



(51) International Patent Classification:

C12N 15/113 (2010.01) A61P 35/00 (2006.01)
A61K 48/00 (2006.01)

(21) International Application Number:

PCT/US2024/041812

(22) International Filing Date:

09 August 2024 (09.08.2024)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/531,993 10 August 2023 (10.08.2023) US

(71) Applicants: **GEORGETOWN UNIVERSITY** [US/US]; 37th & O Streets NW, Washington, District of Columbia 20057 (US). **THE U.S.A., as represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES** [US/US]; OTT-NIH, 6701 Rockledge Drive, Suite 700 MSC 7788, Bethesda, Maryland 20982-7788 (US).

(72) Inventors: **SMITH, Jill P.**; c/o Georgetown University, 37th & O Streets, N.W., Washington, District of Columbia 20057 (US). **STERN, Stephan**; c/o Leidos Biomedical Research Inc., 1050 Boyles Street, P.O. Box B, Frederick, Maryland 21702 (US). **KULARATNE, Ruwanthi**; c/o Leidos Biomedical Research Inc., 1050 Boyles Street, P.O. Box

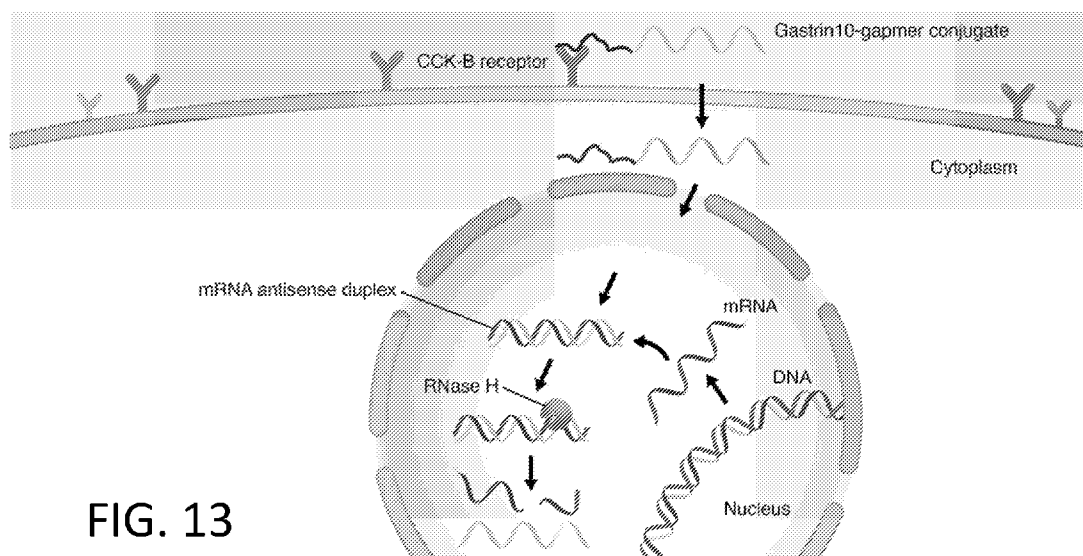
B, Frederick, Maryland 21702 (US). **SHIVAPURKAR, Narayan**; c/o Georgetown University, 37th & O Streets, N.W., Washington, District of Columbia 20057 (US).

(74) Agent: **THIREAULT, Caitlin A.** et al.; Klarquist Sparkman, LLP, One World Trade Center, 121 SW Salmon Street, Suite 1600, Portland, Oregon 97204-2988 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE,

(54) Title: LNA GAPMERS INHIBIT GROWTH OF CCK-BR POSITIVE CANCER



(57) Abstract: An antisense oligonucleotide (ASO) 14 to 20 nucleotides in length that specifically hybridizes with a gastrin mRNA molecule, wherein the ASO comprises at least one locked nucleic acid (LNA) nucleotide at each of the 5' and 3' termini.



SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
- *with sequence listing part of description (Rule 5.2(a))*

LNA GAPMERS INHIBIT GROWTH OF CCK-BR POSITIVE CANCER**CROSS REFERENCE TO RELATED APPLICATIONS**

This application claims the benefit of U.S. Provisional Application No. 63/531,993, filed August 10, 2023, which is incorporated by reference herein in its entirety.

FIELD

This relates to cancer therapies, particularly compositions and methods for the treatment of CCK-BR positive cancer, for example, pancreatic cancer.

ACKNOWLEDGMENT OF GOVERNMENT SUPPORT

This invention was made with government support under CA051008 and 75N91019D00024 awarded by the National Institutes of Health. The government has certain rights in the invention.

INCORPORATION OF ELECTRONIC SEQUENCE LISTING

The Sequence Listing is submitted as an XML file named "Sequence.xml," created on August 8, 2024, (15,709 bytes), which is incorporated herein by reference.

BACKGROUND

The incidence of pancreatic cancer is increasing at an alarming rate. Pancreatic cancer is expected to become the second leading cause of cancer-related deaths within the next 10 years. Pancreatic cancer is considered an immune 'cold tumor,' and does not respond to immune-based therapies. Chemotherapy is the only approved treatment for advanced pancreatic cancer but it suffers from toxicity and poor response. Thus, novel strategies are needed to improve survival of this malignancy.

SUMMARY

Gastrin is made by cancerous cells and is thought to stimulate cancer growth by autocrine fashion through the cholecystokinin-B receptor (CCK-BR). Prior studies have shown that down regulation of gastrin decreases growth of pancreatic cancer. Disclosed herein are antisense oligonucleotides (ASOs) that specifically hybridize with gastrin mRNA. The ASOs disclosed herein include at least one locked nucleic acid (LNA) nucleotide at each of the 5' and 3' termini. In some aspects, the ASOs disclosed herein include a phosphorothioate backbone and/or further comprise a cholecystokinin-B receptor (CCK-BR) targeting moiety coupled to the ASO. The CCK-BR targeting moiety facilitates targeting of the ASOs to pancreatic cancer cells, for example, by binding to CCK-BR. In some examples, the CCK-BR targeting moiety is a gastrin peptide or DNA aptamer that specifically binds to CCK-BR.

The ASOs disclosed herein are useful for treating CCK-BR positive cancer, for example, pancreatic cancer, colon cancer, hepatocellular (liver) cancer, gastric cancer, esophageal cancer, small cell lung cancer,

or medullary thyroid cancer. Pharmaceutical compositions including an ASO disclosed herein and a pharmaceutically acceptable carrier are also disclosed.

Further disclosed are methods of treating a CCK-BR positive cancer or inhibiting metastasis of a CCK-BR positive cancer in a subject, including administering to the subject a therapeutically effective amount of an ASO or pharmaceutical composition disclosed herein. In some aspects, a subject having pancreatic cancer, colon cancer, hepatocellular (liver) cancer, gastric cancer, esophageal cancer, small cell lung cancer, or medullary thyroid cancer is selected for treatment.

The foregoing and other features of this disclosure will become more apparent from the following detailed description of several aspects which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1A-1B: FIG. 1A is a diagram of an exemplary locked nucleic acid (LNA) Gapmer, which includes antisense oligonucleotide DNA flanked by at least one LNA nucleotide on the 3' and 5' end. FIG. 1B shows *in vitro* transfection of two Gapmers targeting an endogenous normalizing gene in human cells (GAPDH).

FIG. 2: Top 11 LNA Gapmers targeting gastrin mRNA and their molecular characteristics.

FIGS. 3A-3F: Effects of anti-gastrin LNA Gapmers on gastrin mRNA expression and cell proliferation in human pancreatic cancer cells *in vitro* using lipofectamine as a transfection reagent. FIG. 1A: Six Gapmers (Gapmers 29, 31, 33, 82, 92, and 90) significantly decreased gastrin expression in AsPC-1 pancreatic cancer cells compared to control cells as determined by qRT-PCR. FIG. 3B: Five anti-gastrin Gapmers (30, 32, 93, 95, and 96) did not statistically decrease gastrin expression in AsPC-1 cells. FIG. 3C: Gapmers that decreased gastrin expression also significantly decreased growth of AsPC-1 cells. FIG. 3D: Gapmers that did not decrease gastrin expression did not have a significant effect on AsPC-1 cell growth. FIG. 3E: Gapmer-90 also significantly decreases gastrin gene expression and cell growth in another human pancreatic cancer cell line, BxPC-3 cancer cells, as compared to controls. FIG. 3F: Treatment of human PANC-1 pancreatic cancer cells with Gapmer-90 significantly decreased cell growth. Significantly different from controls: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$; and **** $P < 0.0001$. ns= not significant.

FIGS. 4A-4D: Cell viability as determined by MTT assay. FIG. 4A: Compared to a scrambled Gapmer negative control, Gapmer-90 decreased growth of human pancreatic cancer AsPC-1 cells. FIG. 4B: Gapmer-90 also decreased growth of human BxPC-3 cells by MTT assay. FIG. 4C: Effects of Gapmer 29 and 90 on growth compared to untreated controls. FIG. 4D: Gapmer 33 inhibits growth but the two Gapmers that did not knock down gastrin mRNA (Gapmers 30 and 32) did not inhibit AsPC-1 growth *in vitro*.

