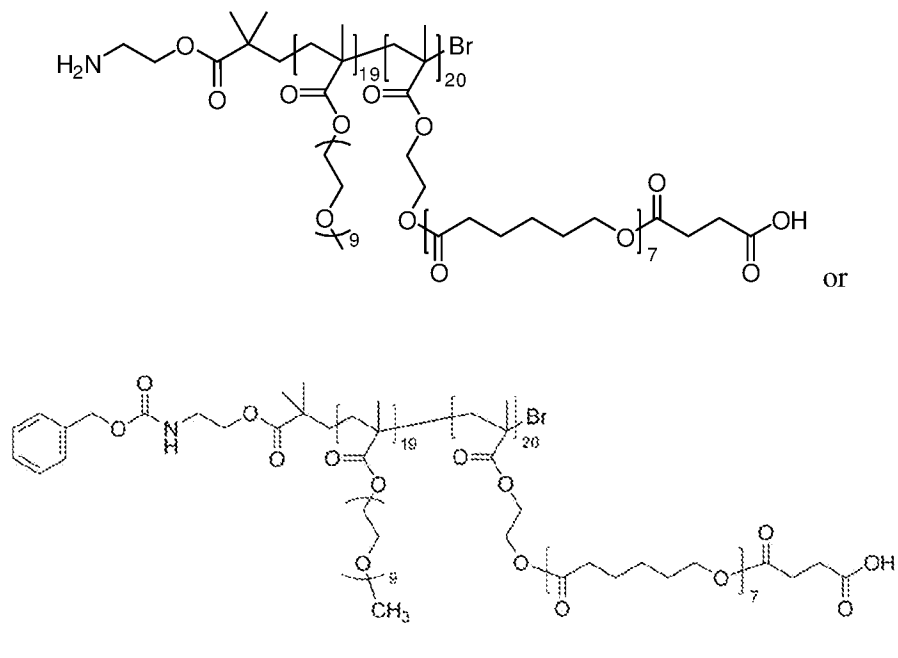
R_3 , R_3' , and, R_3'' are each independently alkyl_(C1-8), aryl_(C3-12), or a substituted version of any of these groups; and

X_1 and X_2 are each independently a monovalent anion;

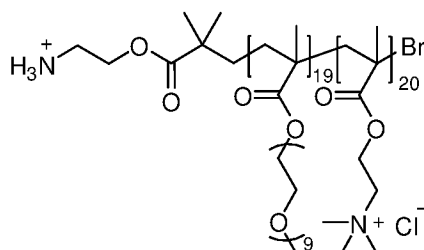
15 or a pharmaceutically salt thereof.

14. The polymer of claim 12 or claim 13, wherein m is 5-25.
15. The polymer of claim 14, wherein m is 8-10.
16. The polymer of claim 12 or claim 13, wherein n is 15-25.
17. The polymer of claim 16, wherein n is 18-20.
- 5 18. The polymer of any one of claims 12, claim 13, or claim 16, wherein p is 5-25.
19. The polymer of claim 18, wherein p is 18-22.
20. The polymer of claim 18, wherein p is 5-15.
21. The polymer of claim 12, wherein q is 5-10.
22. The polymer of claim 12, wherein x is 5-10.
- 10 23. The polymer of claim 22, wherein x is 5.
24. The polymer according to any one of claims 12, 22, and 23, wherein R_3 and R_3' are hydrogen.
25. The polymer of claim 24, wherein R_3 and R_3' are hydrogen and x is 5.
26. The polymer of claim 13, wherein R_1 is ethylene.
- 15 27. The polymer of claim 13, wherein R_2 is ethylene.
28. The polymer of claim 13, wherein R_3 , R_3' , and R_3'' are each methyl.
29. The polymer of claim 13, wherein X_1 is a halide.
30. The polymer of claim 29, wherein X_1 is chloride.
31. The polymer of claim 13, wherein X_2 is a halide.
- 20 32. The polymer of claim 31, wherein X_2 is chloride.

33. The polymer of claim 1 or claim 12, wherein the polymer has the structure:



34. The polymer of claim 13, wherein the second polymer has the structure:



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35. A pharmaceutical composition comprising:

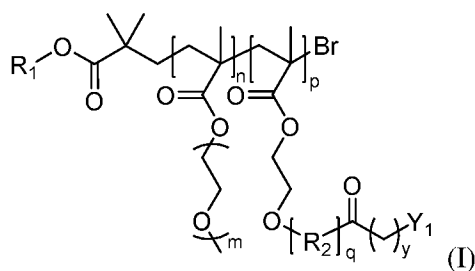
- (a) a micelle, liposome, or nanoparticle; and
- (b) a therapeutic agent;

wherein the micelle, liposome, or nanoparticle comprises a first polymer of any one of claims 1-34, and the micelle, liposome, or nanoparticle encapsulates the therapeutic agent.

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36. The pharmaceutical composition of claim 35, wherein the pharmaceutical composition further comprises an excipient.

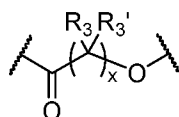
37. The pharmaceutical composition of claim 36, wherein the excipient is a monosaccharide or a disaccharide
38. The pharmaceutical composition of claim 37, wherein the excipient is sucrose.
39. The pharmaceutical composition of claim 35, wherein the micelle, liposome, or nanoparticle further comprises a second polymer.
40. The pharmaceutical composition of claim 39, wherein the first polymer comprises a net anionic charge.
41. The pharmaceutical composition of claim 40, wherein the first polymer is further defined by the formula:



wherein:

R_1 is alkyl_(C1-18), cycloalkyl_(C1-18), aryl_(C1-18), or a substituted version of any of these groups; or an alkyl_(C1-18) substituted with a protected amine group;

R_2 is a group of the formula:



wherein:

R_3 and R_3' are each independently hydrogen, alkyl_(C1-6), or substituted alkyl_(C1-6); and

x is 1-10;

m is 5-40;

n is 10-40;

p is 5-40;

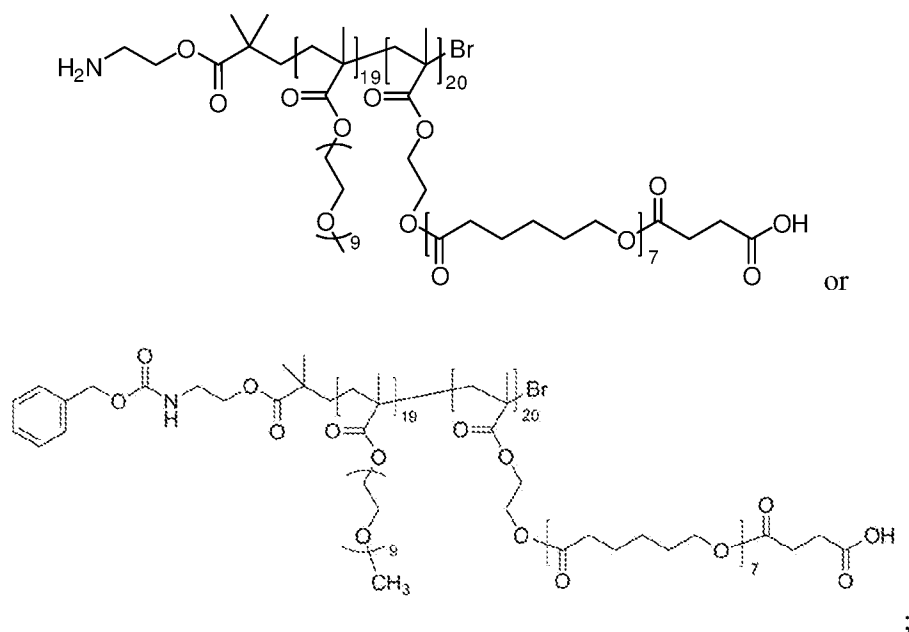
q is 1-20;

y is 1-10; and

5 Y₁ is carboxy, phosphonate, or hydroxysulfonyl;

or a pharmaceutically acceptable salt thereof.

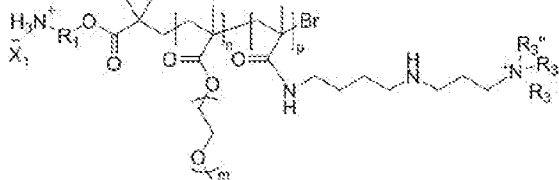
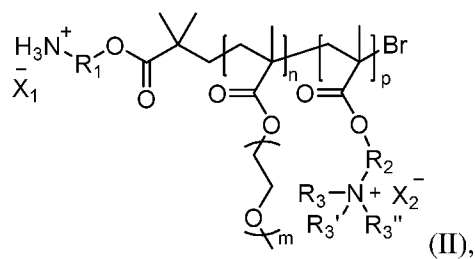
42. The pharmaceutical composition of claim 41, wherein the first polymer is:



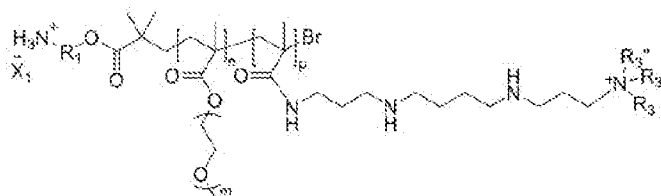
10 or a pharmaceutically acceptable salt thereof.

43. The pharmaceutical composition of any one of claims 39-41, wherein the second polymer comprises a net cationic charge.

44. The pharmaceutical composition of claim 39 or 43, wherein the second polymer is further defined by the structure:



(III), or



(IV)

wherein:

5 m is 5-40;

n is 10-40;

p is 5-30;

R_1 and R_2 are each independently alkanediyl_(C1-8), arenediyl_(C6-12), or a substituted version of either of these groups;

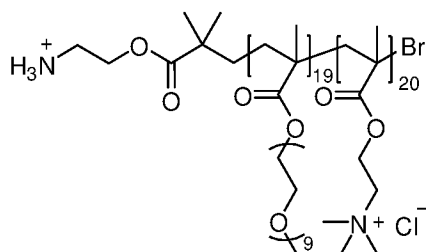
10 R_3 , R_3' , and R_3'' , are each independently alkyl_(C1-8), aryl_(C3-12), or a substituted version of any of these groups; and

X_1 and X_2 are each independently a monovalent anion;

or a pharmaceutically salt thereof.

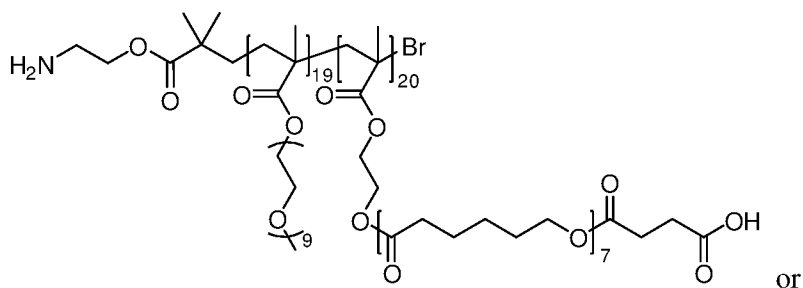
45. The pharmaceutical composition of claim 44, wherein the second polymer is further defined by the formula:

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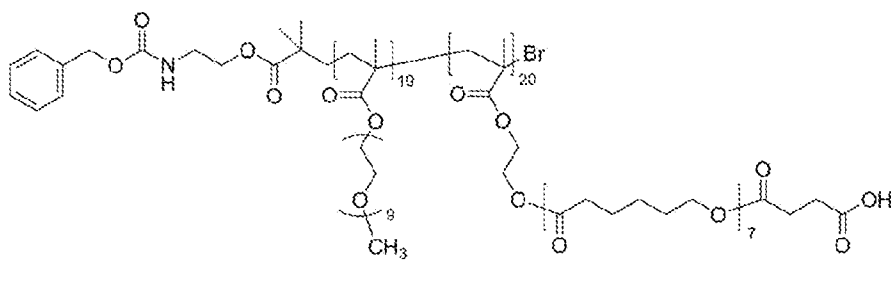


or a pharmaceutically acceptable salt thereof.

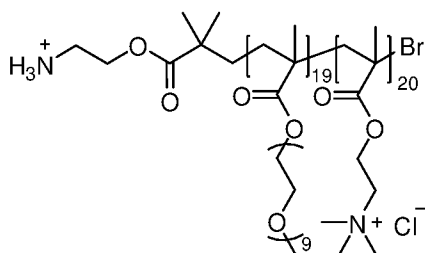
46. The pharmaceutical composition according to any one of claims 39-45, wherein the first polymer has the structure:



or



and wherein the second polymer has the structure:



or a pharmaceutically acceptable salt thereof.

- 10 47. The pharmaceutical composition of any one of claims 39-46, wherein the ratio of the first polymer to the second polymer is from about 100:1 w/w to about 1:10 w/w.

48. The pharmaceutical composition of claim 47, wherein the ratio is from about 100:1 w/w to about 1:1 w/w.
49. The pharmaceutical composition of claim 48, wherein the ratio is about 5:1 w/w.
50. The pharmaceutical composition of claim 48, wherein the ratio is about 50:1 w/w.
- 5 51. The pharmaceutical composition of any one of claims 35-50, wherein the pharmaceutical composition comprises two therapeutic agents that each independently treat or selectively target or bind tumors cells and/or tumor stroma cells.
52. The pharmaceutical composition of any one of claims 35-51, wherein the therapeutic agent is a chemotherapeutic.
- 10 53. The pharmaceutical composition of claim 52, wherein the chemotherapeutic is paclitaxel or cyclopamine.
54. The pharmaceutical composition of claim 53, wherein the pharmaceutical composition comprises paclitaxel and cyclopamine.
55. The pharmaceutical composition of claim 52, wherein the chemotherapeutic is
15 GANT58 or GANT61.
56. The pharmaceutical composition of claim 52, wherein the chemotherapeutic is vitamin D.
57. The pharmaceutical composition of any one of claims 35-56, wherein the pharmaceutical composition comprises: (i) GANT58 and paclitaxel, (ii) GANT61 and
20 paclitaxel, or (iii) vitamin D and paclitaxel.
58. The pharmaceutical composition of claim 52, wherein the chemotherapeutic is 17-N-allylamino-17-demethoxygeldanamycin (17-AAG).
59. The pharmaceutical composition of any one of claims 3-525, wherein the pharmaceutical composition comprises: (iv) GANT58 and 17-AAG, (v) GANT61 and 17-
25 AAG, or (vi) vitamin D and 17-AAG.

60. The pharmaceutical composition of any one of claims 1-55, wherein the pharmaceutical composition is formulated for intravenous, intra-arterial, intraperitoneal, parenteral, subcutaneous, or intra-tumoral administration.
- 5 61. A method of treating a disease in a mammalian subject comprising administering a therapeutically effective amount of a pharmaceutical composition of any one of claims 35-60 to the subject.
62. The method of claim 61, wherein the disease is a cancer.
63. The method of claim 62, wherein the cancer is a pancreatic cancer.
- 10 64. The method of claim 63, wherein the pancreatic cancer is pancreatic ductal adenocarcinoma (PDAC).
65. The method of claim 63, wherein the pancreatic cancer is a pancreatic cancer in which one or more proteins in the sonic hedgehog signaling pathway is defective.
66. The method of claim 65, wherein the pancreatic cancer is a pancreatic cancer in which the sonic hedgehog signaling pathway is upregulated.
- 15 67. The method of claim 65, wherein the pancreatic cancer is a pancreatic cancer which the smoothened receptor (SMO) is misregulated.
68. The method of claim 67, wherein the smoothened receptor is upregulated.
69. The method of claim 62, wherein the cancer is a liver cancer.
70. The method of claim 69, wherein the liver cancer is hepatocellular carcinoma
- 20 71. The method of claim 62, wherein the cancer is ovarian cancer.
72. The method of claim 71, wherein the ovarian cancer is a high grade serous carcinoma.
73. The method of claim 62, wherein the cancer is a basal-cell carcinoma.
74. The method of claim 62, wherein the cancer is a medulloblastoma.
- 25 75. The method of claim 63, wherein the pharmaceutical composition comprises paclitaxel or cyclopamine.

76. The method of claim 75, wherein the pharmaceutical composition comprises paclitaxel and cyclopamine.
77. The method of any one of claims 61-76, wherein the mammalian subject is a human.
78. The method of any one of claims 61-77, wherein the method further comprises
5 administering a checkpoint inhibitor, an immunotherapy, or a radiotherapy to the subject.
79. The method of claims 78, wherein the method further comprises administering a radiotherapy to the subject.

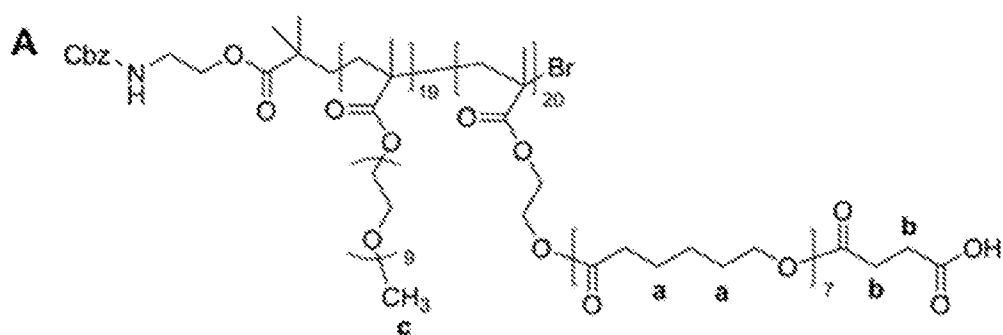


FIG. 1A

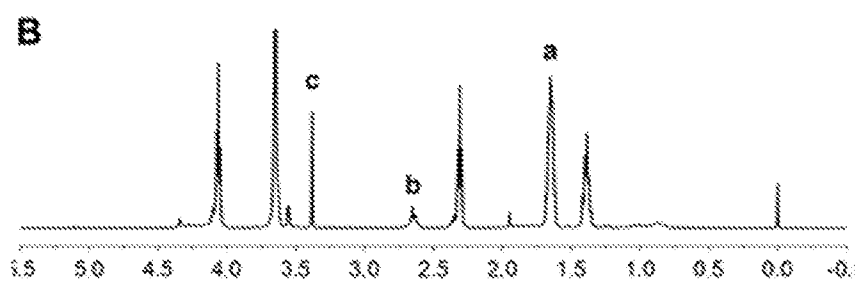


FIG. 1B

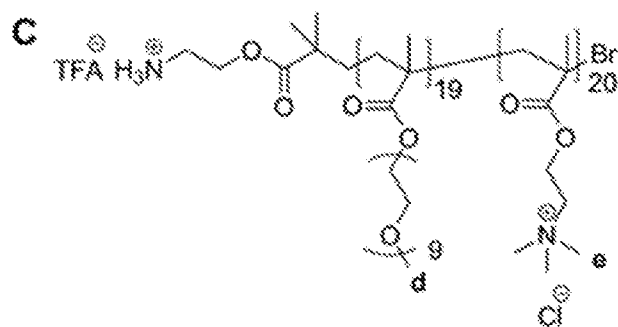


FIG. 1C

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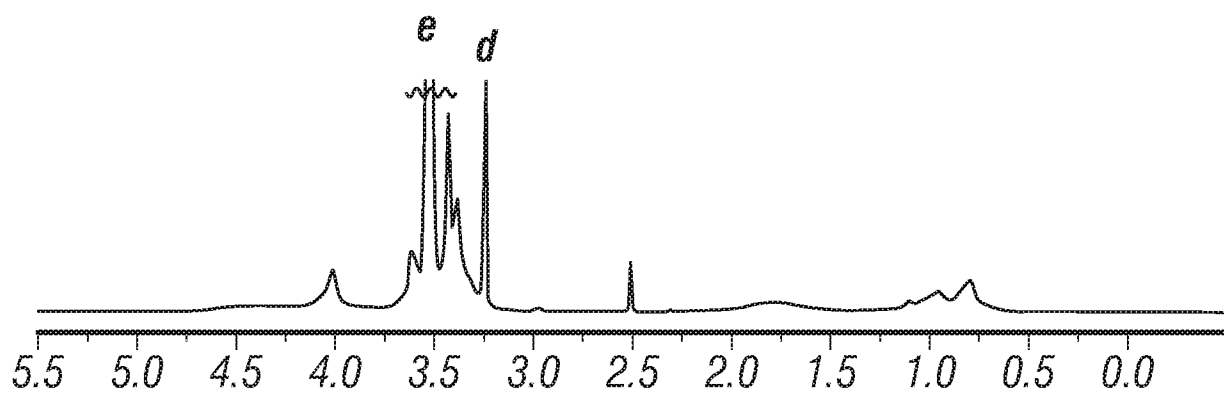


FIG. 1D

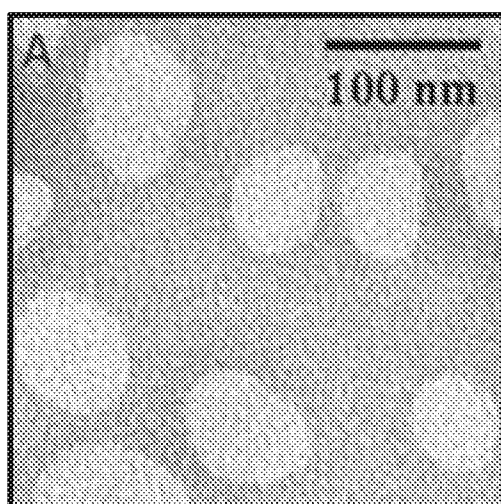


FIG. 2A

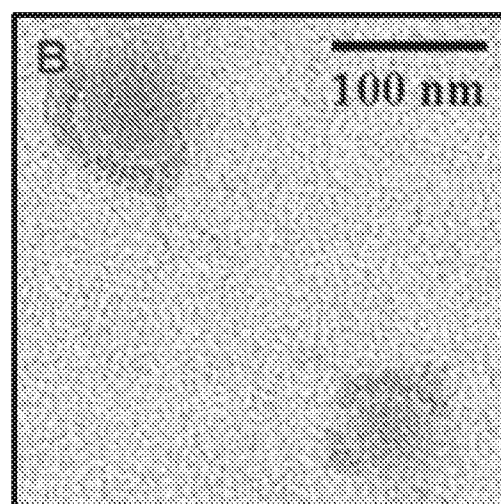


FIG. 2B

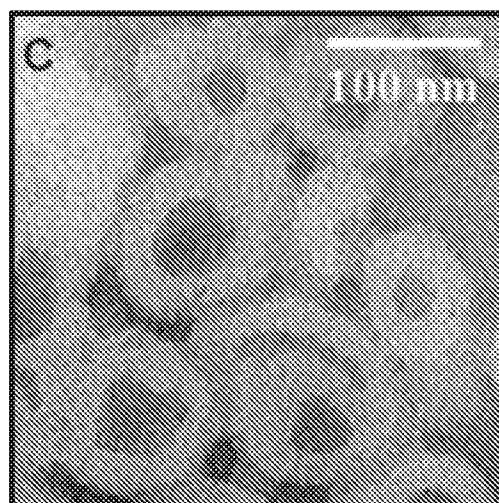


FIG. 2C

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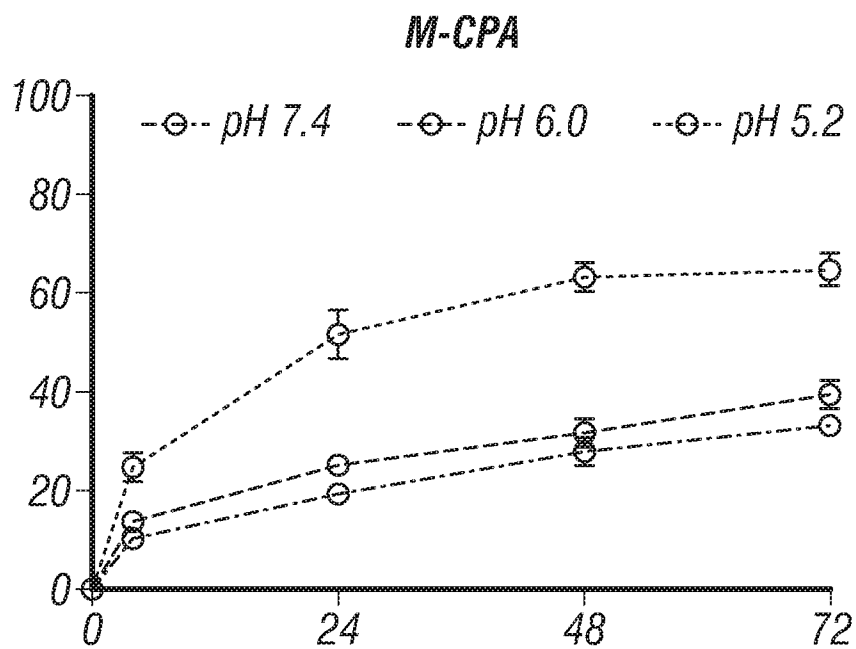


FIG. 2D

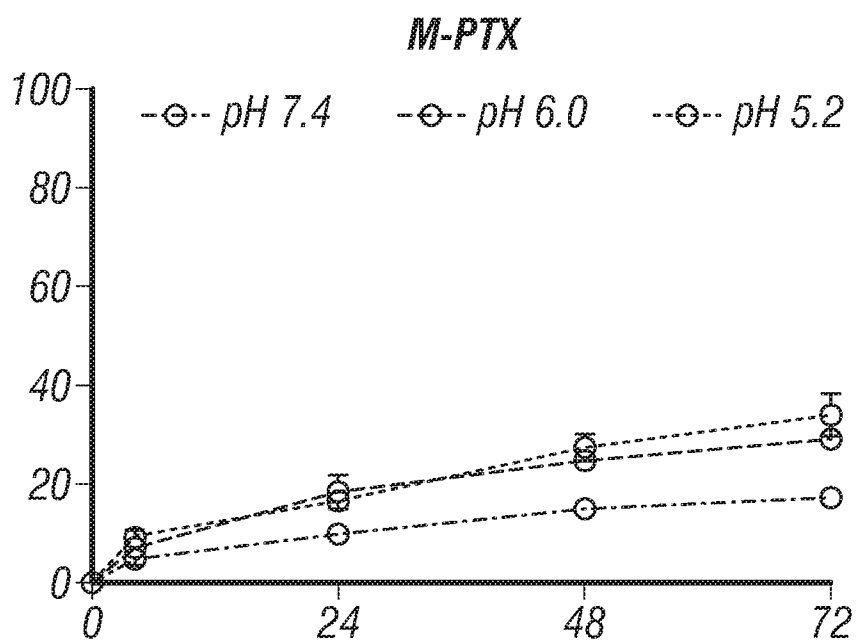


FIG. 2E

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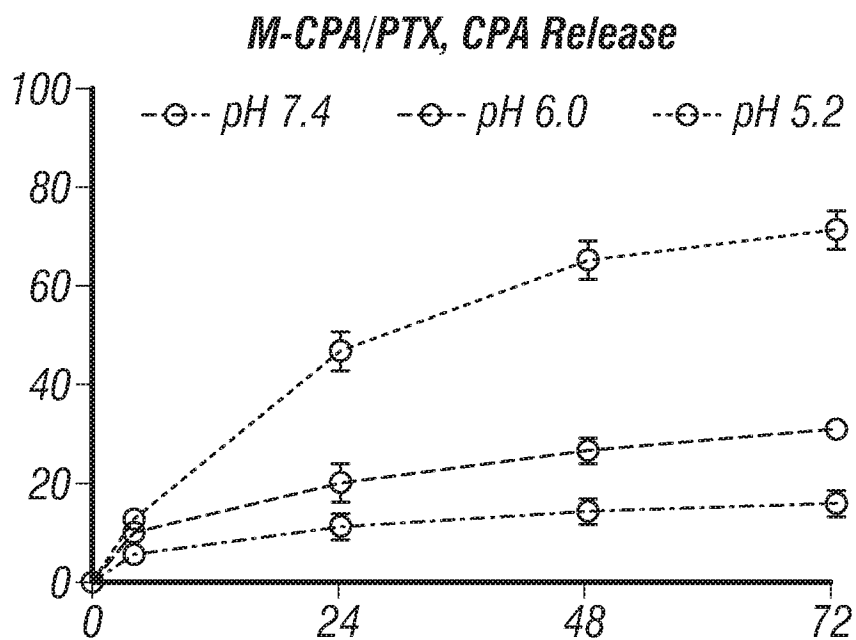


FIG. 2F

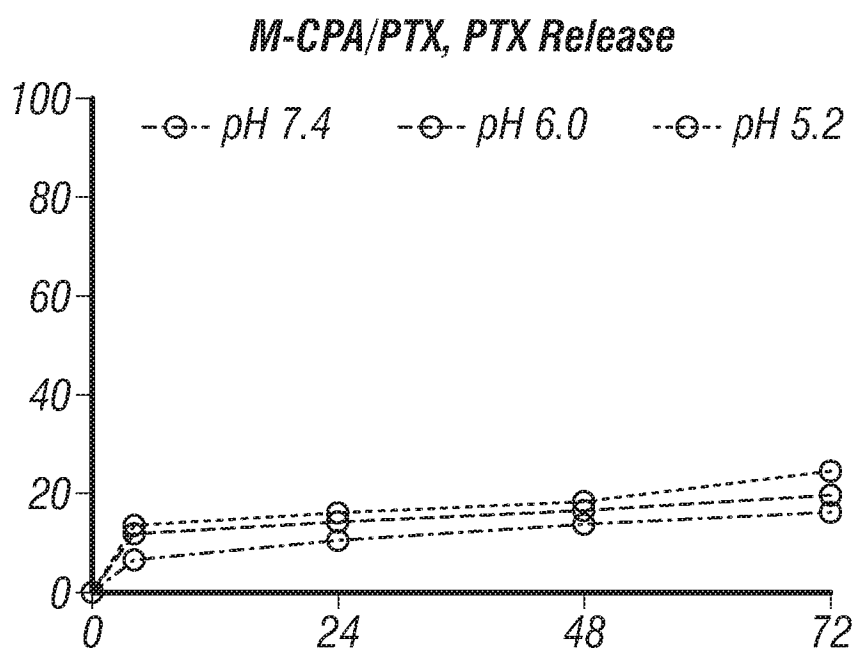


FIG. 2G

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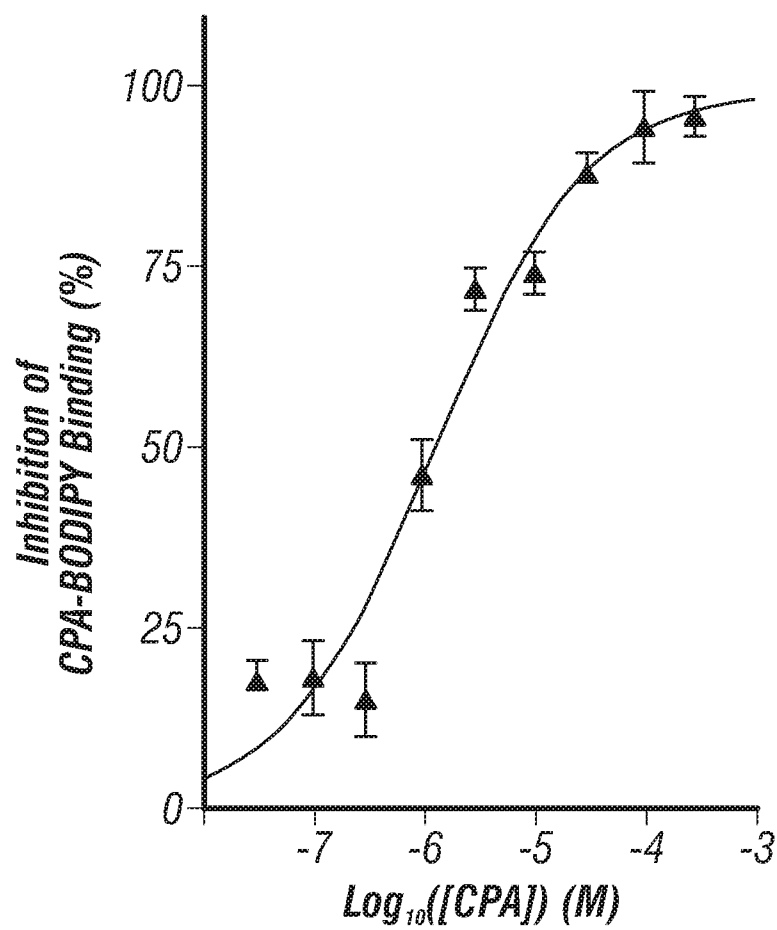


FIG. 3A

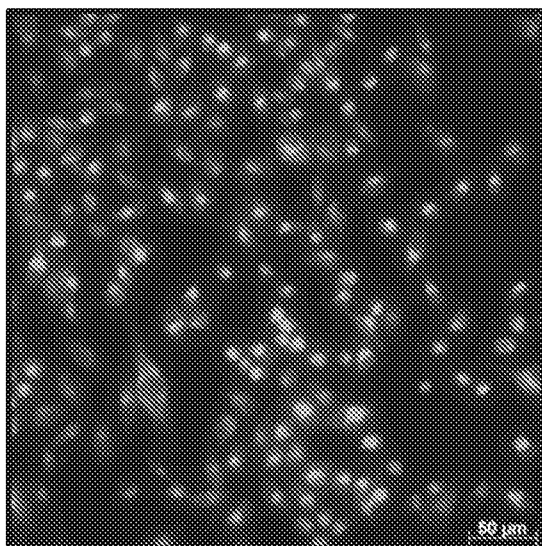


FIG. 3B

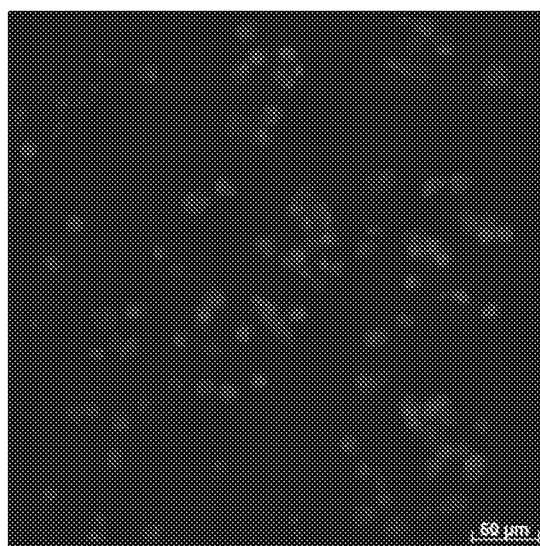


FIG. 3C

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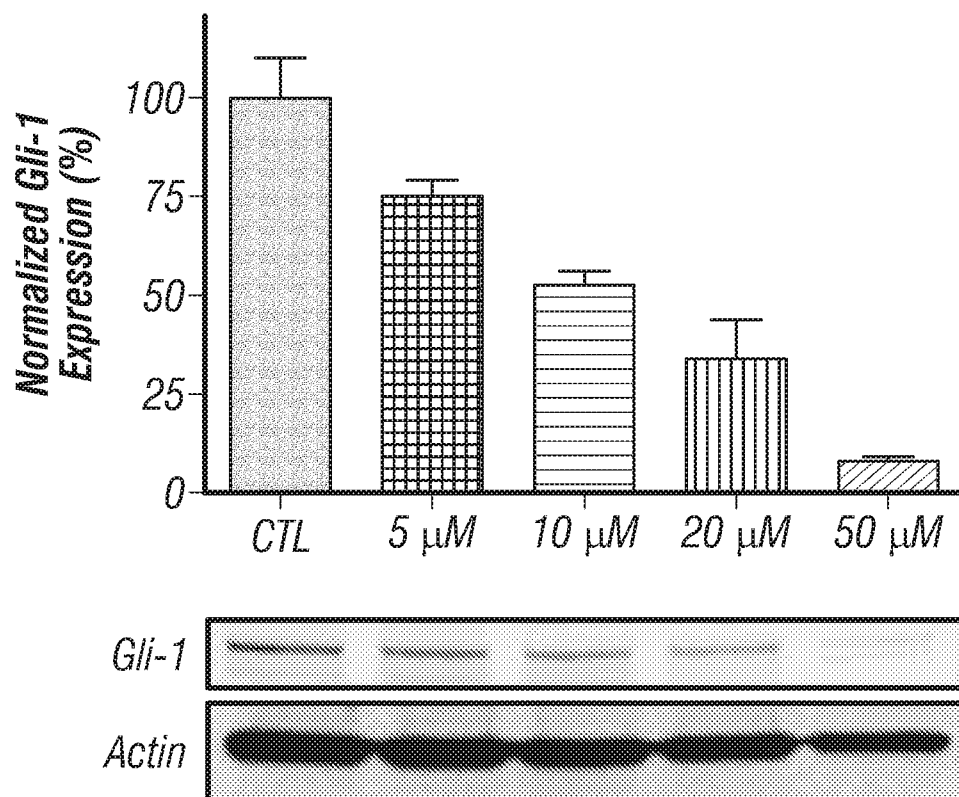