FIGS. 5A-5B: FIG. 5A shows a schematic showing conjugation of a targeting peptide by maleimide-thiol click chemistry to a Gapmer. FIG. 5B shows the structure of an exemplary Gapmer-peptide conjugate (Gapmer-90).

FIGS. 6A-6B: Modification of LNA Gapmer-90 to render it target-specific for the CCK-B receptor. FIG. 6A: Compared to control AsPC-1 pancreatic cancer cells, both the untargeted Gapmer-90 with

Lipofectamine and the Targeted Gapmer-90 without Lipofectamine decrease gastrin mRNA expression.

FIG. 6B: Application of the CCK-B receptor target specific Gapmer-90 to AsPC-1 cells in culture without a transfection reagent show significant decreased growth by MTT assay. **P<0.01.

FIGS. 7A-7B: Gapmer-90 treatment reduces AsPC-1 pancreatic cancer tumor size. FIG. 7A: Final tumor mass in grams at necropsy. FIG. 7B: Representative images from abdominal MRI from mouse from each cohort. The tumor in the control mouse is visibly larger than the tumors in the proglumide-treated or Gapmer-treated mice.

FIGS. 8A-8D: Gapmer-90 treatment reduces metastasis. FIG. 8A: Mean number of metastases per treatment group. There were significantly fewer metastases in the proglumide and Gapmer-90 treated mice compared to controls. FIG. 8B: Metastases were confirmed by histology. AsPC-1 cancer seen invading the kidney capsule. FIG. 8C: Cancer is seen metastasized and invading the mesentery. FIG. 8D: Cancer metastases is seen invading the mouse spleen.

FIGS. 9A-9C: RT-PCR of selective gene mRNA expression from the tumors. FIG. 9A: Gastrin expression is significantly decreased in tumors treated with the LNA Gapmer-90 confirming the Gapmer successfully down-regulated its target gene of interest. FIG. 9B: Transcription factor *ZEB1*, a known regulator of epithelial to mesenchymal transition (EMT) was decreased by both Gapmer-90 and proglumide which may account for the fewer metastases. FIG. 9C: Another EMT transcription factor *ZEB2* was also decreased but did not reach statistical significance. *P<0.05; ** P<0.01; ****P<0.001; ns= not significant.

FIGS. 10A-10K: Effects of LNA Gapmer-90 on growth and metastases of human pancreatic cancer *in vivo*. FIG. 10A: One week after orthotopic tumor implantation, luciferase positive tumors were measured by IVIS and mice were divided into one of four groups with equal bioluminescence as baseline. FIG. 10B: A representative mouse bearing a pancreatic orthotopic tumor shows bioluminescence with luciferase at baseline. FIG. 10C: Final tumor weights in grams at necropsy for each treatment group compared to PBS-treated control mice. FIG. 10D: Number of pancreatic cancer metastases (mean $\pm$ SEM) per mouse in each treatment group compared to controls. FIG. 10E: Representative histologic sections from metastases of various tissues are shown; including spleen, liver, mesentery, intestine, kidney and the primary tumor in the pancreas. Arrows point to the cancer. FIG. 10F: Relative mRNA gastrin expression within tumors as determined by qRT-PCR is shown. FIG. 10G: Relative mRNA expression of transcription factor *ZEB1* as determined by qRT-PCR is shown. FIG. 10H: Relative mRNA expression of transcription factor *ZEB2* as determined by qRT-PCR is shown. FIG. 10I: Serum chemistries (mean $\pm$ SEM) obtained at necropsy for N=10 mice in each treatment group. Columns represent the mean $\pm$ SEM for each treatment group. FIG. 10J: Number of metastases and location per treatment group. FIG. 10K: Quantitative measurement of Gapmer uptake into tumors using a complementary probe. ns = not significant. Significant compared to controls *P<0.05; **P<0.01; ***P<0.005; and ****P<0.001.

FIGS. 11A-11F: LNA Gapmers decrease pancreatic tumor fibrosis, tumor-associated macrophages, and Ki67 index. FIG. 11A: Representative photos from each treatment group stained for intratumoral fibrosis with Masson's trichrome stain. FIG. 11B Quantitative analysis of amount of fibrosis in tumors from

each treatment group is shown. FIG. 11C: Representative images of immunohistochemistry for M2-polarized arginase positive tumor-associated macrophages is shown. FIG. 11D: Quantitative analysis of the number of arginase+ immunoreactive cells per high powered field is shown. FIG. 11E: Representative images are shown from each group of immunohistochemistry of tumors for proliferative index with Ki67 staining. FIG. 11F: Quantitative analysis of the number of immunoreactive Ki67 cells per high powered field is shown. Columns represent the mean $\pm$ SEM for each treatment group. ns = not significant. Significant compared to controls **P<0.01; ***P<0.005; and ****P<0.001.

FIG. 12: An exemplary *in-vivo* experiment. One week after orthotopically injecting 10^6 human pancreatic cancer cells into female athymic nude mice, bioluminescence imaging is performed with IVIS to assure equal tumor size before treatment is started. PBS control or LNA Gapmers are injected intraperitoneally twice weekly for 4 weeks. At the end of week 4, blood is collected for toxicity studies, tumors are excised, and organs are collected for metastases analysis and *ex vivo* studies.

FIG. 13: A proposed mechanism for internalization of CCK-BR targeted Gapmers.

SEQUENCES

The nucleic and amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, and single letter code for amino acids, as defined in C.F.R. 1.822. It is understood that symbol "T" refers to uracil in RNA molecules. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand. In the accompanying sequence listing:

SEQ ID NO: 1 is the nucleotide sequence of Gapmer-90 (test89). TAAGGGTGCATCTGGC

SEQ ID NO: 2 is the nucleotide sequence of Gapmer-29 (test29). AGCCAGTGCAAAGATC

SEQ ID NO: 3 is the nucleotide sequence of Gapmer-33 (test33). CCAGAGCCAGTGCAAA

SEQ ID NO: 4 is an exemplary mRNA sequence encoding human gastrin.

AGATGCAGCGACTATGTGTGTATGTGCTGATCTTTGCACTGGCTCTGGCCGCCTTCTCTGAAGC
TTCTTGGAAGCCCCGCTCCCAGCAGCCAGATGCACCCTTAGGTACAGGGGCCAACAGGGACCT
GGAGCTACCTGGCTGGAGCAGCAGGGCCCAGCCTCTCATCATCGAAGGCAGCTGGGACCCCA
GGGTCCCCACACCTCGTGGCAGACCCGTCCAAGAAGCAGGGACCATGGCTGGAGGAAGAAG
AAGAAGCCTATGGATGGATGGACTTCGGCCGCCGCAGTGCTGAGGATGAGAACTAA

SEQ ID NO: 5 is an exemplary amino acid sequence of a CCK-BR targeting moiety (Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe). EEEAYGWMDF

SEQ ID NO: 6 is an amino acid sequence of human gastrin.

MQRLCVYVLIFALALAAFSEASWKPRSQQPDAPLGTGANRDLELPWLEQQGPASHHRRQLGPQGP
PHLVADPSKKQGPWLEEEEEAYGWMDFGRRSAEDEN

SEQ ID NOs: 7-14 are sequences for Gapmer 30, 31, 32, 92, 93, 95, 96, and 82, respectively (*see*, FIG. 2).

SEQ ID NO: 15 is an exemplary sequence of human gastrin mRNA.

AGAGACCTGAGAGGCCACCAGGCCAGCCGTGGCACCACACACCTCCCAGCTCTGCAGACGAGA
 TGCAGCGACTGTGTGTGTATGTGCTGATCTTTGCACTGGCTCTGGCCGCCTTCTCTGAAGCTTCT
 TGGAAGCCCCGCTCCCAGCAGCCAGATGCACCCTTAGGTACAGGGGCCAACAGGGACCTGGAG
 CTACCCTGGCTGGAGCAGCAGGGCCCCAGCCTTCATCATCGAAGGCAGCTGGGACCCCAGGGT
 5 CCCCCACACCTCGTGGCAGACCCGTCCAAGAAGCAGGGACCATGGCTGGAGGAAGAAGAAGA
 AGCCTATGGATGGATGGACTTCGGCCGCCGCACTGCTGAGGATGAGAACTAACAATCCTAGAA
 CCAAGCTTCAGAGCCTAGCCACCTCCCACCCCACTCCAGCCCTGTCCCCTGAAAACTGATCAA
 AAATAAACTAGTTTCCAGTGGA

10 **SEQ ID NO: 16** is an exemplary DNA aptamer that targets CCK-BR (AP1153).

CATGGTGCAGGTGTGGCTGGGATTCAATTTGCCGGTGCTGGTGCGTCCGCGGCCGCTAATCCTGT
 TC

DETAILED DESCRIPTION

15 I. Introduction

“Gapmers” are synthetic, single-stranded antisense oligonucleotides (ASOs) that are typically 16–20 nucleotides long with a central stretch (or gap) of 8–13 DNA monomers flanked by one or more “locked nucleic acids” (LNAs). Sugar modifications present in LNA increases nuclease resistance, protects the ends of the gapmers, avoids or reduces immune responses to gapmers, and increases binding affinity to
 20 complementary sequences. Due to the stability and long half-life of gapmers *in vivo*, these oligonucleotides are being developed for *in vivo* gene modification.