FIG. 3D

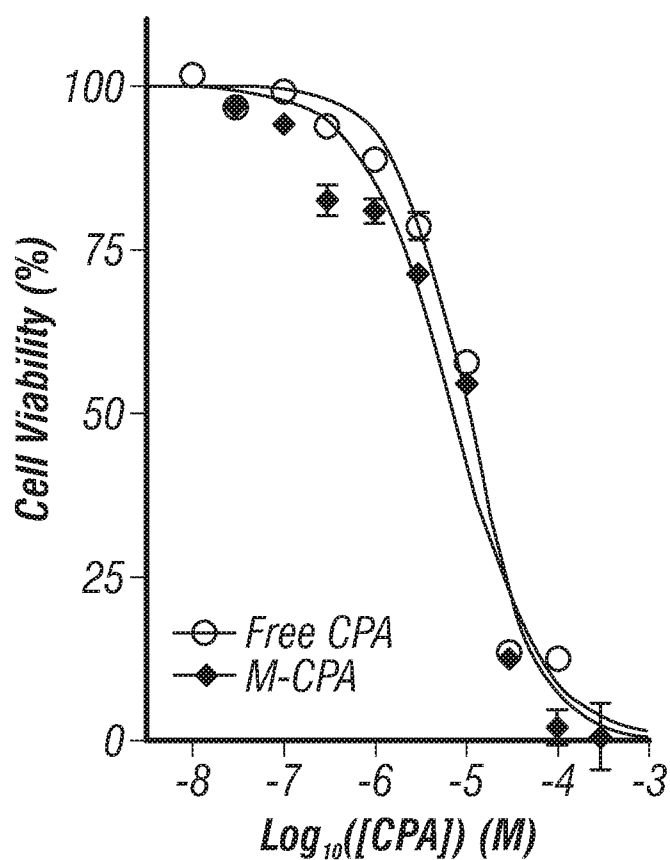


FIG. 3E

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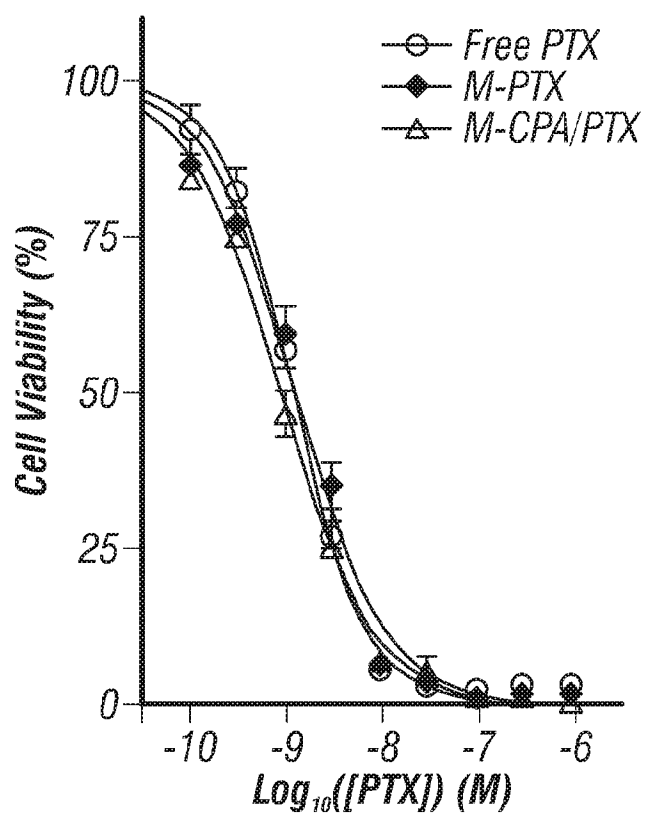


FIG. 3F

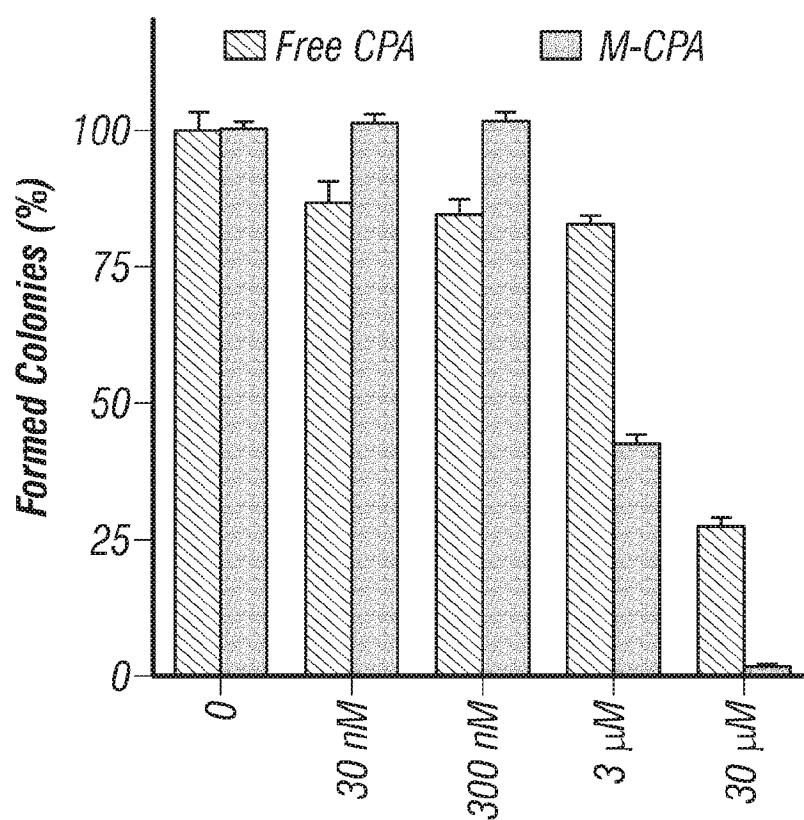


FIG. 3G

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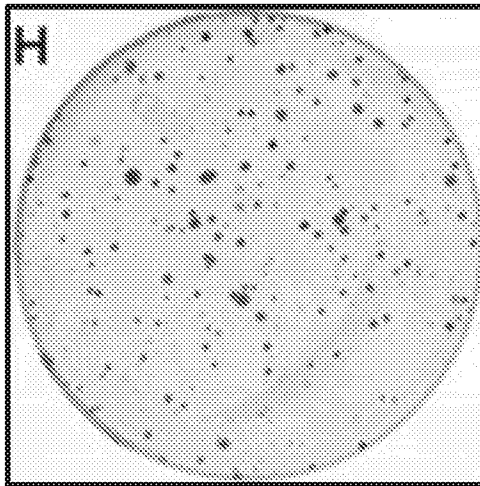


FIG. 3H

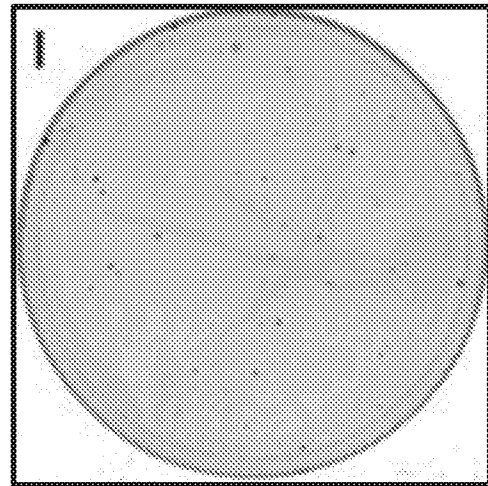


FIG. 3I

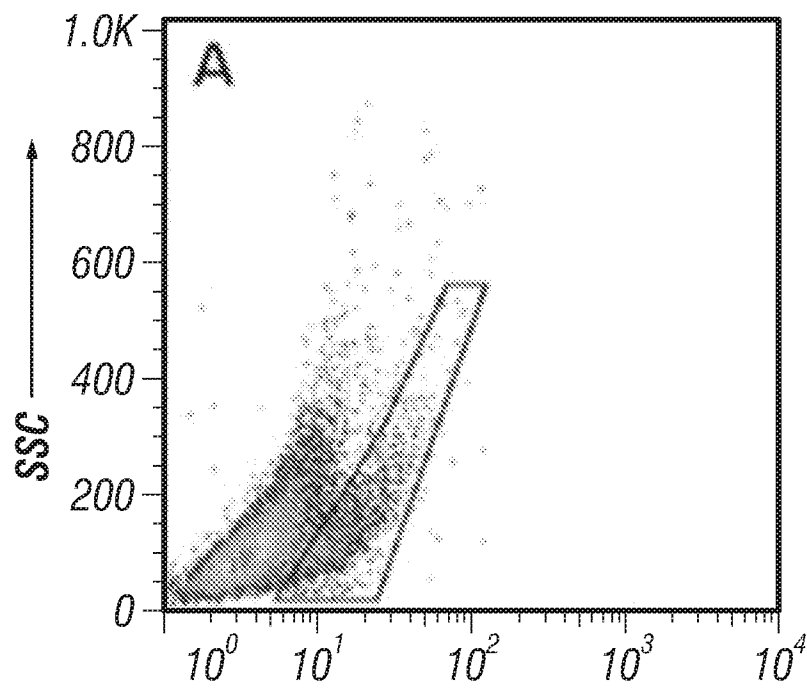


FIG. 4A

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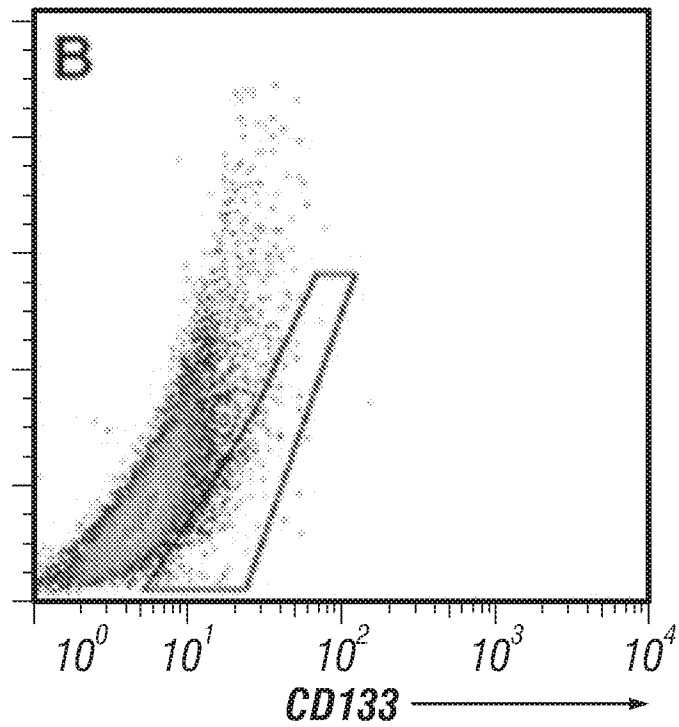


FIG. 4B

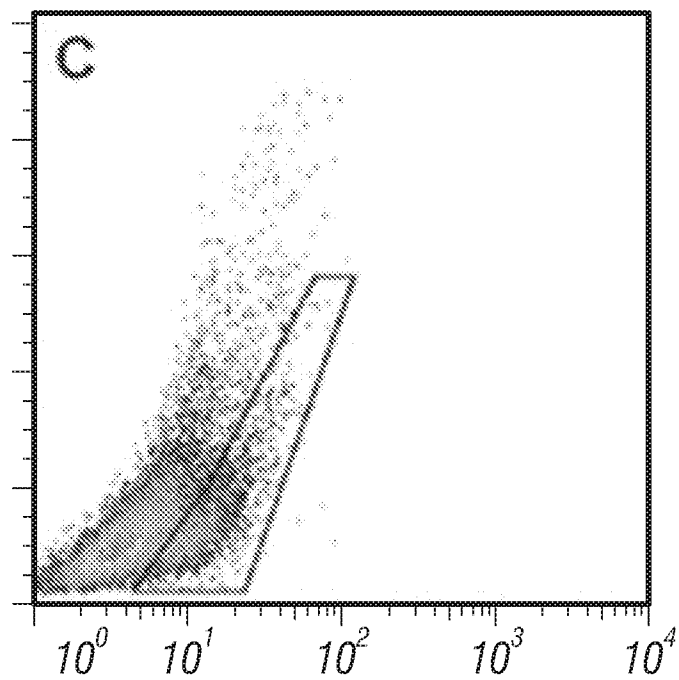


FIG. 4C

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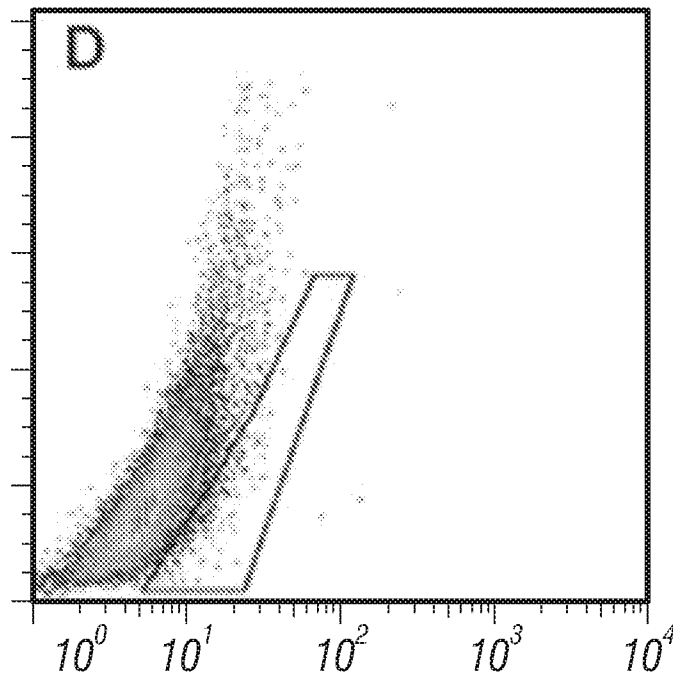


FIG. 4D

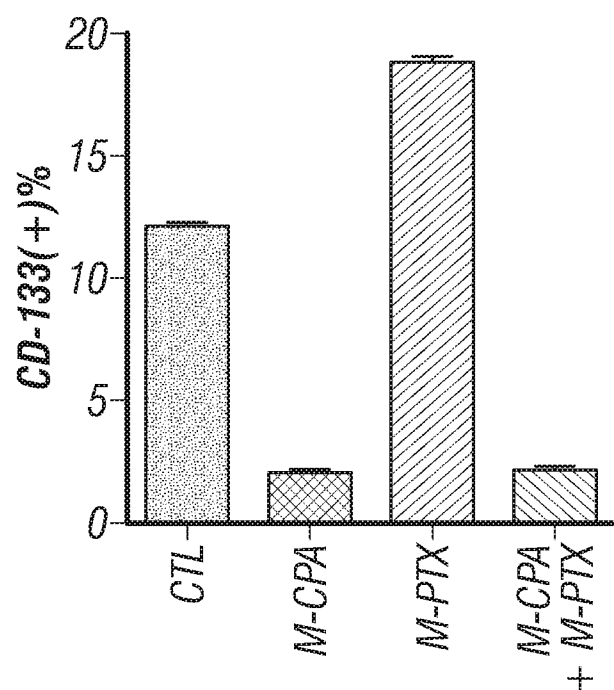


FIG. 4E

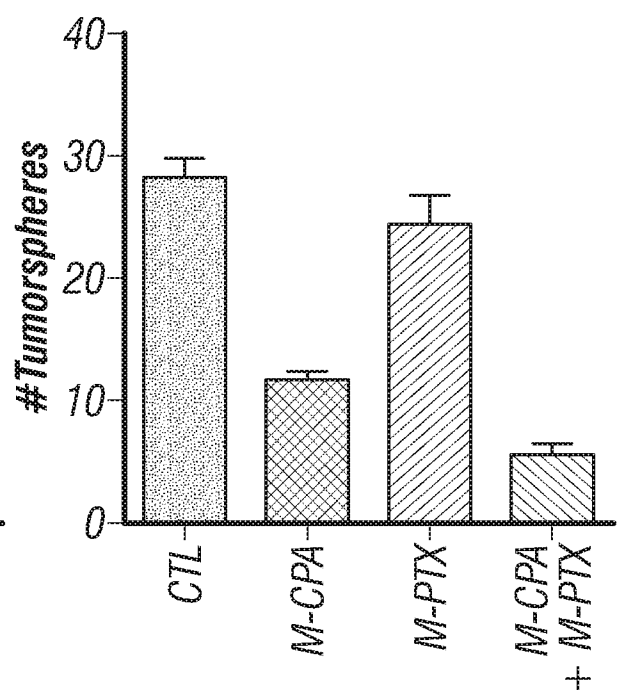


FIG. 4F

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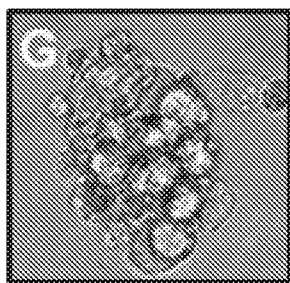


FIG. 4G

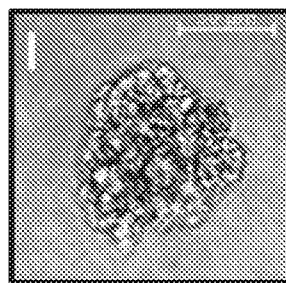


FIG. 4I

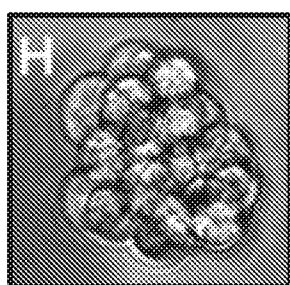


FIG. 4H

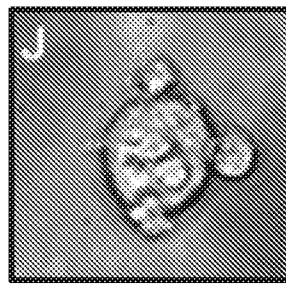


FIG. 4J

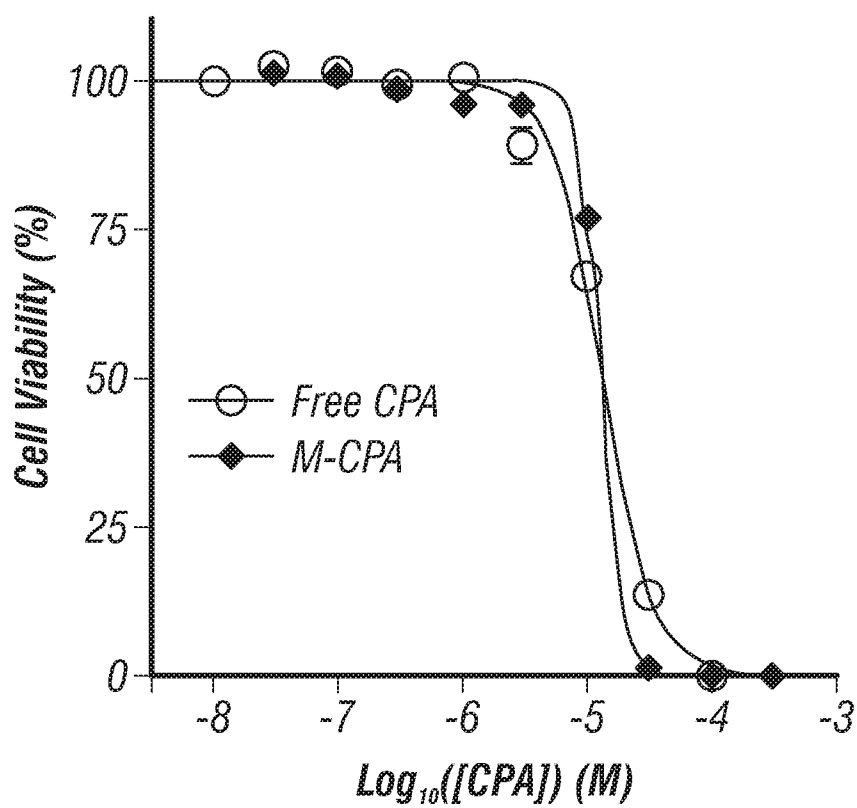


FIG. 5A

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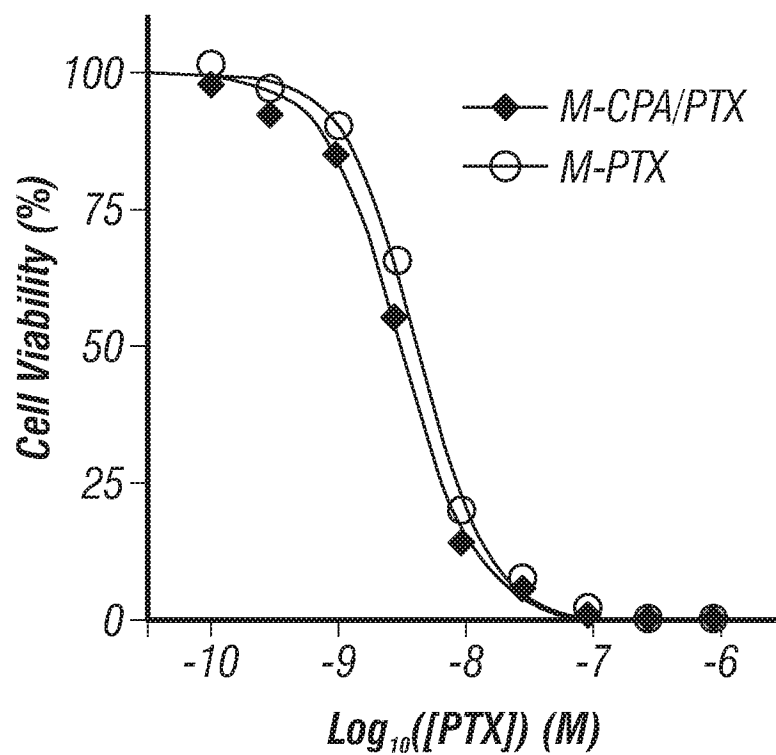


FIG. 5B

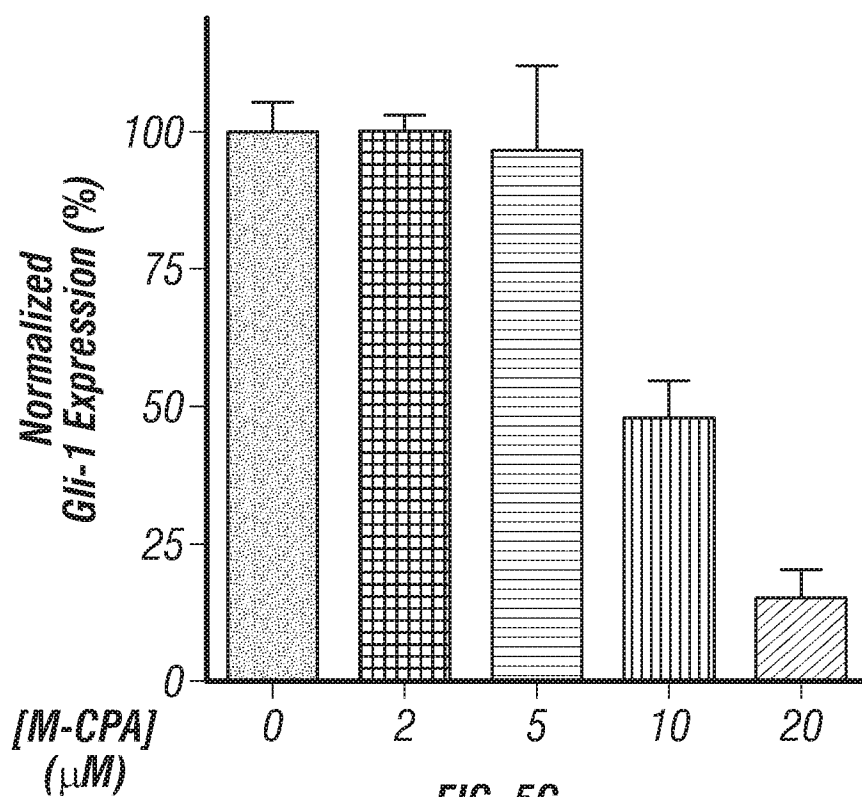


FIG. 5C

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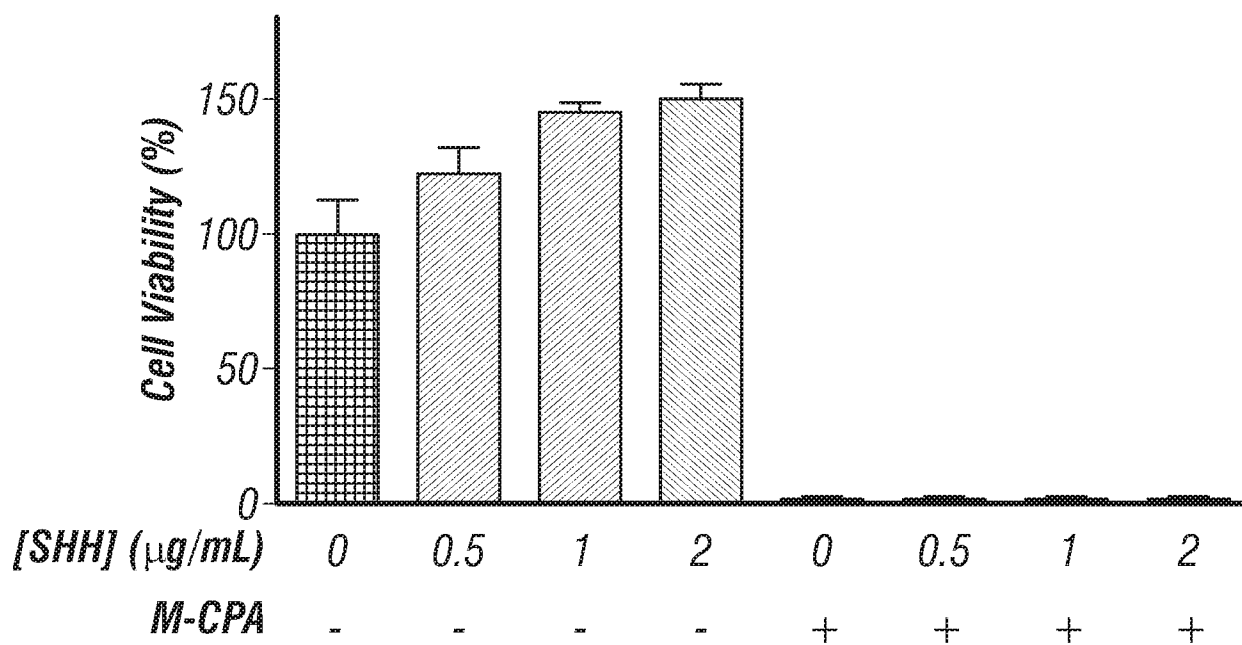


FIG. 5D

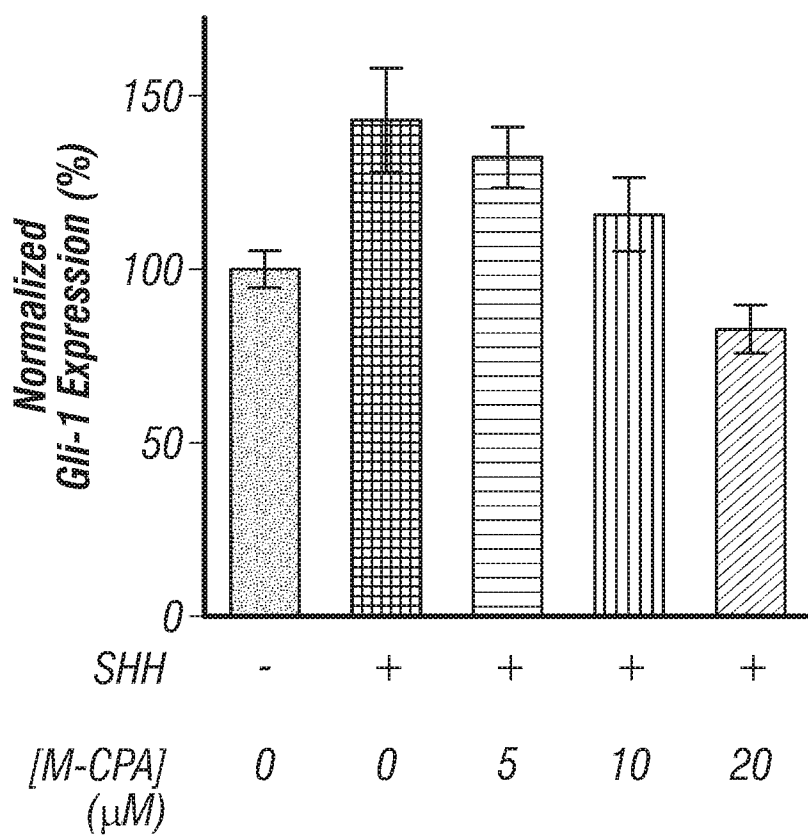


FIG. 5E

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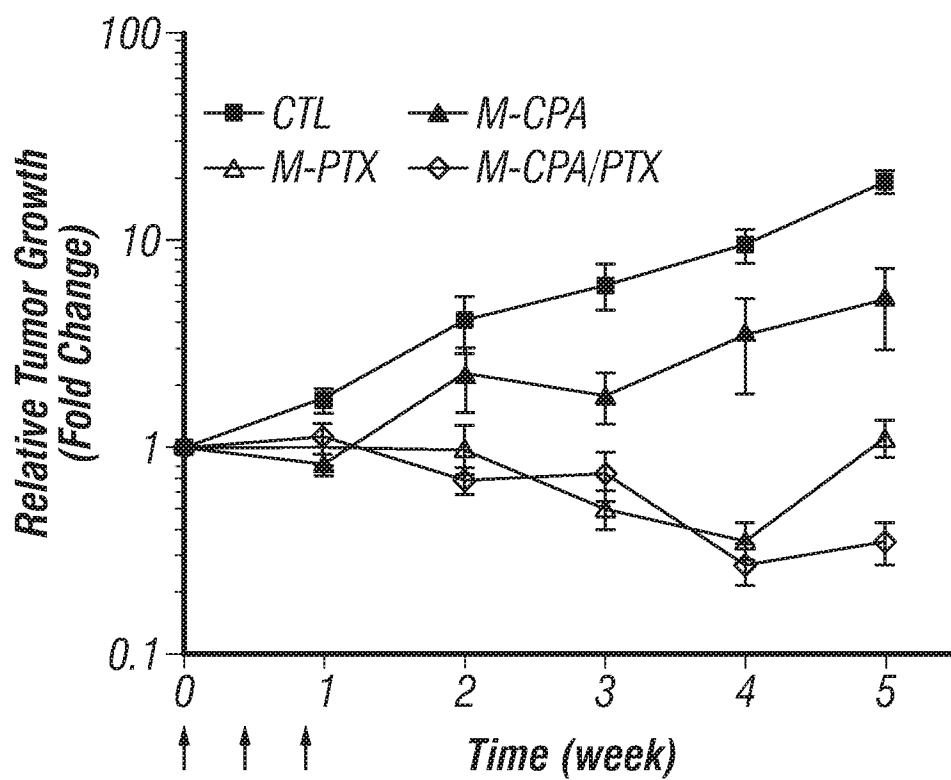


FIG. 6A

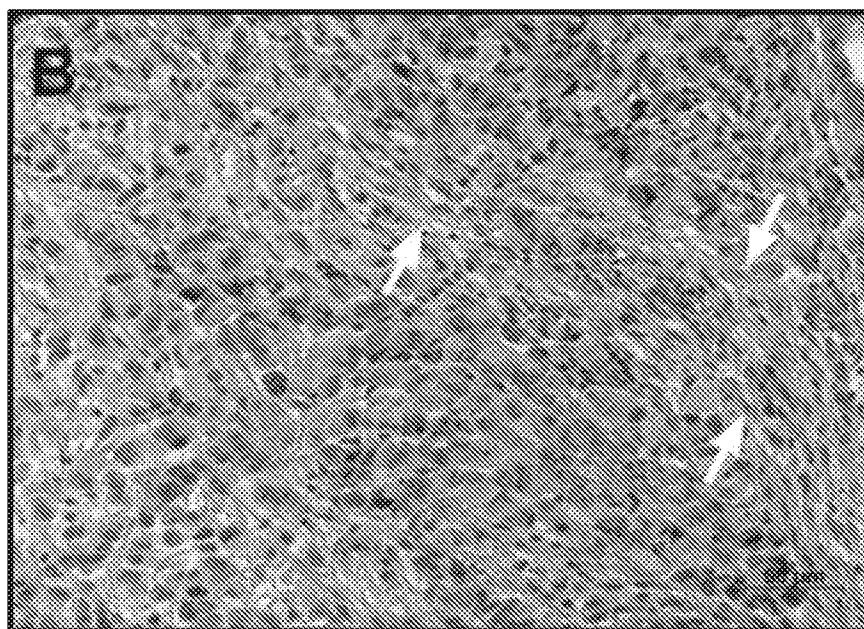


FIG. 6B

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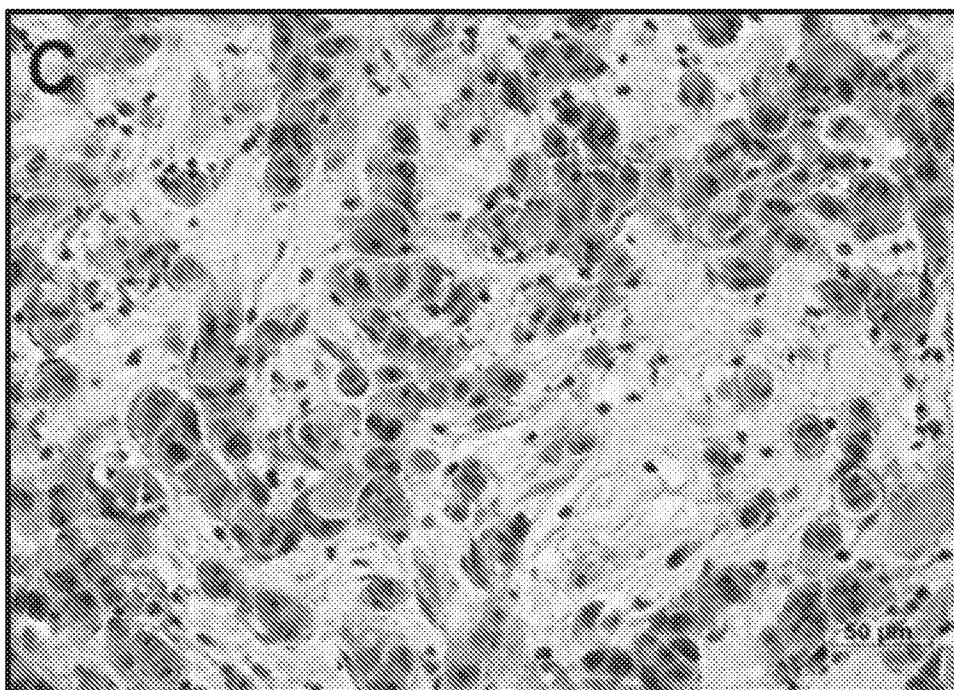