Here, ninety-six gapmers were designed targeting different sites of gastrin mRNA (human). The efficacy of several gapmers were tested, including, Gapmer-29, Gapmer-30, Gapmer-31, Gapmer-32, Gapmer-33, Gapmer-82, Gapmer-90, Gapmer-92, Gapmer-93, Gapmer-95, and Gapmer-96. It is shown that
 25 several Gapmers successfully knock down gastrin mRNA and decrease cancer cell growth in one or more pancreatic cancer cell lines. Interestingly, the Gapmers were found to be highly specific and varying the sequence by a single nucleotide, in some instances, could result in a loss of activity. For example, Gapmers-29 and 31 significantly decreased gastrin expression and growth of pancreatic cancer cells *in vitro*, yet Gapmer-30 (designed with a sequence shift of one nucleotide) was not effective.

30 Three separate human pancreatic cancer cell lines were used for *in vitro* testing and two for *in vivo*. These cell lines were selected since they range in surface membrane expression of CCK-BR, with PANC-1 having the highest number of receptors (283 ± 68 fmol/mg protein; as determined by radioactive receptor ligand assay), followed by BxPC-3 (125 ± 44 fmol/mg protein; as determined by radioactive receptor ligand assay), and finally AsPC-1 (as determined by qRT-PCR). AsPC-1, however, has the highest gastrin
 35 expression, followed by BxPC-3 and PANC-1. The varying expression of gastrin mRNA and the range in the quantity of CCK-BR is expected to be representative of what may be found in human subjects with pancreatic cancer.

Although LNA Gapmers can effectively penetrate the cell membranes to downregulate selective genes *in vivo*, it was hypothesized that efficacy could be enhanced and off-target toxicity diminished by
 40 selectively targeting gastrin receptor CCK-BR, which is specifically expressed on cancerous cells. One gapmer (Gapmer-90), the most potent Gapmer tested *in vitro*, was conjugated to a 10 amino acid peptide of

gastrin to achieve specific targeting of pancreatic cancer cells through CCK-BR. The CCK-BR targeted Gapmer-90 is shown herein to decrease growth and metastases of human pancreatic tumors in mice, as well as decrease tumoral Ki67 proliferative index scores. It was further confirmed that this modification of Gapmer-90 increased the concentration of Gapmer measured within the tumors as compared to untargeted Gapmers of the same concentration. The effect of the anti-gastrin Gapmer on pancreatic tumor metastases was striking. Without being bound by any particular theory, one mechanism that could account for the decreased metastasis is inhibition of transcription factors regulating epithelial to mesenchymal transition (EMT), *e.g.*, *ZEB1* and *ZEB2*. Gastrin has also been shown to increase β -catenin and VEGF-A expression in cancers, thus promoting the EMT.

An interesting finding was the effect of LNA Gapmers on pancreatic tumoral fibrosis. Without being bound by any particular theory, one mechanism that could account for decreased fibrosis in the tumors may be related to decreasing cross-communication between the cancer epithelial cells and cancer-associated fibroblasts. CCK receptors have been described on pancreatic stellate cells and stimulation of these receptors produces collagen in the extracellular matrix. Since we also observed a moderate decrease of fibrosis in the tumors of the mice treated with the untargeted Gapmer, the decreased fibrosis may be secondary to the inhibition of endogenous gastrin production from the cancer epithelial cells rather than blockade of the CCK-B receptors on the fibroblasts. In addition, there was a decrease of immunosuppressive M2-polarized tumor associated macrophages in the tumor microenvironment. This decrease may be contributed to decreased primary tumor size and/or remodeling of the tumor microenvironment with less fibrosis.

The Gapmers disclosed herein are a form of "Precision Medicine" as they specifically target a gene that is over-expressed in CCK-BR positive cancers (*e.g.*, pancreatic cancer). Precision medicine and genomic profiling target-specific therapy directed to cancer-cell receptors has improved the outcome of many recalcitrant cancers. Pancreatic cancer has a dismal prognosis with a 5-year survival of just 11% and even with the best chemotherapy regimens; the median survival is still typically less than one year for most subjects. Thus, there is a desperate need for novel cancer therapeutics to improve survival of this devastating disease. The therapy disclosed herein is stable, has a long duration of action, and limited off-target toxicity. Moreover, manufacturing Gapmers is much less complex than monoclonal antibodies, CAR-T cells, or other biological agents, and therefore offers a promising treatment strategy for improving the survival of pancreatic cancer.

II. Summary of Terms

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of many common terms in molecular biology may be found in Krebs *et al.* (eds.), *Lewin's genes XII*, published by Jones & Bartlett Learning, 2017. As used herein, the singular forms "a," "an," and "the," refer to both the singular as well as plural, unless the context clearly indicates otherwise. For example, the term "an oligonucleotide" includes singular or plural oligonucleotides and can be considered equivalent to the phrase "at least one oligonucleotide." As used herein the term "about" refers to a range of 5% of a reference value

(e.g., “about 100” refers to the range of 95 to 105), unless otherwise indicated. As used herein, the term “comprises” means “includes.” It is further to be understood that any and all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for descriptive purposes, unless otherwise indicated. Although many methods and materials similar or equivalent to those described herein can be used, particular suitable methods and materials are described herein. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. To facilitate review of the various aspects, the following explanations of terms are provided:

Administration: To provide or give a subject an agent, such as a therapeutic agent (e.g., an ASO or pharmaceutical composition disclosed herein) by any effective route. Exemplary routes of administration include, but are not limited to, injection or infusion (e.g., subcutaneous, intramuscular, intradermal, intraperitoneal, intrathecal, intravenous, intracerebroventricular, intrastriatal, intracranial, or into the spinal cord), oral, intraductal, sublingual, rectal, transdermal, intranasal, and inhalation routes.

Antisense oligonucleotide (ASO): A synthetic, single-stranded nucleic acid-based oligomer that is complementary to a target nucleic acid molecule, such as a target RNA. An antisense oligonucleotide can include one or more chemical modifications to the sugar, base, and/or internucleoside linkages. Exemplary modifications to the sugar include, but are not limited to, LNA, 2'-O-methyl, 2'-O-methoxy-ethyl (MOE), 2'-fluoro, cEt and tc-DNA. Modifications to the internucleoside linkages include, for example, phosphorothioate and phosphoramidate. One example of a modified base is 5-methylcytosine.

Complementarity: The ability of a nucleic acid to form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick base pairing or other non-traditional types. A percent complementarity indicates the percentage of residues in a nucleic acid molecule that can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9, and 10 out of 10 being 50%, 60%, 70%, 80%, 90%, and 100% complementarity, respectively).

Contacting: Placing an agent in direct physical association, including both in solid and liquid form, and can take place either *in vivo* or *in vitro*. Contacting includes contacting a cell by placing an agent (e.g., an ASO or pharmaceutical composition disclosed here) in direct physical association with the cell.

Control: A reference standard. A control can be a positive or negative control. In some examples, the control is a measurement (e.g., a tumor measurement, or quantification of gene expression) obtained prior to administering an agent to a subject or cell (e.g., pharmaceutical composition disclosed herein). In some examples, the control is a historical control or a standard reference or range (e.g., a typical measurement or range observed for a particular population, for example, a human population either having pancreatic cancer or not having pancreatic cancer).

Degenerate variant: A nucleic acid sequence variant due to degeneracy (redundancy) of the genetic code. While the nucleic acid sequence of a degenerate variant differs from a reference sequence, the degenerate variant and reference sequence encode the same amino acid sequence.

Exogenous: Originating from outside a reference organism source. For example, a nucleic acid molecule that is exogenous to a cell is a nucleic acid that originated from a source other than the cell itself (*e.g.*, an artificial nucleic acid, such as a recombinant nucleic acid).

Hybridization or hybridize: The binding of a nucleic acid molecule to another nucleic acid molecule, for example the binding of an ASO to another nucleic acid, such as a gastrin mRNA, thereby forming a duplex molecule. The ability of one nucleic acid molecule to bind to another nucleic acid molecule can depend upon the complementarity between the nucleotide sequences of two nucleic acid molecules, and the stringency of the hybridization conditions. Methods of performing nucleic acid hybridization are known (*see, e.g.*, Green and Sambrook (2012) *Molecular Cloning: A Laboratory Manual*, fourth edition, Cold Spring Harbor Laboratory, Plainview, NY; ISBN 978-1-936113-42-2).

In some aspects, moderately stringent hybridization conditions are when the hybridization is performed at about 42°C in a hybridization solution containing 25 mM KPO₄ (pH 7.4), 5X SSC, 5X Denhart's solution, 50 µg/mL denatured, sonicated salmon sperm DNA, 50% formamide, 10% dextran sulfate, and 1-15 ng/mL oligonucleotide (about 5x10⁷ cpm/µg), while the washes are performed at about 50°C with a wash solution containing 2X SSC and 0.1% sodium dodecyl sulfate. In some aspects, highly stringent hybridization conditions include when the hybridization is performed at about 42°C in a hybridization solution containing 25 mM KPO₄ (pH 7.4), 5X SSC, 5X Denhart's solution, 50 µg/mL denatured, sonicated salmon sperm DNA, 50% formamide, 10% dextran sulfate, and 1-15 ng/mL oligonucleotide (about 5x10⁷ cpm/µg), while the washes are performed at about 65°C with a wash solution containing 0.2X SSC and 0.1% sodium dodecyl sulfate.