FIG. 6C

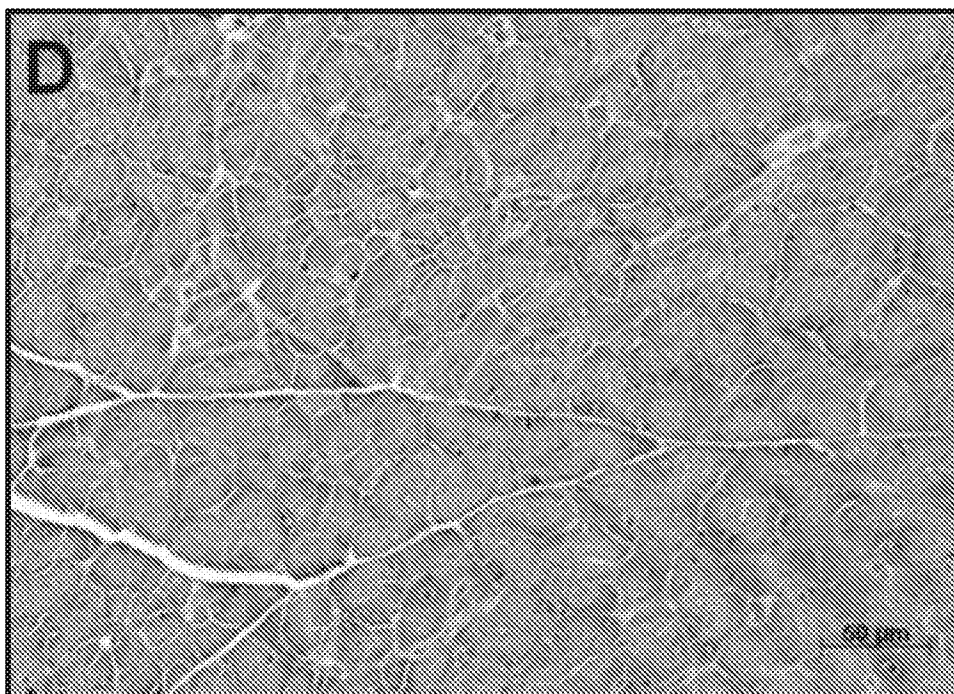
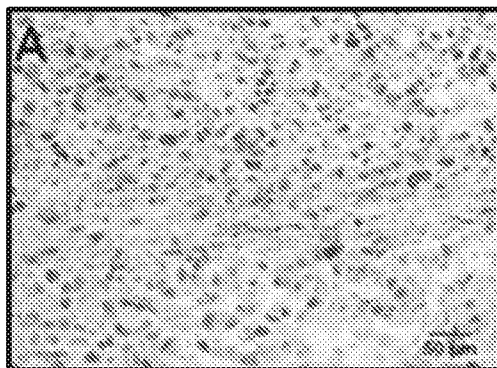
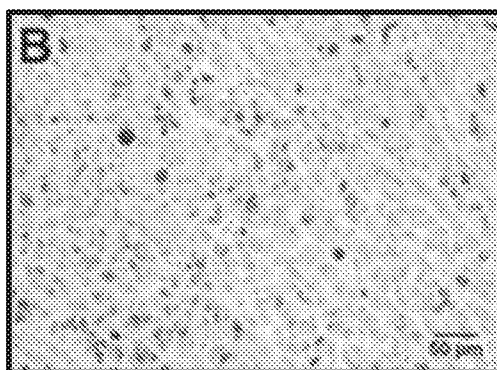
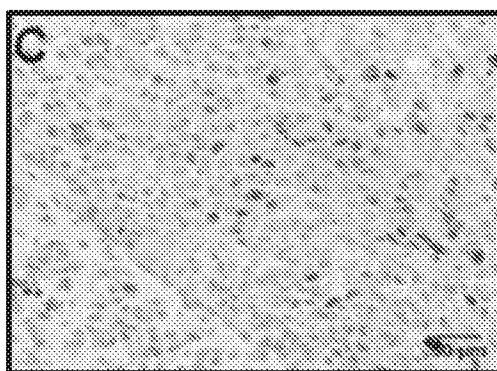
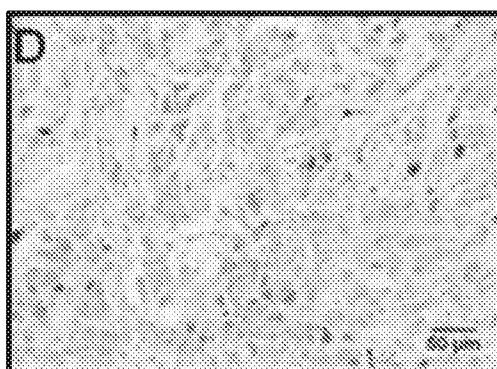
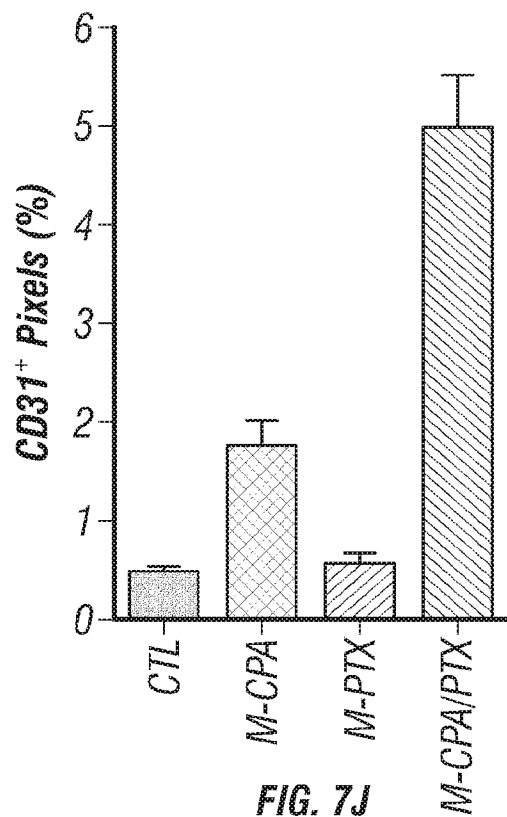
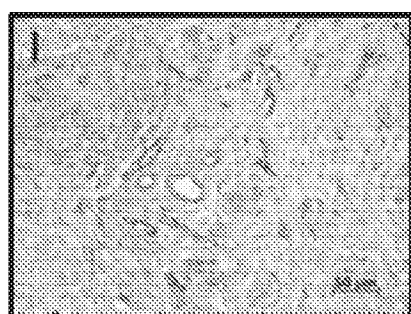
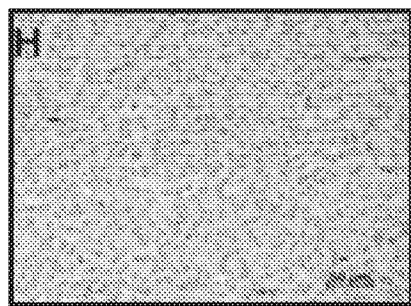
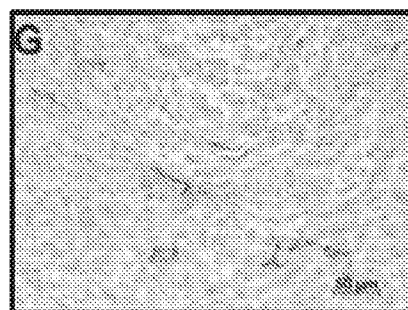
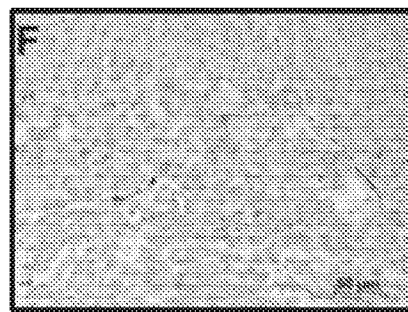
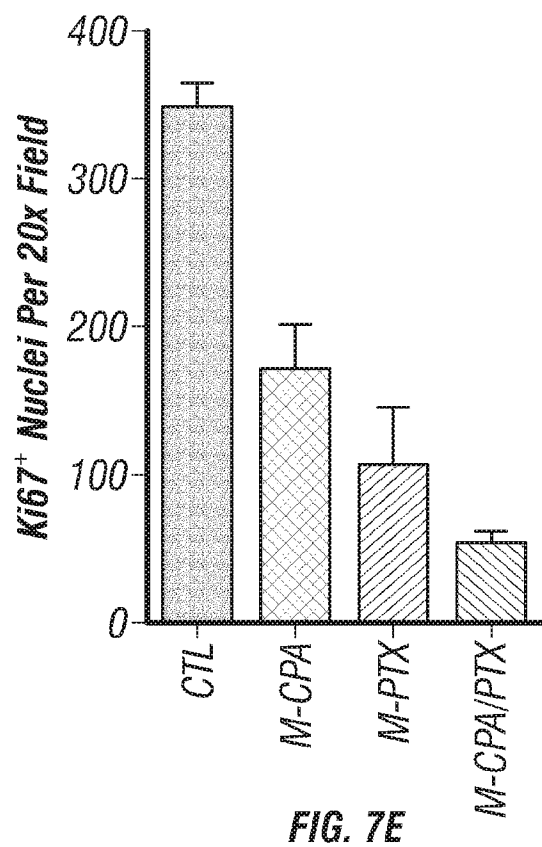


FIG. 6D

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*FIG. 7A**FIG. 7B**FIG. 7C**FIG. 7D*

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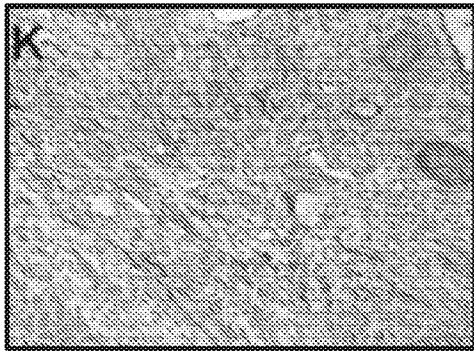


FIG. 7K

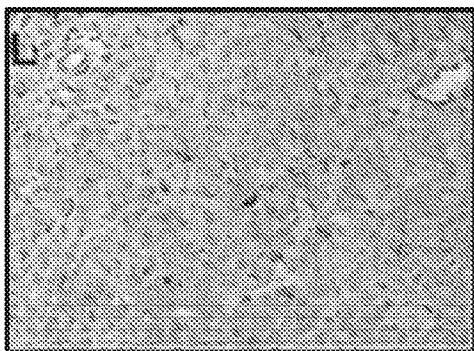


FIG. 7L

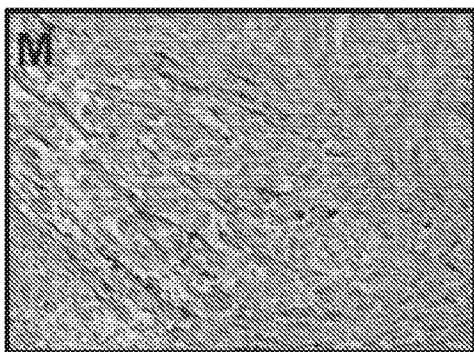


FIG. 7M

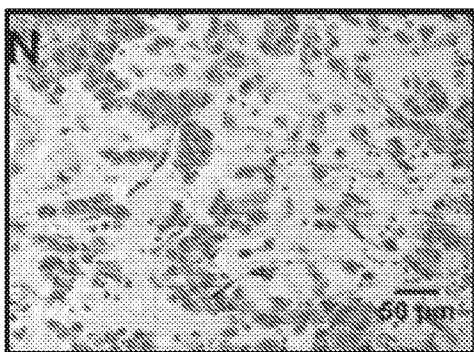
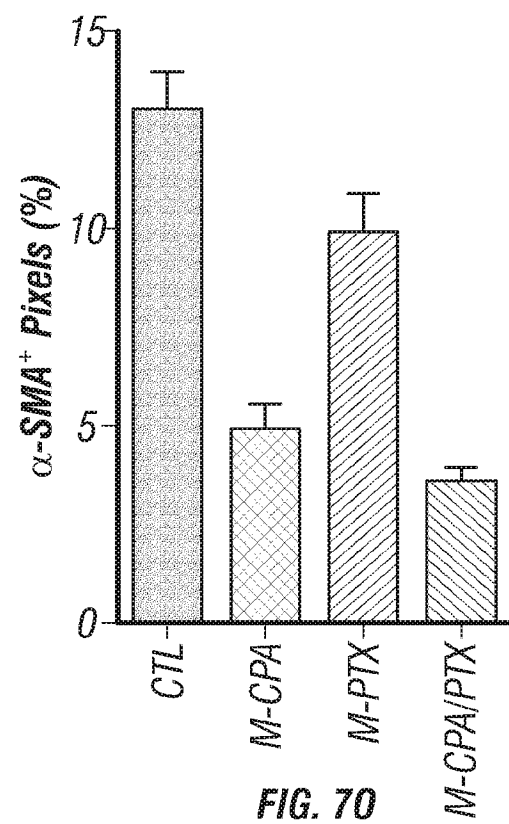


FIG. 7N



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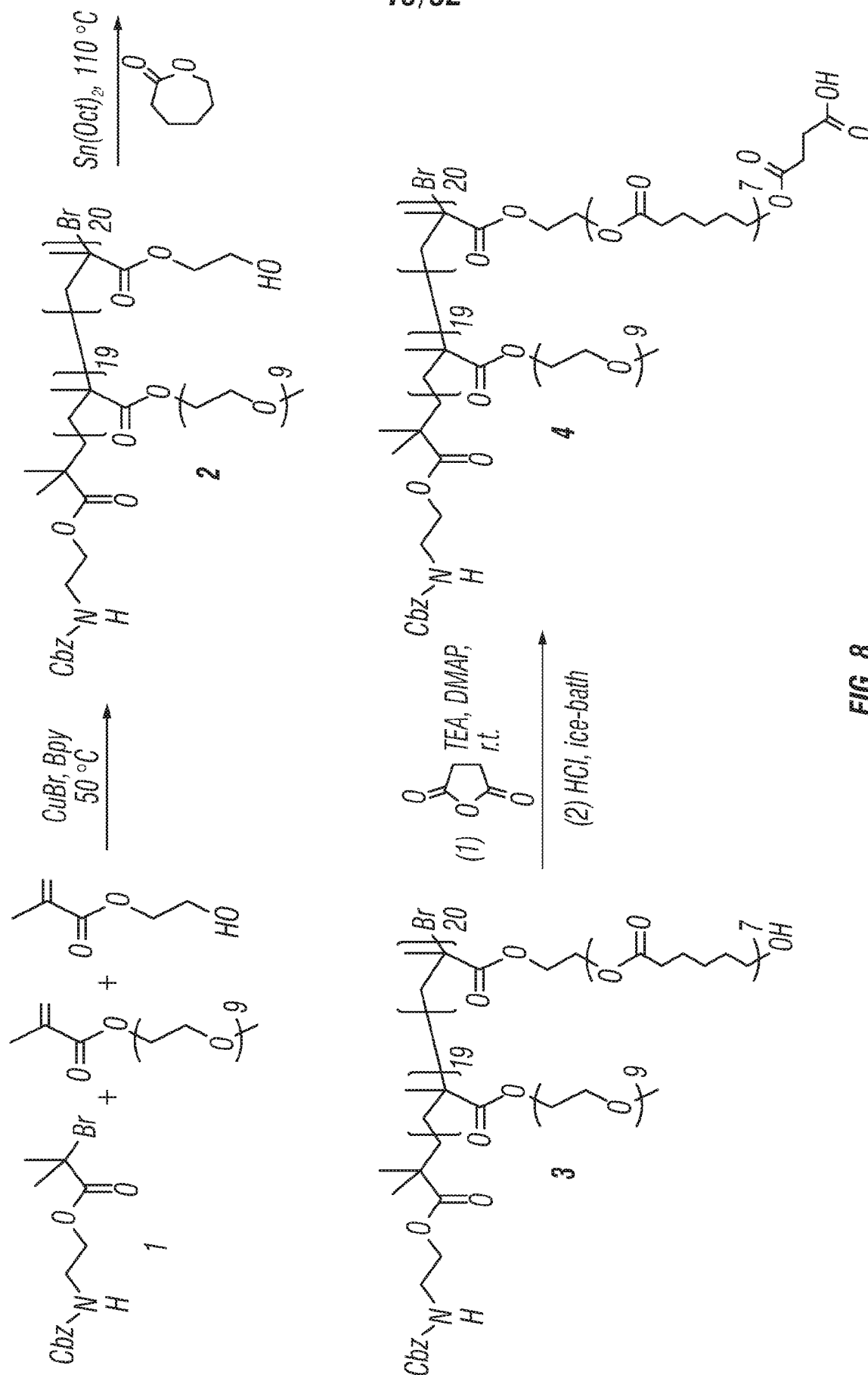


FIG. 8

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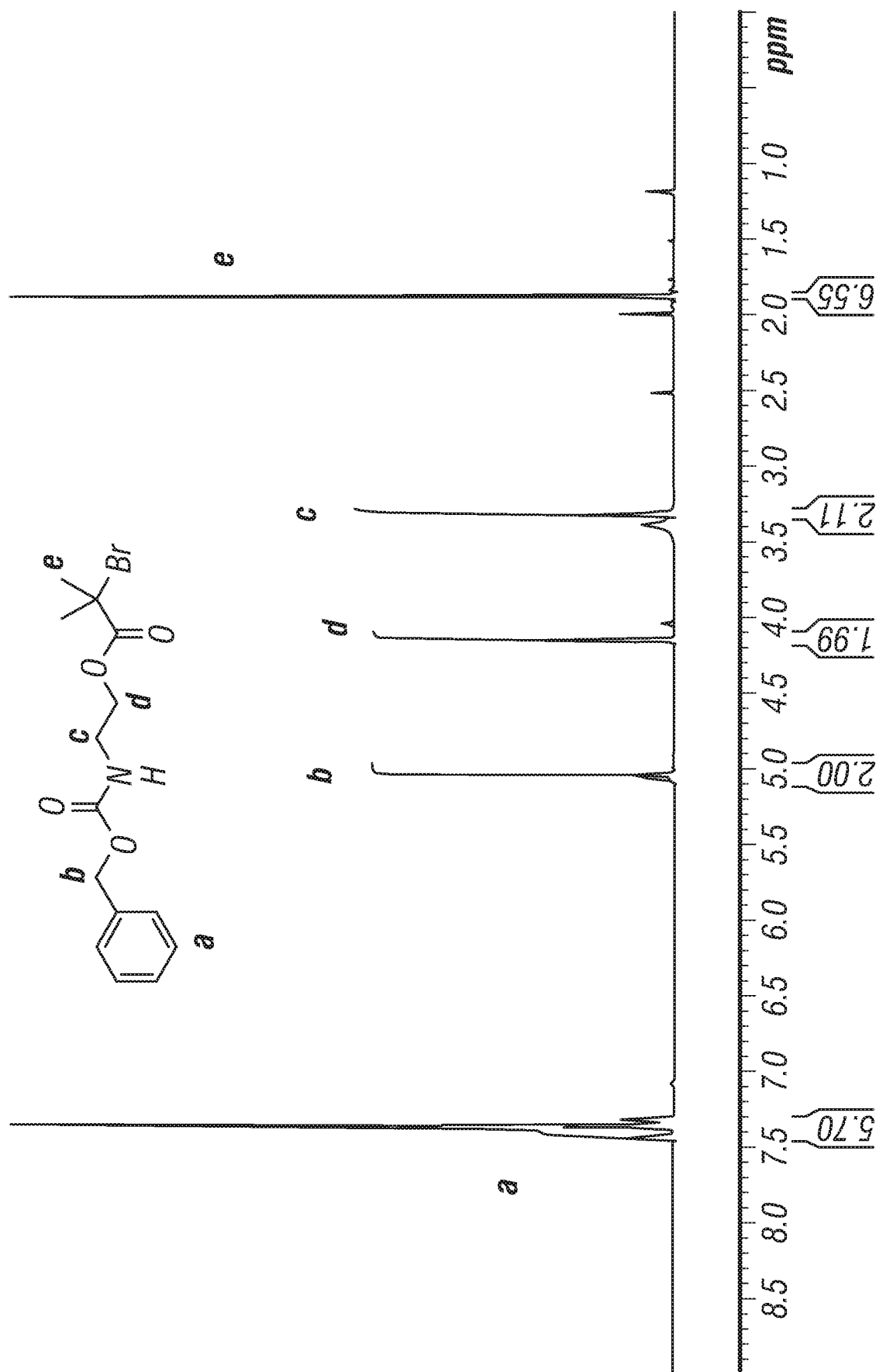


FIG. 9

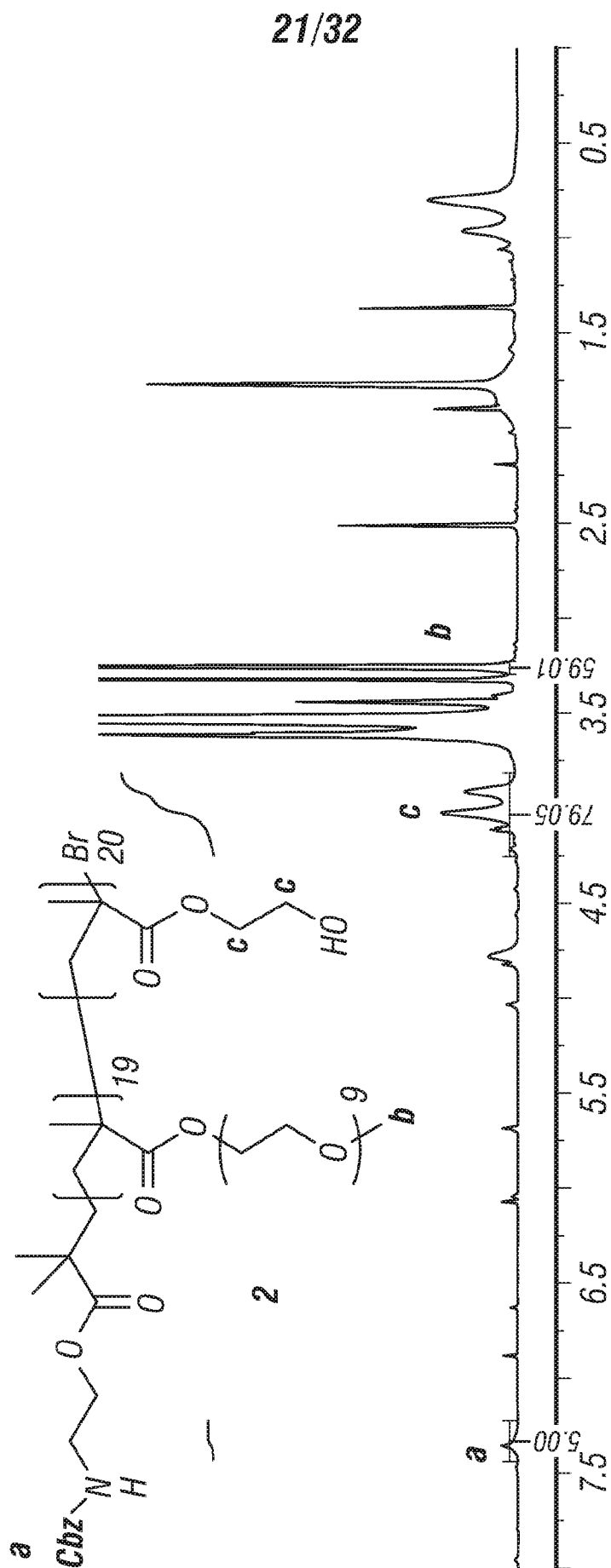


FIG. 10

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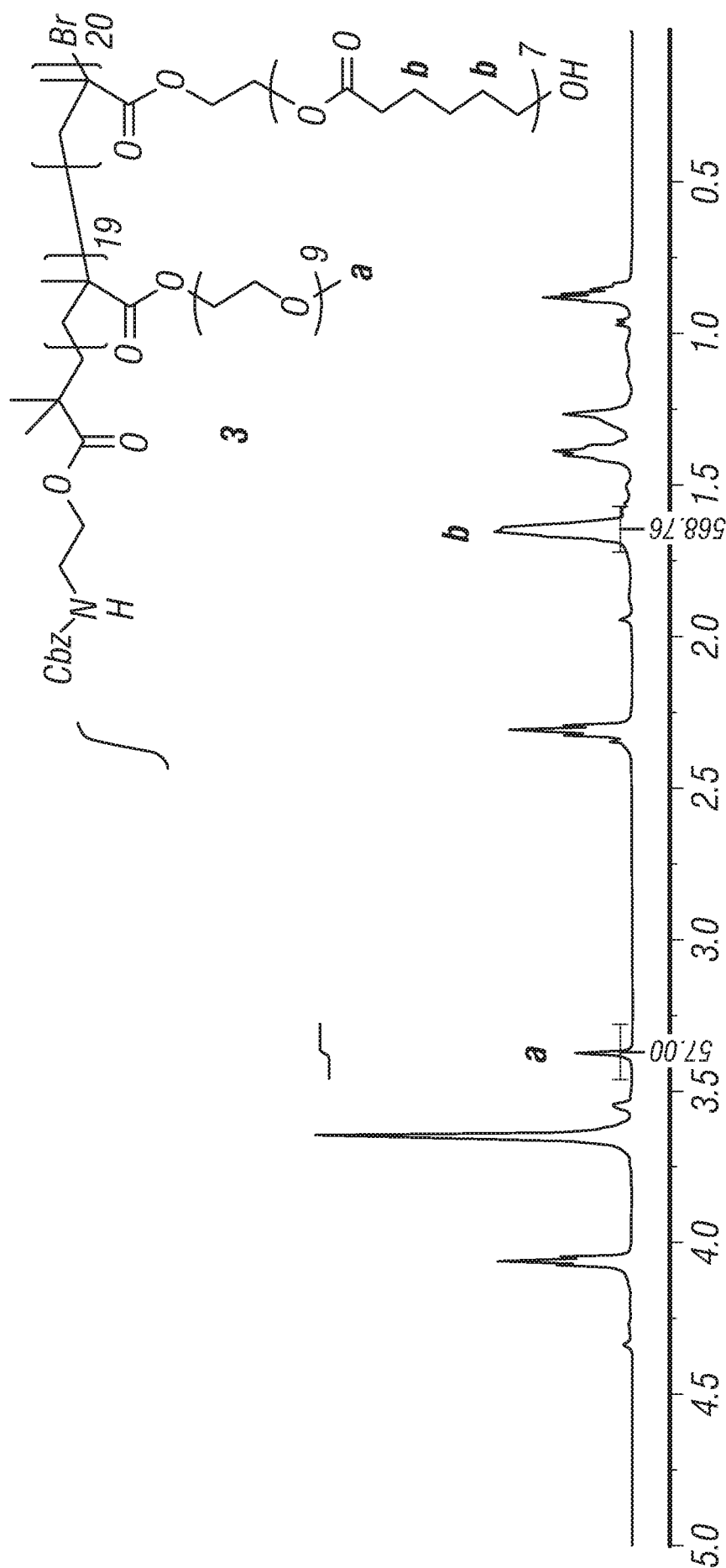
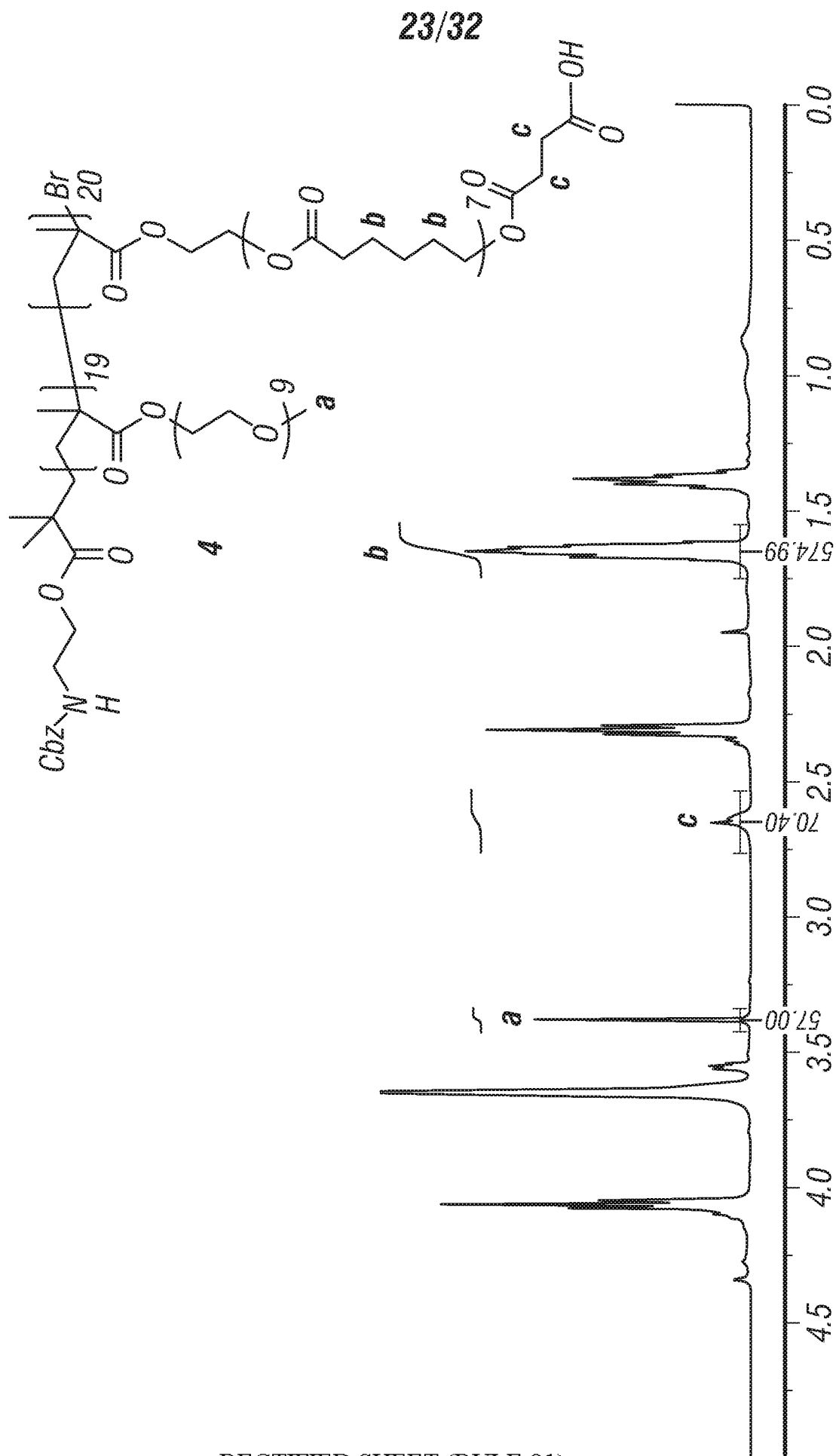
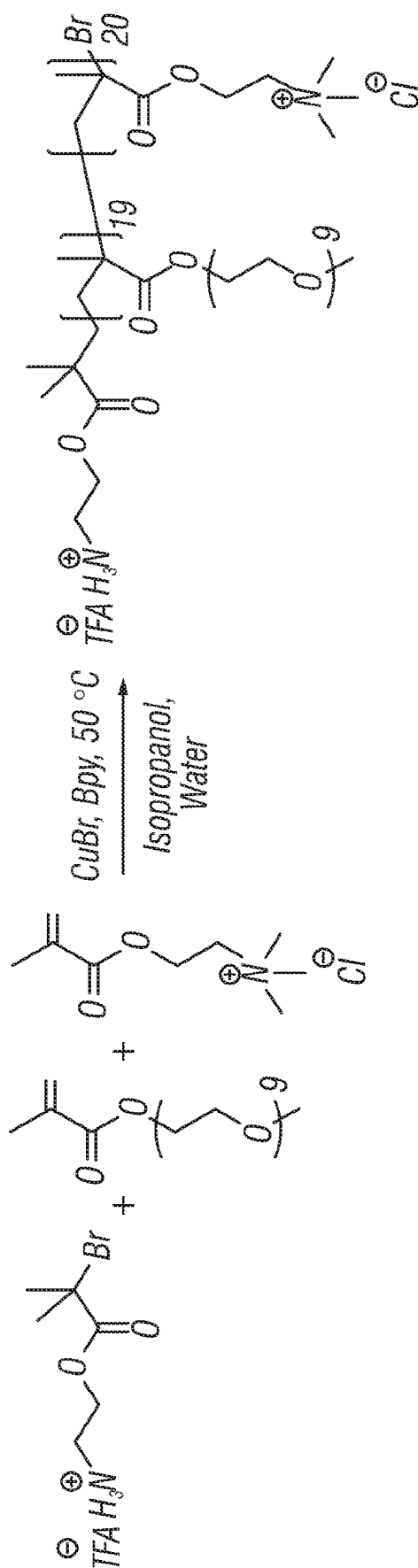