Increase or Decrease: A positive or negative change, respectively, relative to a reference value (*e.g.*, a control). A difference can be a qualitative or quantitative. In some examples, the difference is statistically significant (*e.g.*, P-value less than 0.05 or 0.01). In some examples, the difference is an increase relative to a control of at least 5%, such as at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 150%, at least 200%, at least 250%, at least 300%, at least 350%, at least 400%, at least 500%, or greater than 500%. In some examples, the difference is a decrease relative to a control of at least 5%, such as at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or 100%.

Inhibiting or treating a disease: Treatment refers to a therapeutic intervention that ameliorates a sign or symptom of a disease or condition (*e.g.*, pancreatic cancer) after the disease or condition has begun to develop. The term "ameliorating," with reference to a disease or condition, refers to any observable beneficial effect of the treatment. The beneficial effect can be evidenced, for example, by a delayed onset of symptoms in a subject, a reduction in severity of some or all symptoms (*e.g.*, number and/or size of tumors), a slower progression, an improvement in the quality of life of the subject, or by other parameters that are specific to the particular disease or condition. Treatment may be assessed by objective or subjective parameters, including the results of a physical examination, clinical tests, or self-evaluations by the subject.

Inhibiting a disease or condition includes decreasing risk of the disease or condition, for example, decreasing the risk of developing cancer or cancer metastasis. A “prophylactic” treatment is a treatment administered to a subject who does not exhibit signs of a disease, or exhibits only early signs, for the purpose of decreasing the risk of further developing a disease or condition. In some examples, the disclosed methods are prophylactic and/or therapeutic. In some examples, the disclosed methods are therapeutic and not prophylactic.

Isolated or Purified: An “isolated” or “purified” biological component (such as a nucleic acid or protein) is one that has been substantially separated from other biological components in the environment in which the component occurs, *e.g.*, separated from other chromosomal and extra-chromosomal DNA and RNA, proteins and/or cells. Nucleic acids and proteins that have been “isolated” include nucleic acids and proteins purified by standard purification methods. The term embraces nucleic acids and proteins prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids.

Absolute purity or isolation is not required, it is intended as a relative term. Thus, for example, a purified/isolated protein, nucleic acid, or cell preparation is one in which the protein, nucleic acid, or cell is more enriched than the protein, nucleic acid, or cell is in its initial environment. In one example, a preparation is purified/isolated such that the protein, nucleic acid, or cell represents at least 50% of the total content of the preparation. In some examples, an isolated nucleic acid (or vector) is at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% pure. In a specific, non-limiting example, an isolated nucleic acid (or vector) is 90% free of other components.

Locked nucleic acid (LNA): A modified nucleotide. The ribose moiety of an LNA nucleotide is modified with an extra bridge connecting the 2' oxygen and the 4' carbon.

Pharmaceutically acceptable carriers: The pharmaceutically acceptable carriers of use in this disclosure are conventional. *Remington's Pharmaceutical Sciences*, 23rd Edition, Academic Press, Elsevier, (2020) describes compositions and formulations suitable for pharmaceutical delivery of ASOs and other compositions disclosed herein. In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (such as powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

Recombinant: A molecule made by artificially combining two molecules that do not naturally occur together (*e.g.*, a “chimera”).

Sequence identity: The degree of similarity between amino acid or nucleic acid sequences. Sequence identity is frequently measured in terms of percentage identity (or percent identity); the higher the percentage,

the more similar the two sequences are. Homologs of a polypeptide (or nucleotide sequence) will possess a relatively high degree of sequence identity when aligned using standard methods.

Methods of alignment of sequences for comparison have been described. The NCBI Basic Local Alignment Search Tool (BLAST®) tool is often used and is available from several sources, including the National Center for Biotechnology Information (blast.ncbi.nlm.nih.gov/Blast.cgi). Various types of BLAST® are available, for example, blastp, blastn, blastx, tblastn, tblastx, and psiblast. A description of how to determine sequence identity using this program is available on the NCBI website and other resources. In some examples, percent sequence identity is determined using default query parameters, for example, parameters provided below is Table 1.

Table 1: Exemplary default parameters for BLAST programs.

	BLASTN	BLASTP	BLASTX	TBLASTN	TBLASTX	PSIBLAST
Gap Opening Penalty	5	11	11	11	11	5
Gap Extension Penalty	2	1	1	1	1	2
Nucleic Match	1	n/a	n/a	n/a	n/a	1
Nucleic Mismatch	-3	n/a	n/a	n/a	n/a	-3

Subject: Any mammal, such as humans, non-human primates, domestic dogs, domestic cats, rodents, pigs, sheep, cows, and the like which is to be the recipient of a treatment provided herein. In some examples, the subject is a veterinary subject (*e.g.*, non-human primate, domestic cat, domestic dog, mouse, or rat). In a non-limiting example, the subject is a human subject, for example, one with pancreatic cancer.

Therapeutically effective amount: The amount of a therapeutic agent (*e.g.*, a pharmaceutical composition disclosed herein) sufficient to achieve a desired effect (*e.g.*, decrease in tumor load or inhibition of metastasis) in a subject being treated with the agent. In some examples, the desired effect is decreasing tumor volume, weight, and/or metastasis. In some examples, the desired effect is decreasing expression of gastrin, *ZEB1*, and/or *ZEB2* in tumor cells. The exact amount and timing of administration can depend on a number of factors, including the subject being treated, the severity and type of the condition being treated, and the manner of administration. A therapeutically effective amount can be determined by a practitioner, for example, through clinical trials or various *in vitro*, *in vivo* or *in situ* assays.

III. Antisense Oligonucleotides

Disclosed are antisense oligonucleotides (ASOs) that specifically hybridize with gastrin mRNA. An ASO that specifically binds gastrin mRNA preferentially binds gastrin mRNA with no or insignificant binding to off-target mRNA. In some examples, the ASO specifically binds human gastrin mRNA. In some aspects, the disclosed ASOs include at least one locked nucleic acid (LNA) nucleotide at each of the 5' and 3' termini of the ASO.

The ASOs disclosed herein are 12 to 30 nucleotides in length, for example, 12 to 25, 12 to 20, 12 to 15, 14 to 30, 14 to 25, 14 to 20, 14 to 18, 14 to 17, 14 to 16, 15 to 30, 15 to 25, 15 to 20, 15 to 18, 15 to 17, 15 to 16, 16 to 30, 16 to 25, 16 to 20, or 16 to 18. In some aspects, an ASO disclosed herein is 15 to 17 nucleotides in length. In some aspects, an ASO disclosed herein is 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length. In some aspects, an ASO disclosed herein is 16 nucleotides in length (*e.g.*, a “16mer”).

The nucleotide sequences of the ASOs disclosed herein are complementary to gastrin mRNA (*e.g.*, the ASO sequence is antisense relative to a sequence of gastrin mRNA, thereby allowing hybridization of the ASO to gastrin mRNA). 100% complementarity to gastrin mRNA is not required, only sufficient complementarity to achieve specific hybridization with gastrin mRNA (*e.g.*, preferential binding to gastrin mRNA with no or insignificant off-target binding). In some examples, an ASO disclosed herein has at least 80% (*e.g.*, 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) complementarity to a gastrin mRNA. In some examples, an ASO disclosed herein has at least 93% or 95% complementarity to a gastrin mRNA. In a further example, an ASO disclosed herein has 100% complementarity to a gastrin mRNA.

Gastrin mRNA sequences have been described and are publicly available, *e.g.*, *see*, NCBI Reference Sequence: NM_000805.5 (human gastrin; ncbi.nlm.nih.gov/nuccore/NM_000805.5); *see also*, NCBI Gene ID: 2520 (human gastrin; ncbi.nlm.nih.gov/gene/2520). Exemplary mRNA sequences encoding human gastrin are also disclosed herein (*see*, SEQ ID NOS: 4 and 15). In some aspects, the ASOs disclosed herein are complementary to a mammalian gastrin mRNA. In a non-limiting example, the ASOs disclosed herein are complementary to a human gastrin mRNA.