FIG. 11

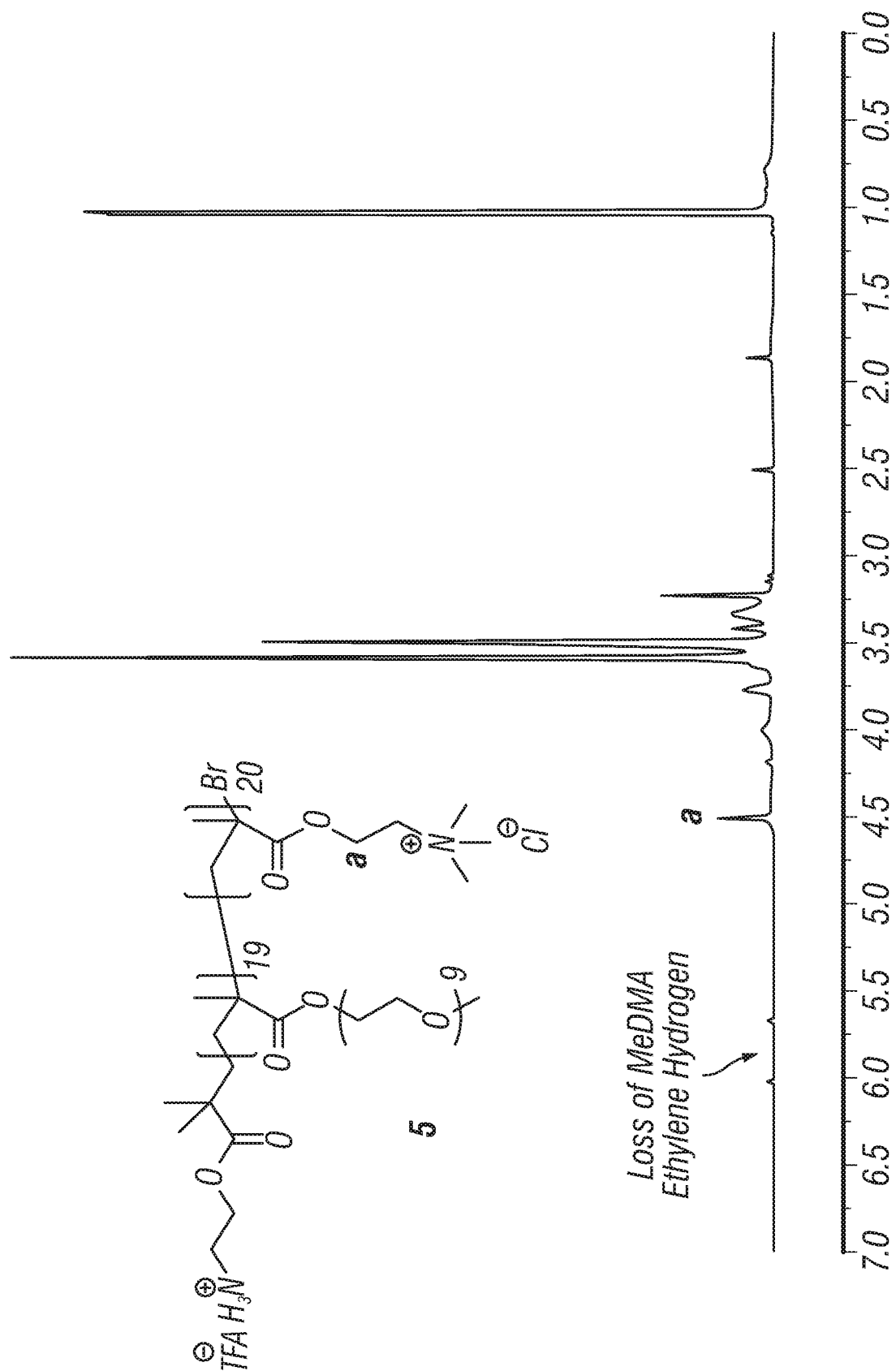


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FIG. 13



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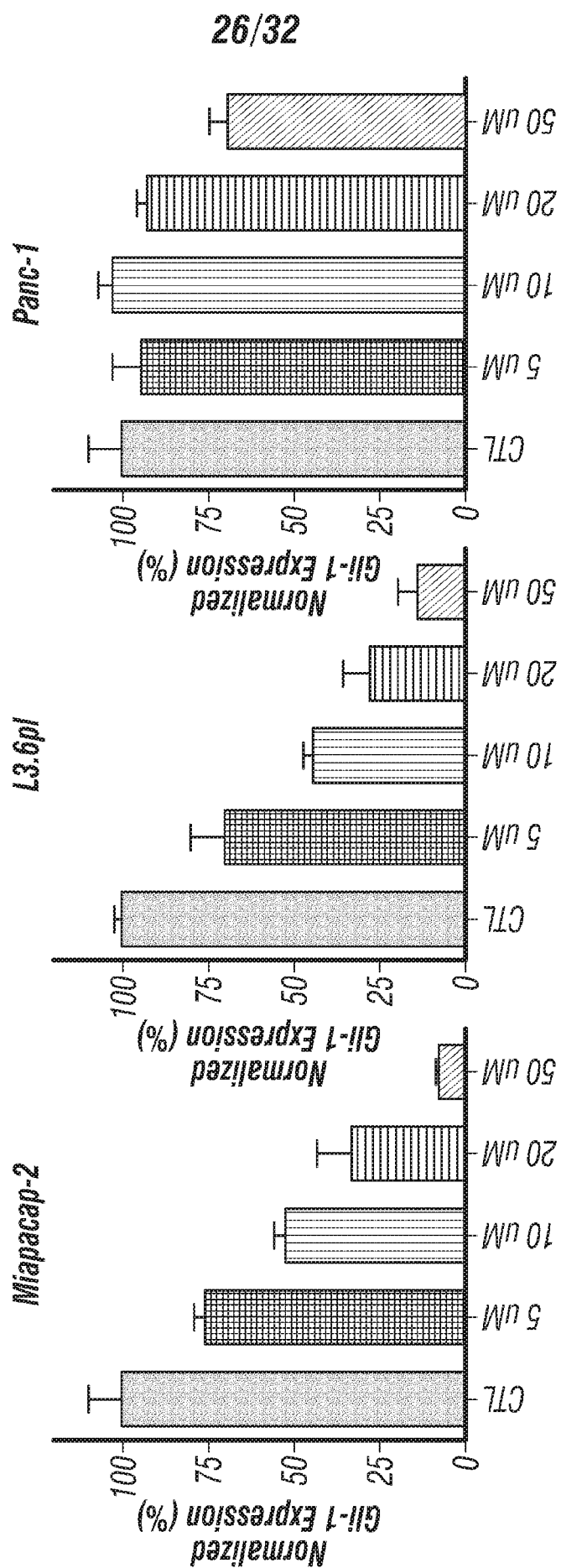
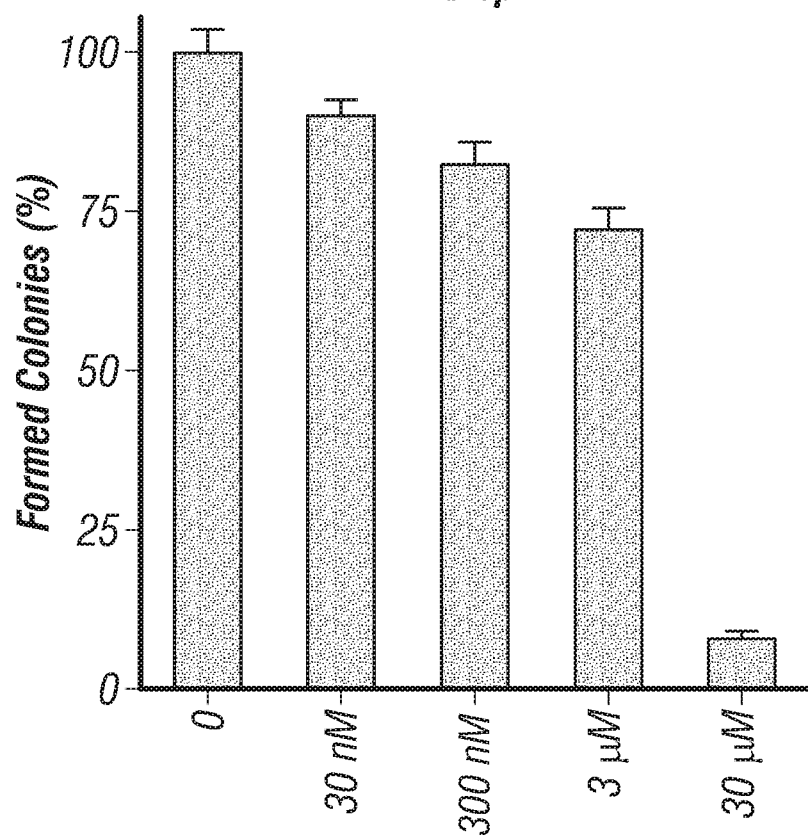
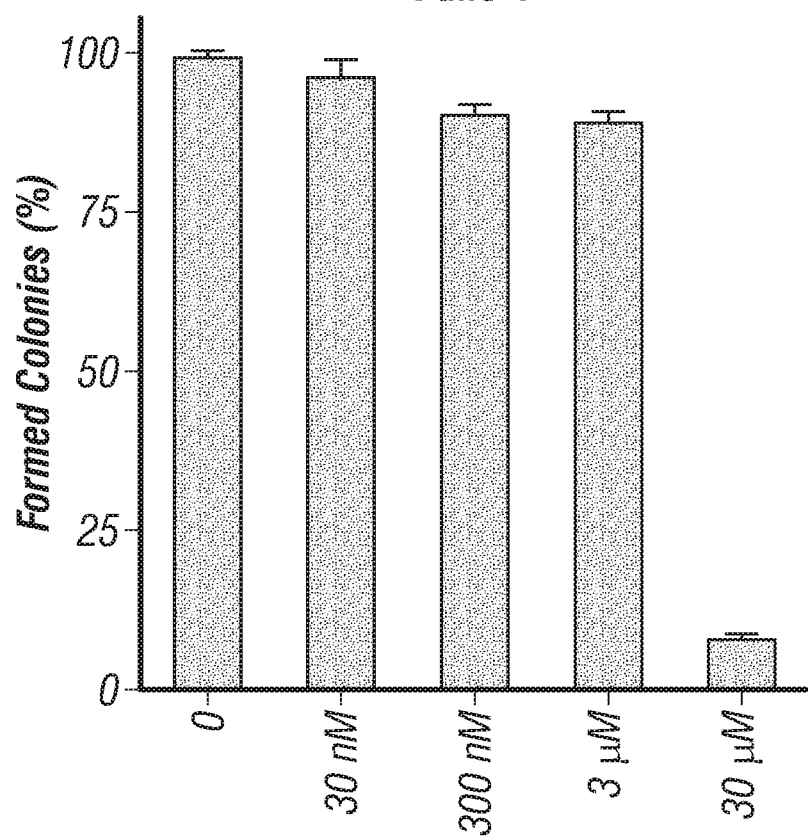
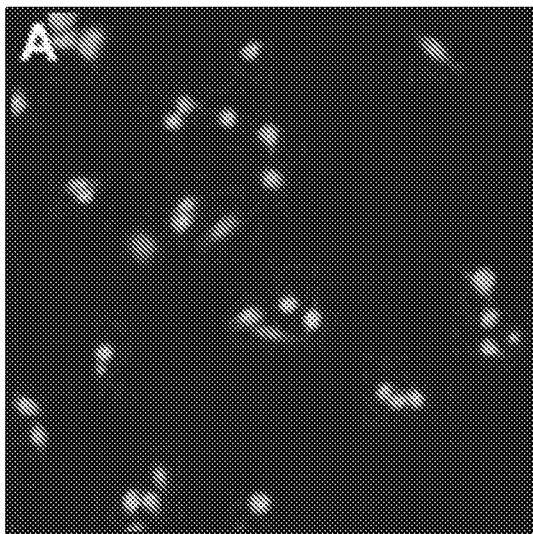
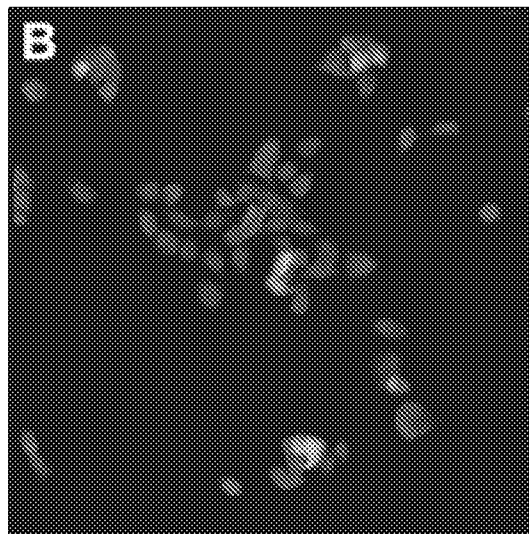
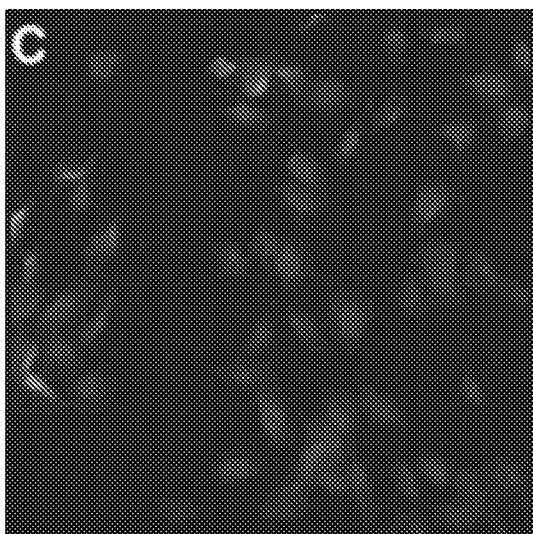
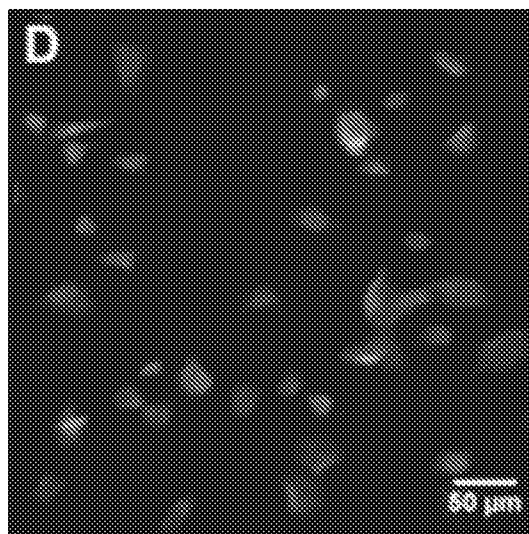


FIG. 15

27/32**L3.6pl****FIG. 16A****Panc-1****FIG. 16B**

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*FIG. 17A**FIG. 17B**FIG. 17C**FIG. 17D*

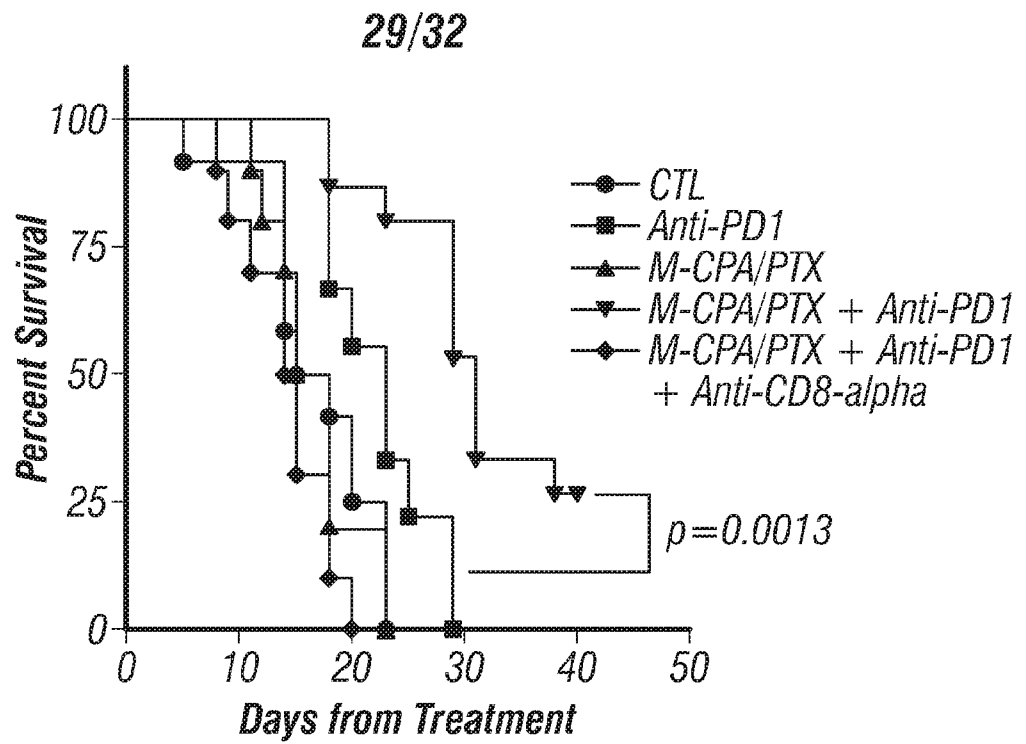


FIG. 18

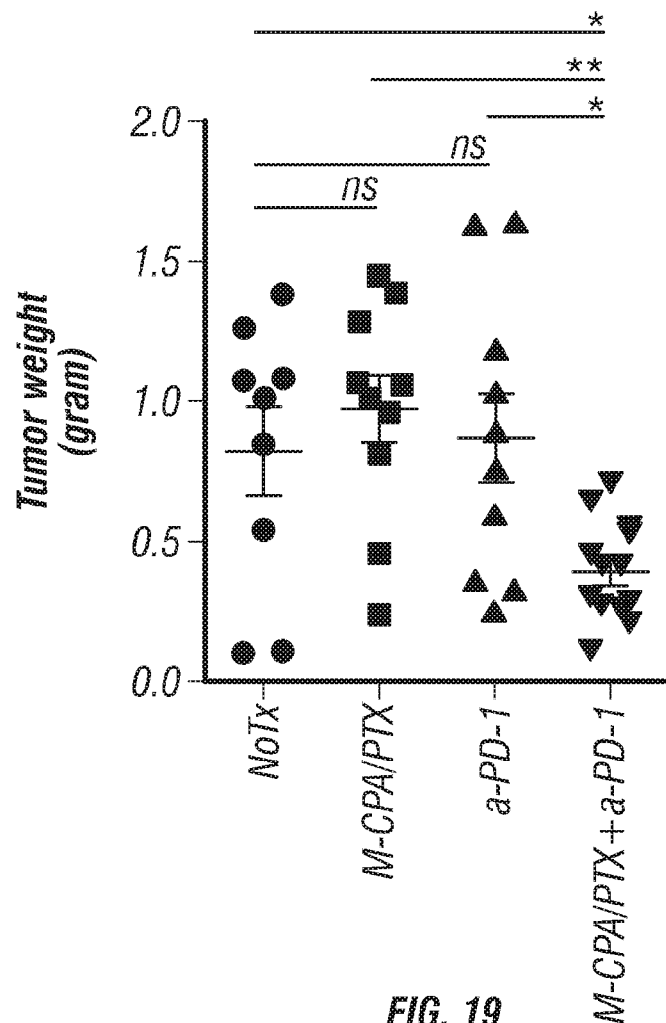


FIG. 19

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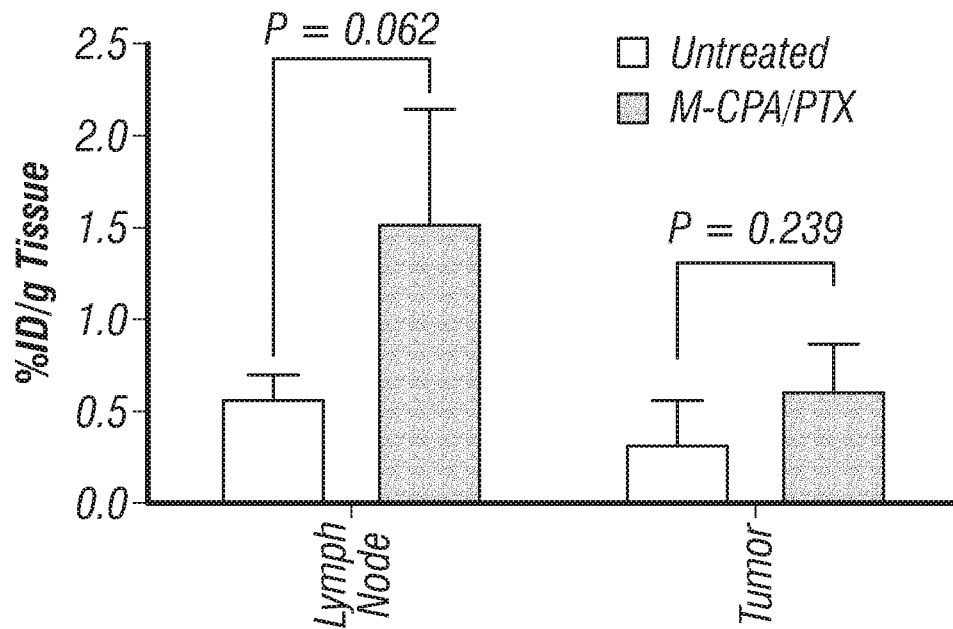