Exemplary sequences of human gastrin mRNA are provided herein as SEQ ID NOS: 4 and 15. In some examples, an ASO disclosed herein specifically hybridizes to SEQ ID NO: 4 or 15, or a degenerate variant thereof. In some examples, an ASO disclosed herein specifically hybridizes to a 12 to 30 (*e.g.*, 12 to 25, 12 to 20, 12 to 15, 14 to 30, 14 to 25, 14 to 20, 14 to 18, 14 to 17, 14 to 16, 15 to 30, 15 to 25, 15 to 20, 15 to 18, 15 to 17, 15 to 16, 16 to 30, 16 to 25, 16 to 20, or 16 to 18) contiguous nucleotide sequence that has at least 80% sequence identity (*e.g.*, 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) to SEQ ID NO: 4 or 15. In some examples, an ASO disclosed herein specifically hybridizes to a 12 to 30 (*e.g.*, 12 to 25, 12 to 20, 12 to 15, 14 to 30, 14 to 25, 14 to 20, 14 to 18, 14 to 17, 14 to 16, 15 to 30, 15 to 25, 15 to 20, 15 to 18, 15 to 17, 15 to 16, 16 to 30, 16 to 25, 16 to 20, or 16 to 18) contiguous nucleotide sequence that has at least 93% sequence identity to SEQ ID NO: 4 or 15. In some examples, an ASO disclosed herein specifically hybridizes to a 12 to 30 (*e.g.*, 12 to 25, 12 to 20, 12 to 15, 14 to 30, 14 to 25, 14 to 20, 14 to 18, 14 to 17, 14 to 16, 15 to 30, 15 to 25, 15 to 20, 15 to 18, 15 to 17, 15 to 16, 16 to 30, 16 to 25, 16 to 20, or 16 to 18) contiguous nucleotide sequence of SEQ ID NO: 4 or 15.

In some examples, an ASO disclosed herein specifically hybridizes to a 14 to 20 contiguous nucleotide sequence that has at least 80% sequence identity (*e.g.*, 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) to SEQ ID NO: 4 or 15. In some examples, an ASO disclosed herein

specifically hybridizes to a 14 to 20 contiguous nucleotide sequence that has at least 93% sequence identity to SEQ ID NO: 4 or 15. In some examples, an ASO disclosed herein specifically hybridizes to a 14 to 20 contiguous nucleotide sequence of SEQ ID NO: 4 or 15. In some examples, an ASO disclosed herein specifically hybridizes to a 15 to 17 contiguous nucleotide sequence that has at least 80% sequence identity (e.g., 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) to SEQ ID NO: 4 or 15. In some examples, an ASO disclosed herein specifically hybridizes to a 15 to 17 contiguous nucleotide sequence that has at least 93% sequence identity to SEQ ID NO: 4 or 15. In some examples, an ASO disclosed herein specifically hybridizes to a 15 to 17 contiguous nucleotide sequence of SEQ ID NO: 4 or 15.

In some examples, an ASO disclosed herein specifically hybridizes to a 16 contiguous nucleotide sequence that has at least 80% sequence identity (e.g., 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) to SEQ ID NO: 4 or 15. In some examples, an ASO disclosed herein specifically hybridizes to a 16 contiguous nucleotide sequence that has at least 93% sequence identity to SEQ ID NO: 4 or 15. In some examples, an ASO disclosed herein specifically hybridizes to a 16 contiguous nucleotide sequence of SEQ ID NO: 4 or 15.

The disclosed ASOs include at least one locked nucleic acid (LNA) nucleotide at each of the 5' and 3' termini of the ASO. In some examples, a disclosed ASO includes no more than 8 LNA nucleotides at the 5' or 3' terminus of the ASO, for example, no more than 7, 6, 5, 4, 3, or 2 LNA nucleotides at the 5' or 3' terminus of the ASO. In some examples, a disclosed ASO includes no more than 1 or 2 LNA nucleotides at the 5' terminus of the ASO. In some examples, a disclosed ASO includes no more than 2 or 3 LNA nucleotides at the 3' terminus of the ASO. In some examples, the ASO includes 1 to 8 LNA molecules at the 5' and/or 3' terminus of the ASO, for example, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, 1 to 2, 2 to 8, 2 to 7, 2 to 6, 2 to 5, 2 to 4, 2 to 3, 3 to 8, 3 to 7, 3 to 6, 3 to 5, 3 to 4, 4 to 8, 4 to 6, or 6 to 8 LNA molecules. In some examples, the ASO includes 1 to 4 LNA molecules at the 5' and/or 3' terminus of the ASO. In some examples, the ASO includes 1, 2, 3, 4, 5, 6, 7, or 8 LNA nucleotide at the 5' terminus. In some examples, the ASO includes one LNA nucleotide at the 5' terminus. In some examples, the ASO includes 1, 2, 3, 4, 5, 6, 7, or 8 LNA nucleotide at the 3' terminus. In some examples, the ASO includes two LNA nucleotides at the 3' terminus.

In some aspects, an ASO disclosed herein includes at least 80% sequence identity (e.g., 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) to any ASO sequence disclosed in FIG. 2. In some examples, an ASO disclosed herein includes at least 93% sequence identity to any ASO sequence disclosed in FIG. 2. In some examples, an ASO disclosed herein includes or consists of any ASO sequence disclosed in FIG. 2. In some aspects, an ASO disclosed herein includes at least 80% sequence identity (e.g., 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) to any one of SEQ ID NOs: 1-3, 8, 10, or 14. In some examples, an ASO disclosed herein includes at least 93% sequence identity to any one of SEQ ID NOs: 1-3, 8, 10, or 14. In some examples, an ASO disclosed herein includes or consists of any one of SEQ ID NOs: 1-3, 8, 10, or 14.

In some examples, an ASO disclosed herein includes at least 93% sequence identity to SEQ ID NO:

1. In some examples, an ASO disclosed herein includes at least 93% sequence identity to SEQ ID NO: 2. In some examples, an ASO disclosed herein includes at least 93% sequence identity to SEQ ID NO: 3. In some examples, an ASO disclosed herein includes at least 93% sequence identity to SEQ ID NO: 8. In some examples, an ASO disclosed herein includes at least 93% sequence identity to SEQ ID NO: 10. In some examples, an ASO disclosed herein includes at least 93% sequence identity to SEQ ID NO: 14. In some examples, an ASO disclosed herein includes or consists of SEQ ID NO: 1. In some examples, an ASO disclosed herein includes or consists of SEQ ID NO: 2. In some examples, an ASO disclosed herein includes or consists of SEQ ID NO: 3. In some examples, an ASO disclosed herein includes or consists of SEQ ID NO: 8. In some examples, an ASO disclosed herein includes or consists of SEQ ID NO: 10. In some examples, an ASO disclosed herein includes or consists of SEQ ID NO: 14.

In some aspects, an ASO disclosed herein includes a phosphorothioate backbone modification. A phosphorothioate backbone refers to a modification made to a DNA backbone in which the nonbridging oxygen in the phosphate moiety of the DNA sugar-phosphate backbone is replaced with sulfur. This modification renders internucleotide linkages resistant to nuclease degradation. Including phosphorothioate bonds between the last 3-5 nucleotides at the 5' and/or 3' end of an oligo is sufficient to inhibit exonuclease degradation of the modified oligo. Including phosphorothioate bonds throughout the entire oligo also confers resistance to endonucleases. In some examples, at least 3 bases (*e.g.*, at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 bases) of an ASO disclosed herein includes a phosphorothioate backbone. In some examples, at least 15 bases of an ASO disclosed herein includes a phosphorothioate backbone. In some examples, all of the internucleotide linkages of the ASO include a phosphorothioate modification. Methods of modifying an oligo backbone to a phosphorothioate backbone are known, and commercial services offer artificial synthesis of oligos including backbone modifications, *e.g.*, a phosphorothioate backbone (*see, e.g.*, Integrated DNA Technologies® (IDT®) website: [idtdna.com/site/catalog/modifications/category/8](https://www.idtdna.com/site/catalog/modifications/category/8)).

In some aspects, an ASO disclosed herein is coupled to a cholecystokinin-B receptor (CCK-BR) targeting moiety (*e.g.*, a gastrin peptide or DNA aptamer targeting gastrin). The CCK-BR targeting moiety can be attached, for example, to the ASO on the 5' or 3' end. In some aspects, the CCK-BR targeting moiety is coupled to the 5' thiol of the ASO. In some aspects, the CCK-BR targeting moiety is coupled to the 3' end of the ASO. In a non-limiting example, 3,3'-disulfanediylbis(propan-1-ol) is used to modify the 3' end of the ASO, which is then reacted with a maleimide-CCK-BR targeting moiety to couple to the 3' end.

In some aspects, the CCK-BR receptor targeting moiety is a peptide that binds CCK-BR. In some aspects, the peptide that binds CCK-BR includes at least 5 amino acids, for example, at least 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids. In some aspects, the peptide that binds CCK-BR includes no more than 50 amino acids, for example, no more than 45, 40, 35, 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, or 10 amino acids. In some aspects, the peptide that binds CCK-BR includes a number of amino acids within a range of any two values disclosed above.

In some aspects, the peptide that binds CCK-BR includes 5 to 20 amino acids, for example, 5 to 17, 5 to 15, 5 to 12, 5 to 10, 5 to 8, 8 to 15, 8 to 12, 8 to 10, 10 to 15, or 10 to 12 amino acids. In some examples, the peptide that binds CCK-BR includes 8 to 17 amino acids. In some examples, the peptide that binds CCK-BR includes 8 to 12 amino acids. In some examples, the peptide that binds CCK-BR includes 10 to 16 or 10 to 17 amino acids. In some examples, the peptide that binds CCK-BR includes or consists of 10 amino acids. In some examples, the peptide that binds CCK-BR is a gastrin peptide fragment, or is derived from gastrin (*e.g.*, an artificial variant thereof). In some examples, the peptide that binds CCK-BR is a human gastrin peptide fragment, or is derived from human gastrin (*e.g.*, an artificial variant thereof).