FIG. 20A

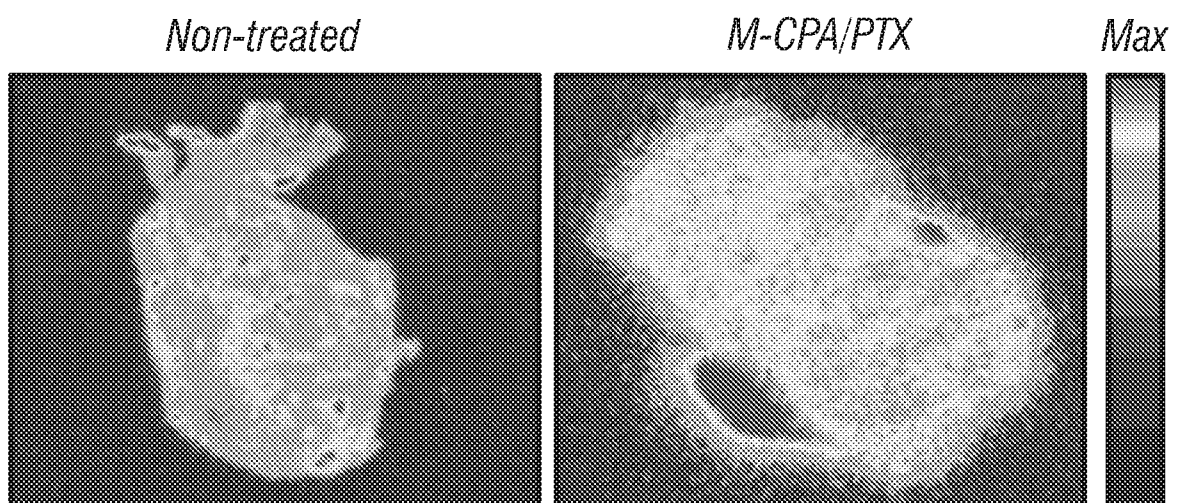
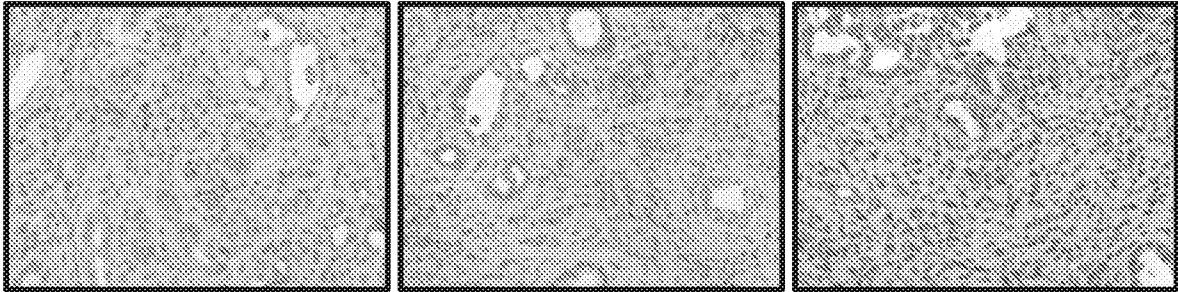
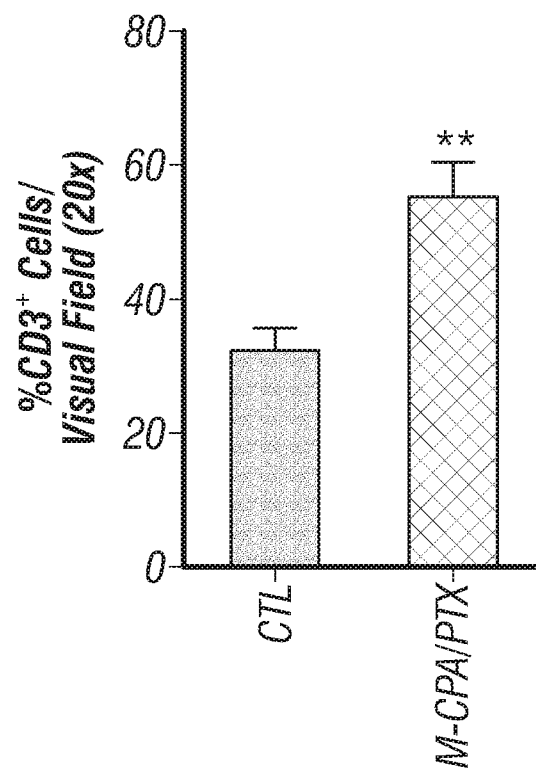
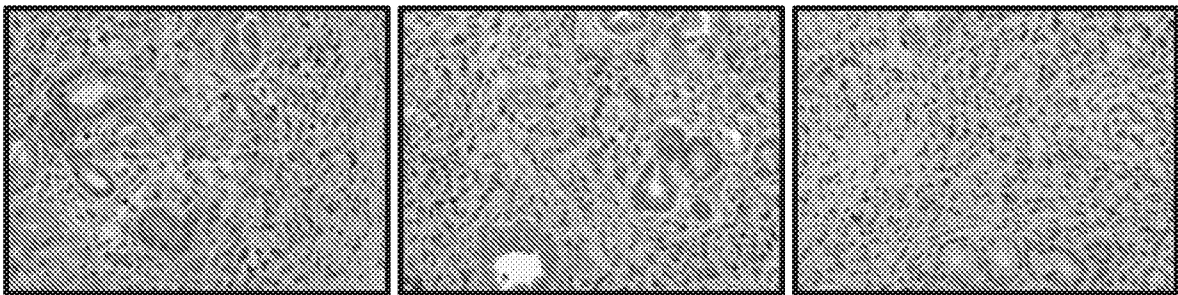


FIG. 20B

Untreated CTL*Treated (6 doses of M-CPA/PTX in 2 weeks)***FIG. 21**

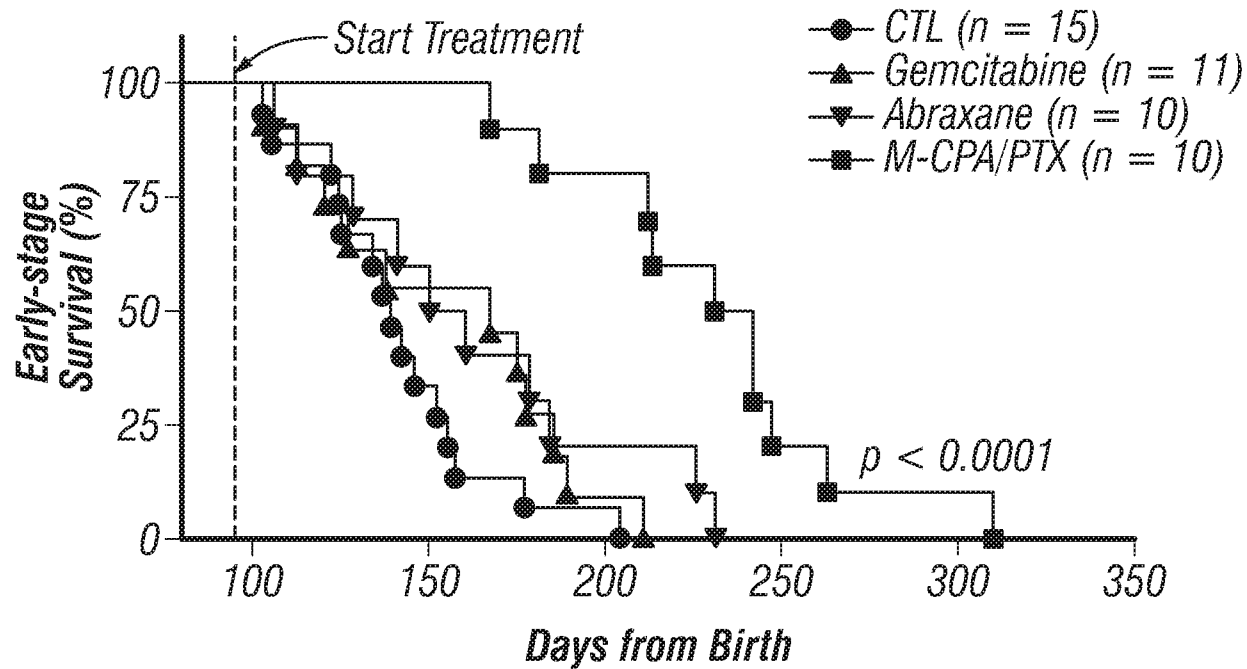


FIG. 22

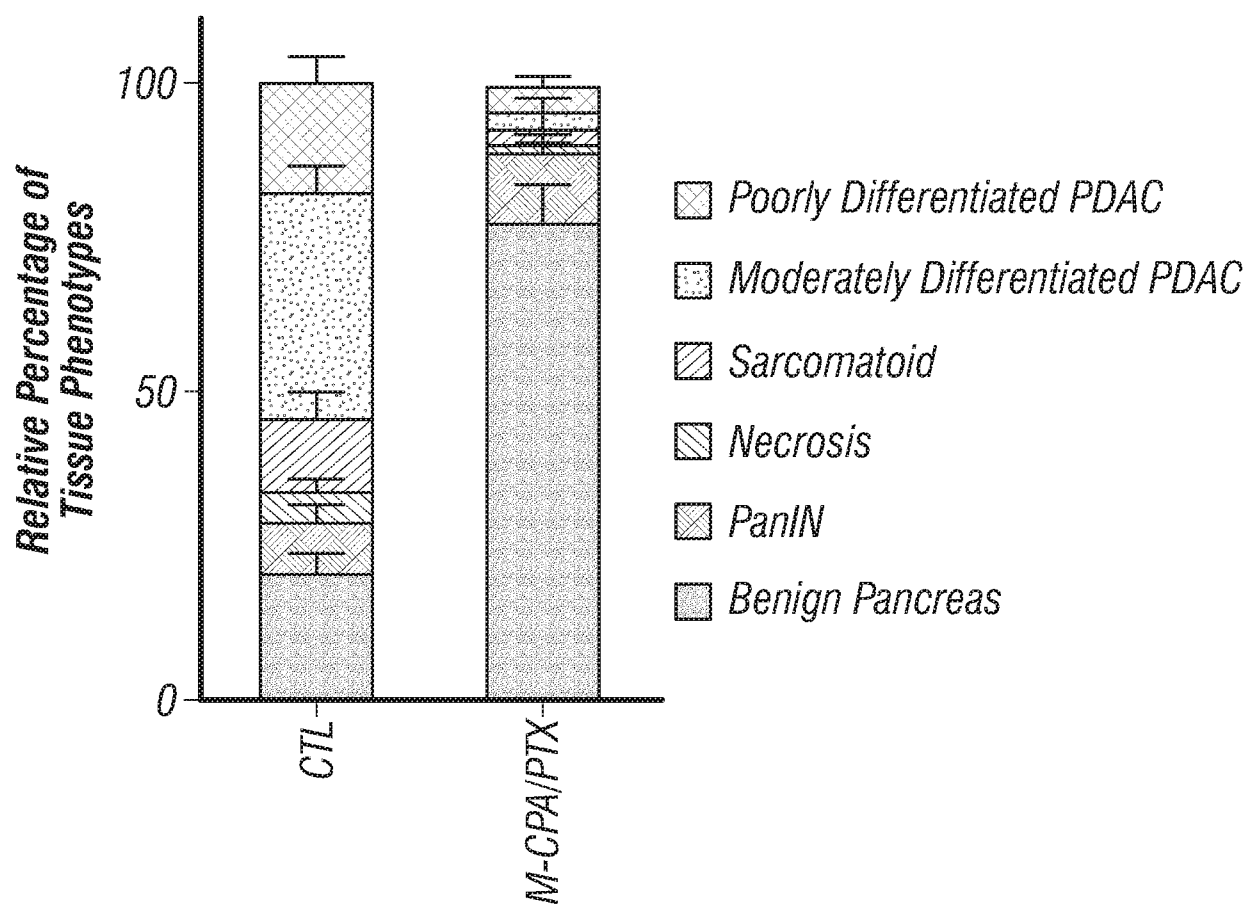


FIG. 23

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/65763

A. CLASSIFICATION OF SUBJECT MATTER
 IPC(8) - A61K 47/48, A61P 35/00, C08F 8/42 (2017.01)
 CPC - A61K 31/382, A61K 33/24, A61K 47/489

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 -- Y	CHEN et al. "Antibacterial polymeric nanostructures for biomedical applications", Chem. Commun., 2014. Vol. 50, pp 14482-14493, entire document, especially: pg 14487, Figure 7A, PEO-b-PCL-b-PTA triblock copolymer; pg 14487, Figure 8a, PMEO2MA-b-PTA diblock copolymer; pg 14489, col 1, Figure 10a	1-7, 9-11 ----- 8, 12, 14, 24, 33
Y	YOSHIDA et al. "pH- and ion-sensitive polymers for drug delivery", Expert Opin Drug Deliv. 2013. Vol. 10(11), pp 1497-1513, 28 pages, entire document, especially: pg 3, para 2, HPMC acetate succinate (HPMC-AS); pg 26, Table 2, Poly(methacrylic acid-co-methyl methacrylate), Edragit S	8
Y	DU et al. "Evaluation of Polymeric Micelles from Brush Polymer with Poly(E-caprolactone)-b-Poly(ethylene glycol) Side Chains as Drug Carrier", Biomacromolecules. 2009. Vol. 10, pp 2169-2174, entire document, especially: abstract; pg 2171, Scheme 1, PHEMA-g-(PCL-b-PEG)	12, 14, 24, 33

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

20 February 2017

Date of mailing of the international search report

13 APR 2017

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
 P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

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PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/65763

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.: 18-20, 35-79
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:
--See attached extra sheet--

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.: 1-12, 14 (in part), 24 and 33

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.

--Box III--

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Group I+: Claims 1-17 and 21-34 directed to a polymer, wherein the polymer is a block copolymer comprising a polyacrylic acid backbone with a plurality of PEG sidechains and a plurality of E-caprolactone sidechains. The claimed block copolymer will be searched to the extent that said polymer encompasses the polymer further defined by the formula I (claim 12); wherein R1 is alkyl(C1-18) R2 is a group of the formula indicated wherein: R3 and R3' are each independently hydrogen; and x is 1; m is 5; n is 10; p is 5; q is 1; y is 1; and Y1 is carboxy; or a pharmaceutically acceptable salt thereof. It is believed that claims 1-12, 14 (in part), 24 and 33 read on this first named invention, and thus these claims will be searched without fee to the extent that they encompass the polymer defined by the formula I (claim 12) as specified above. Applicant is invited to elect additional methods of improving the efficacy and/or reduce the side effects of the administration of a substituted hexitol derivative, wherein each method elected will require one additional invention fee. Applicants must specify the claims that encompass any additionally elected method. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the '+' group(s) will result in only the first claimed invention to be searched. Additionally, an exemplary election wherein different actual variables are selected is suggested. An exemplary election would be wherein said polymer encompasses the polymer further defined by the formula I (claim 12); wherein R1 is alkyl(C1-18) R2 is a group of the formula indicated wherein: R3 and R3' are each independently hydrogen; and x is 5; m is 5; n is 10; p is 5; q is 1; y is 1; and Y1 is carboxy; or a pharmaceutically acceptable salt thereof (i.e., claims 1-12, 14 (in part), 24 and 33).

The groups of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I+ includes the technical feature of a unique polymer of formula I, not required by any other invention of Group I+.

Common technical features:

The inventions of Group I+ share the technical feature of a core compound of formula I in claim 1

This shared technical feature, however, does not provide a contribution over the prior art, as being obvious over the article entitled "ChemInform Abstract: Antibacterial Polymeric Nanostructures for Biomedical Applications" by Chen, et al. published in August 2014 (hereinafter 'Chen'), which discloses a polymer which is highly similar to the polymer of formula I as specified above (pg 14487, Figure 8a), but Chen does not teach the oxygen atom at the end of the ethylene moiety on the acrylic acid monomer closest to the bromine atom in the backbone. However, it would have been obvious to a person having ordinary skill in the art to prepare the polymer with an oxygen atom instead of a nitrogen atom by routine experimentation in order to expand the range of substituents that can be attached to explore different properties and identify the preferred formulation.

As said methods were known in the art at the time of the invention these cannot be considered special technical features that would otherwise unify the inventions of Group I+

The inventions of Group I+ thus lack unity under PCT Rule 13.