An exemplary CCK-BR targeting moiety that is a peptide that binds CCK-BR is provided as SEQ ID NO: 5. In some aspects, an ASO disclosed herein is coupled to a peptide that binds CCK-BR that has at least 80% (*e.g.*, 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to SEQ ID NO: 5. In some examples, an ASO disclosed herein is coupled to a peptide that binds CCK-BR that has at least 90% sequence identity to SEQ ID NO: 5. In some examples, an ASO disclosed herein is coupled to a peptide that binds CCK-BR that includes or consists of SEQ ID NO: 5. In some aspects, the peptide that binds CCK-BR is gastrin-16 (G16) peptide. G16 peptide is commercially available, *see, e.g.*, “Gastrin-16 peptide” Catalog Number 312893 available from NovoPro® (novoprolabs.com/p/gastrin-16-312893.html). In some aspects, an ASO disclosed herein is coupled to a peptide that binds CCK-BR and has at least 80% (*e.g.*, 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to the amino acid sequence of G16 peptide. In some examples, an ASO disclosed herein is coupled to a peptide that binds CCK-BR and has at least 90% sequence identity to the amino acid sequence of G16 peptide. In some examples, an ASO disclosed herein is coupled to a peptide that binds CCK-BR and includes or consists of the amino acid sequence of G16. In some aspects, a CCK-BR peptide targeting moiety includes a C-terminal maleimide moiety (*e.g.*, 3-maleimido-propionyl moiety).

In some aspects, the cholecystokinin-B receptor (CCK-BR) targeting moiety is a DNA aptamer that binds CCK-BR, for example, a DNA aptamer described in Clawson *et al. Nucleic Acid Ther*, 27(1):23-35, 2017. In some aspects, the DNA aptamer that binds CCK-BR is at least 20 nucleotides in length, for example, at least 30, at least 40, at least 50, or at least 60 nucleotides in length. In some aspects, the DNA aptamer that binds CCK-BR is no more than 100 nucleotides in length, for example, no more than 90 nucleotides, no more than 80 nucleotides, or no more than 70 nucleotides. In some aspects, the DNA aptamer that binds CCK-BR is 20 to 100 nucleotides in length, for example, 20 to 90, 20 to 80, 20 to 70, 20 to 60, 20 to 50, 30 to 100, 30 to 90, 30 to 80, 30 to 70, 30 to 60, 30 to 50, 40 to 100, 40 to 90, 40 to 80, 40 to 60, 40 to 50, 50 to 100, 50 to 90, 50 to 80, 50 to 70, 50 to 60, 60 to 100, 60 to 90, 60 to 80, 60 to 70, 65 to 100, 65 to 90, 65 to 80, 65 to 75, 65 to 70, or 65 to 67 nucleotides in length. In some examples, the DNA aptamer that binds CCK-BR is 60 to 70 nucleotides in length. In some examples, the DNA aptamer that binds CCK-BR is 64 to 68 nucleotides in length. In some examples, the DNA aptamer that binds CCK-BR is AP1153 (*see, Clawson et al. Nucleic Acid Ther*, 27(1):23-35, 2017; *see also*, SEQ ID NO: 16).

In some aspects, an ASO disclosed herein is coupled to a DNA aptamer that binds CCK-BR and has at least 80% (e.g., 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to SEQ ID NO: 16. In some examples, an ASO disclosed herein is coupled to a DNA aptamer that binds CCK-BR and has at least 90% sequence identity to SEQ ID NO: 16. In some examples, an ASO disclosed herein is coupled to a DNA aptamer that binds CCK-BR and includes or consists of SEQ ID NO: 16.

The ASOs disclosed herein can be prepared using any suitable method, including, for example, by direct chemical synthesis. Many methods for chemical synthesis of nucleic acids are known, for example, the phosphotriester method of Narang *et al.*, *Meth. Enzymol.* 68:90-99, 1979; the phosphodiester method of Brown *et al.*, *Meth. Enzymol.* 68:109-151, 1979; the diethylphosphoramidite method of Beaucage *et al.*, *Tetra. Lett.* 22:1859-1862, 1981; the solid phase phosphoramidite triester method described by Beaucage & Caruthers, *Tetra. Letts.* 22(20):1859-1862, 1981; or the solid support method described in U.S. Patent No. 4,458,066. Methods using automated synthesizers have also been described, for example as described in Needham-VanDevanter *et al.*, *Nucl. Acids Res.* 12:6159-6168, 1984. *See also*, Letsinger and Mahadevan "Oligonucleotide Synthesis on a Polymer Support." *J Am Chem Soc.* 87:3526-3527, 1965; Letsinger. "Nucleotide chemistry. XIII. Synthesis of oligothymidylates via phosphotriester intermediates." *J Am Chem Soc.* 91(12):3350-3355, 1969; Agarwal *et al.* "Total synthesis of the gene for an alanine transfer ribonucleic acid from yeast." *Nature.* 227(5253):27-34, 1970; Letsinger *et al.* "Letter: Phosphite coupling procedure for generating internucleotide links." *J Am Chem Soc.* 97(11):3278-3279, 1975; Letsinger and Lunsford. "Synthesis of thymidine oligonucleotides by phosphite triester intermediates." *J Am Chem Soc.* 98(12):3655-3661, 1976; and McBride "An investigation of several deoxynucleoside phosphoramidites useful for synthesizing deoxyoligonucleotides." *Tetrahedron Letters.* 24(3):245-248, 1982.

Also disclosed are pharmaceutical compositions including an ASO disclosed herein and a pharmaceutically acceptable carrier. Suitable pharmaceutically acceptable carriers include, but are not limited to, liquids such as water, saline, phosphate-buffered saline (PBS), dextrose solution, sucrose solution, glycerol, and ethanol. Pharmaceutically acceptable carriers can include pharmaceutical salts, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. Pharmaceutically acceptable carriers can include auxiliary substances, such as wetting or emulsifying agents, stabilizing agents, pH buffering substances, and the like. In some aspects, a pharmaceutical composition disclosed herein includes trehalose, for example, as a cryoprotectant if the composition is lyophilized. A suitable carrier can be readily determined by a practitioner in view of the teachings here and other available sources of information, for example, a thorough discussion of pharmaceutically acceptable carriers/excipients is available in *Remington's Pharmaceutical Sciences*, 23rd Edition, Academic Press, Elsevier, (2020).

Pharmaceutical carriers can include additional excipients. In some examples, the pharmaceutical carrier confers a protective effect on the ASOs disclosed herein such that potential damage from formulation procedures, packaging, storage, transport, and the like, is minimized. Exemplary excipients that can be

included in a pharmaceutically acceptable carrier to help protect nucleic acids from degradative conditions include, but are not limited to, proteins (*e.g.*, ovalbumin and bovine serum albumin), amino acids (*e.g.*, glycine), polyhydric and dihydric alcohols, such as but not limited to polyethylene glycols (PEG) of varying molecular weights, such as PEG-200, PEG-400, PEG-600, PEG-1000, PEG-1450, PEG-3350, PEG-6000, PEG-8000 and any molecular weights in between these values, propylene glycols (PG), and sugar alcohols, such as a carbohydrate, preferably, sorbitol. Protective excipients are commercially available from a number of vendors, such Sigma® (St. Louis, Mo).

The exact concentration of various excipients will vary, however, generally excipients are present at a concentration between 0.05 weight (wt) % to 20 weight (wt) %. In some examples a protein excipient, such as BSA, is present at a concentration of between 1.0 weight (wt) % to about 20 wt %, preferably 10 wt %. If an amino acid, *e.g.* glycine, is used in the formulation, it can be present at a concentration of about 1 wt. % to about 5 wt. %. A carbohydrate, *e.g.* sorbitol, if present, can be present at a concentration of about 0.1 wt % to about 10 wt. %, such as between about 0.5 wt. % to about 15 wt. %, or about 1 wt. % to about 5 wt. %. In some examples, the pharmaceutically acceptable carrier includes or consists of about 0.1% to 2% saline, for example about 0.5%, about 0.7%, about 0.8%, about 9%, about 1%, or about 1.5% saline. In a non-limiting example, the pharmaceutically acceptable carrier includes or consists of about 0.9% saline. In some examples, the pharmaceutically acceptable carrier includes or consists of about 2% to 20% dextrose or sucrose, for example about 2%, about 5%, about 10%, about 12%, about 15%, or about 18% dextrose or sucrose.

In some aspects, a pharmaceutical composition disclosed herein includes an ASO disclosed herein at a concentration of 5nM to 500µM, for example, 10nM to 500µM, 50nM to 500µM, 1µM to 500µM, 10µM to 500µM, 100µM to 500µM, 200µM to 500µM, 300µM to 500µM, 400µM to 500µM, 10nM to 400µM, 50nM to 400µM, 1µM to 400µM, 10µM to 400µM, 100µM to 400µM, 200µM to 400µM, 300µM to 400µM, 10nM to 300µM, 50nM to 300µM, 1µM to 300µM, 10µM to 300µM, 100µM to 300µM, 200µM to 300µM, 10nM to 200µM, 50nM to 200µM, 1µM to 200µM, 10µM to 200µM, 100µM to 200µM, 10nM to 100µM, 50nM to 100µM, 1µM to 100µM, 10µM to 100µM, 10nM to 10µM, 50nM to 10µM, 1µM to 10µM, 10nM to 1000nM, 20nM to 1000nM, 30nM to 1000nM, 40nM to 1000nM, 50nM to 1000nM, 60nM to 1000nM, 70nM to 1000nM, 80nM to 1000nM, 90nM to 1000nM, 100nM to 1000nM, 10nM to 500nM, 20nM to 500nM, 30nM to 500nM, 40nM to 500nM, 50nM to 500nM, 60nM to 500nM, 70nM to 500nM, 80nM to 500nM, 90nM to 500nM, 100nM to 500nM, 10nM to 200nM, 20nM to 200nM, 30nM to 200nM, 40nM to 200nM, 50nM to 200nM, 60nM to 200nM, 70nM to 200nM, 80nM to 200nM, 90nM to 200nM, 100nM to 200nM, 10nM to 120nM, 20nM to 120nM, 30nM to 120nM, 40nM to 120nM, 50nM to 120nM, 60nM to 120nM, 70nM to 120nM, 80nM to 120nM, 90nM to 120nM, or 100nM to 120nM. In some aspects, a pharmaceutical composition disclosed herein includes an ASO disclosed herein at a concentration of 60nm to 120nm.

In some examples, an ASO disclosed herein is lyophilized for storage and suspended, for example in a pharmaceutically acceptable carrier (*e.g.*, saline, PBS, dextrose or sucrose solution), when needed for

administration. In some examples, the pharmaceutical composition is in a concentrated form for transport and/or storage and is diluted in a suitable pharmaceutically acceptable carrier prior to administration to a subject. Generally, an ASO disclosed herein is present in a pharmaceutical composition in an amount sufficient to provide a therapeutic effect when administered in one or more doses to a subject.

5

IV. Methods

Also disclosed are methods of treating cancer (*e.g.*, a CCK-BR positive cancer) or inhibiting metastasis (*e.g.*, metastasis of a CCK-BR positive cancer), said methods including administering an effective amount of an ASO or pharmaceutical composition disclosed herein to a subject in need thereof. In some aspects, a subject having a cancer that expresses CCK-BR (a CCK-BR positive cancer) is selected for treatment. Exemplary CCK-BR positive cancers include, but are not limited to, pancreatic cancer, colon cancer, hepatocellular (liver) cancer, gastric cancer, esophageal cancer, small cell lung cancer, and medullary thyroid cancer. In some aspects, a subject having pancreatic cancer is selected for treatment. In some aspects, a subject having pancreatic cancer resistant to standard immunotherapy and/or chemotherapy treatment is selected for treatment. In some aspects, a subject having a cancer that expresses gastrin is selected for treatment. In some aspects, administering an ASO or pharmaceutical composition disclosed herein decreases one or more sign or symptom of cancer, for example (and without limitation), decreases tumor size or weight, tumor fibrosis, or metastasis.

The ASOs or pharmaceutical compositions disclosed herein can be administered by any suitable route. In some examples, the ASO or pharmaceutical composition is administered orally, parenterally, topically, intranasally, by inhalation, or by local injection. Parenteral administration includes subcutaneous, intravenous, intramuscular, intraperitoneal, intrapleural, and intracisternal injections, or infusion techniques. Administration can be systemic (*e.g.*, parenteral administration), or local to a target tumor (*e.g.*, local injection). In a non-limiting example, the pharmaceutical composition is administered parenterally (*e.g.*, intraperitoneally, subcutaneously, or intravenously). In a further non-limiting example, the pharmaceutical composition is administered intravenously. The administration route may vary, for example, based on the age or condition of the patient, or severity of disease symptoms (*e.g.*, metastasis).

The exact dose of an ASO or pharmaceutical composition administered to a subject may vary depending on multiple factors, including the timing of administration, the subject being treated, the severity of the condition being treated, and the manner of administration. Generally, an appropriate dose is used that is sufficient to treat cancer while minimizing unwanted side effects. In some aspects, 1mg to 5000 mg of an ASO or pharmaceutical composition disclosed herein is administered to the subject per dose, for example, 10mg to 5000mg, 100mg to 5000mg, 200mg to 5000mg, 300mg to 5000mg, 400mg to 5000mg, 500mg to 5000mg, 600mg to 5000mg, 700mg to 5000mg, 800mg to 5000mg, 900mg to 5000mg, 1000mg to 5000mg, 2000mg to 5000mg, 3000mg to 5000mg, 4000mg to 5000mg, 10mg to 4000mg, 100mg to 4000mg, 200mg to 4000mg, 300mg to 4000mg, 400mg to 4000mg, 500mg to 4000mg, 600mg to 4000mg, 700mg to 4000mg, 800mg to 4000mg, 900mg to 4000mg, 1000mg to 4000mg, 2000mg to 4000mg, 3000mg to

4000mg, 10mg to 3000mg, 100mg to 3000mg, 200mg to 3000mg, 300mg to 3000mg, 400mg to 3000mg, 500mg to 3000mg, 600mg to 3000mg, 700mg to 3000mg, 800mg to 3000mg, 900mg to 3000mg, 1000mg to 3000mg, 2000mg to 3000mg, 10mg to 2000mg, 100mg to 2000mg, 200mg to 2000mg, 300mg to 2000mg, 400mg to 2000mg, 500mg to 2000mg, 600mg to 2000mg, 700mg to 2000mg, 800mg to 2000mg, 900mg to 2000mg, 1000mg to 2000mg, 10mg to 1000mg, 100mg to 1000mg, 200mg to 1000mg, 300mg to 1000mg, 400mg to 1000mg, 500mg to 1000mg, 600mg to 1000mg, 700mg to 1000mg, 800mg to 1000mg, 900mg to 1000mg, 1mg to 900mg, 10mg to 900mg, 100mg to 900mg, 200mg to 900mg, 300mg to 900mg, 400mg to 900mg, 500mg to 900mg, 600mg to 900mg, 700mg to 900mg, 800mg to 900mg, 1mg to 800mg, 10mg to 800mg, 100mg to 800mg, 200mg to 800mg, 300mg to 800mg, 400mg to 800mg, 500mg to 800mg, 600mg to 800mg, 700mg to 800mg, 1mg to 700mg, 10mg to 700mg, 100mg to 700mg, 200mg to 700mg, 300mg to 700mg, 400mg to 700mg, 500mg to 700mg, 600mg to 700mg, 1mg to 600mg, 10mg to 600mg, 100mg to 600mg, 200mg to 600mg, 300mg to 600mg, 400mg to 600mg, 500mg to 600mg, 1mg to 500mg, 10mg to 500mg, 100mg to 500mg, 200mg to 500mg, 300mg to 500mg, 400mg to 500mg, 1mg to 400mg, 10mg to 400mg, 100mg to 400mg, 200mg to 400mg, 300mg to 400mg, 1mg to 300mg, 10mg to 300mg, 100mg to 300mg, 200mg to 300mg, 1mg to 200mg, 10mg to 200mg, 100mg to 200mg, 1mg to 100mg, or 10mg to 100mg per dose of an ASO or pharmaceutical composition disclosed herein.

In some aspects, 0.1mg to 100 mg per kg (of the subject's weight) of an ASO or pharmaceutical composition disclosed herein is administered to the subject per dose, for example, 1mg to 100mg, 10mg to 100mg, 20mg to 100mg, 30mg to 100mg, 40mg to 100mg, 50mg to 100mg, 60mg to 100mg, 70mg to 100mg, 80mg to 100mg, 90mg to 100mg, 0.1mg to 90mg, 1mg to 90mg, 10mg to 90mg, 20mg to 90mg, 30mg to 90mg, 40mg to 90mg, 50mg to 90mg, 60mg to 90mg, 70mg to 90mg, 80mg to 90mg, 0.1mg to 80mg, 1mg to 80mg, 10mg to 80mg, 20mg to 80mg, 30mg to 80mg, 40mg to 80mg, 50mg to 80mg, 60mg to 80mg, 70mg to 80mg, 0.1mg to 70mg, 1mg to 70mg, 10mg to 70mg, 20mg to 70mg, 30mg to 70mg, 40mg to 70mg, 50mg to 70mg, 60mg to 70mg, 0.1mg to 60mg, 1mg to 60mg, 10mg to 60mg, 20mg to 60mg, 30mg to 60mg, 40mg to 60mg, 50mg to 60mg, 0.1mg to 50mg, 1mg to 50mg, 10mg to 50mg, 20mg to 50mg, 30mg to 50mg, 40mg to 50mg, 0.1mg to 40mg, 1mg to 40mg, 10mg to 40mg, 20mg to 40mg, 30mg to 40mg, 0.1mg to 30mg, 1mg to 30mg, 10mg to 30mg, 20mg to 30mg, 0.1mg to 20mg, 1mg to 20mg, 10mg to 20mg, 0.1mg to 10mg, 1mg to 10mg, 1mg to 5mg, 0.1mg to 5mg, 0.1mg to 2mg, or 0.1 to 0.5mg per kg (of the subject's weight) of an ASO or pharmaceutical composition disclosed herein is administered to the subject per dose. A dose can be administered, for example and without limitation, as a single administration (*e.g.*, single injection), over multiple administrations (*e.g.*, multiple injections), or as a continuous infusion (*e.g.* IV) over a set period of time. In some examples, a pump is used to control infusion rate and to minimize cellular damage.

Administration includes single or multiple administrations, for example, an ASO or pharmaceutical composition disclosed here can be administered once, or may be administered periodically until a therapeutic result is achieved or until side effects warrant discontinuation of therapy. In some examples, the ASO or pharmaceutical composition is administered once, twice, three times, four times, or more per day

during a treatment course. A treatment course can last one or more weeks, for example, 1 to 12 weeks (*e.g.*, 1 to 10, 1 to 8, 1 to 6, 1 to 5, 1 to 4, 1 to 3, 1 to 2, 2 to 12, 2 to 10, 2 to 8, 2 to 6, 2 to 5, 2 to 4, 2 to 3, 3 to 12, 3 to 10, 3 to 8, 3 to 6, 3 to 5, 3 to 4, 4 to 12, 4 to 10, 4 to 8, 4 to 6, 6 to 12, 6 to 10, or 6 to 8 weeks). In an example, an ASO or pharmaceutical composition disclosed herein is administered to a subject once a day for 1 to 6 weeks. In a further example, an ASO or pharmaceutical composition disclosed herein is administered to a subject twice a day for 1 to 6 weeks. In a further example, an ASO or pharmaceutical composition disclosed herein is administered to a subject twice a day for 2 to 6 weeks. In a further example, an ASO or pharmaceutical composition disclosed herein is administered to a subject twice a day for 1 to 4 weeks. In a non-limiting example, an ASO or pharmaceutical composition disclosed herein is administered to a subject twice a day for 4 weeks. A subject can undergo multiple treatment courses until a desired therapeutic effect is achieved.

In some aspects, an ASO or pharmaceutical composition disclosed herein is administered in an amount effective to decrease tumor size or weight. In some aspects, tumor size or weight is decreased by at least 5%, at least 8%, at least 10%, at least 15%, at least 20%, at least 25%, at least 50%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% as compared to a suitable control (*e.g.*, tumor size or weight prior to the treatment). In some aspects, an ASO or pharmaceutical composition disclosed herein is administered in an amount effective to decrease the number of tumors in a subject. In some aspects, tumor number is decreased by at least 5%, at least 8%, at least 10%, at least 15%, at least 20%, at least 25%, at least 50%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% as compared to a suitable control (*e.g.*, number of tumors prior to the treatment). In some aspects, an ASO or pharmaceutical composition disclosed herein is administered in an amount effective to decrease or inhibit metastasis in a subject. In some aspects, the rate of metastasis is decreased and/or the risk of metastasis is decreased by at least 5%, at least 8%, at least 10%, at least 15%, at least 20%, at least 25%, at least 50%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% as compared to a suitable control (*e.g.*, typical metastasis rates in subjects not receiving treatment with an ASO disclosed herein).

In some aspects, an ASO or pharmaceutical composition disclosed herein is administered in an amount effective to decrease tumor fibrosis in a subject. In some aspects, tumor fibrosis is decreased by at least 5%, at least 8%, at least 10%, at least 15%, at least 20%, at least 25%, at least 50%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% as compared to a suitable control (*e.g.*, fibrosis in subjects not receiving treatment with an ASO disclosed herein or a fibrosis measurement in the subject prior to treatment). In some aspects, an ASO or pharmaceutical composition disclosed herein is administered in an amount effective to decrease M2-polarized macrophages in a tumor of the subject. In some aspects, M2-polarized macrophages are decreased by at least 5%, at least 8%, at least 10%, at least 15%, at least 20%, at least 25%, at least 50%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% as compared to a suitable control (*e.g.*, M2-polarized macrophages in subjects

not receiving treatment with an ASO disclosed herein or a measurement of M2-polarized macrophages in the subject prior to treatment).

In some aspects, an ASO or pharmaceutical composition disclosed herein is administered in an amount effective to decrease gastrin, ZEB1, or ZEB2 expression in tumor cells. In some examples, expression is decreased by at least 5%, at least 8%, at least 10%, at least 15%, at least 20%, at least 25%, at least 50%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% as compared to a suitable control (*e.g.*, expression prior to the treatment). In some aspects, an ASO or pharmaceutical composition disclosed herein is administered in an amount effective to decrease gastrin expression in tumor cells. In some examples, expression is decreased by at least 5%, at least 8%, at least 10%, at least 15%, at least 20%, at least 25%, at least 50%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% as compared to a suitable control (*e.g.*, gastrin expression prior to the treatment).

Following administration of the ASO or pharmaceutical composition, the subject can be evaluated for response to treatment, such as evaluating tumor burden (*e.g.*, measuring number of tumors, size, and/or weight) through an MRI, ultrasound, or other imaging technique (the RESIST criteria is typically used). In some examples, tumor expression of gastrin, ZEB1, and/or ZEB2 is measured in tumors. Methods of detecting target nucleic acids (*e.g.*, gastrin mRNA) have been described, and include (but are not limited to) sequencing methods (*e.g.*, high through-put sequencing and pyrosequencing), PCR-methods (*e.g.*, qPCR), and probe-based method (*e.g.*, microarrays).

In some examples, the subject receives a cancer treatment in addition to an ASO or pharmaceutical composition disclosed herein, such as one or more of surgery, radiation, chemotherapy, biologic therapy, immunotherapy, or other therapeutic. Administration of a second or additional cancer treatment may be before, after, or substantially simultaneously with the administration of an ASO or pharmaceutical composition disclosed herein. A skilled clinician can select appropriate additional therapies (from those listed here or other known therapies) for the subject, depending on factors, for example, the cancer being treated, treatment history, clinical stage and/or grade of the disease, patient condition, *etc.*

Exemplary chemotherapeutic agents include (but are not limited to) alkylating agents, such as nitrogen mustards (such as mechlorethamine, cyclophosphamide, melphalan, uracil mustard or chlorambucil), alkyl sulfonates (such as busulfan), nitrosoureas (such as carmustine, lomustine, semustine, streptozocin, or dacarbazine); antimetabolites such as folic acid analogs (such as methotrexate), pyrimidine analogs (such as 5-FU or cytarabine), and purine analogs, such as mercaptopurine or thioguanine; or natural products, for example vinca alkaloids (such as vinblastine, vincristine, or vindesine), epipodophyllotoxins (such as etoposide or teniposide), antibiotics (such as dactinomycin, daunorubicin, doxorubicin, bleomycin, plicamycin, or mitomycin C), and enzymes (such as L-asparaginase). Additional agents include platinum coordination complexes (such as cis-diamine-dichloroplatinum II, also known as cisplatin), substituted ureas (such as hydroxyurea), methyl hydrazine derivatives (such as procarbazine), and adrenocortical suppressants (such as mitotane and aminoglutethimide); hormones and antagonists, such as adrenocorticosteroids (such as

prednisone), progestins (such as hydroxyprogesterone caproate, medroxyprogesterone acetate, and magesrol acetate), estrogens (such as diethylstilbestrol and ethinyl estradiol), antiestrogens (such as tamoxifen), and androgens (such as testosterone propionate and fluoxymesterone). Examples of the most commonly used chemotherapy drugs include adriamycin, melphalan (Alkeran®) Ara-C (cytarabine), carmustine, busulfan, 5-lomustine, carboplatinum, cisplatin, cyclophosphamide (Cytoxan®), daunorubicin, dacarbazine, 5-fluorouracil, fludarabine, hydroxyurea, idarubicin, ifosfamide, methotrexate, mithramycin, mitomycin, mitoxantrone, nitrogen mustard, paclitaxel (or other taxanes, such as docetaxel), vinblastine, vincristine, VP-16, while newer drugs include gemcitabine (Gemzar®), trastuzumab (Herceptin®), irinotecan (CPT-11), leustatin, navelbine, rituximab (Rituxan®) imatinib (STI-571), Topotecan (Hycamtin®), capecitabine, 10-ibrutinomab (Zevalin®), and calcitriol.

In some aspects, a topyrimidine analog (*e.g.*, 5-FU or gemcitabine), a platinum anti-neoplastic agent (for example, cis-diamine-dichloroplatinum II, also known as cisplatin and oxaliplatin), or paclitaxel is administered in addition to an ASO or pharmaceutical composition disclosed herein. In some examples, a FOLFIRINOX regimen (a combination treatment of leucovorin calcium (folinic acid), fluorouracil, 15-irinotecan hydrochloride, and oxaliplatin) is administered in addition to an ASO or pharmaceutical composition disclosed herein.

Exemplary immunotherapies include, for example, monoclonal antibody cancer immunotherapies (*e.g.*, anti-CTLA-4, anti-PD1, or anti-PDL1), T cell agonist antibodies, oncolytic viruses, adoptive cell transfer (ACT) therapies (*e.g.*, CAR T cells, CAR NK cells, TCRs, TILs *etc.*), or any combination of two or 20-more thereof. In some examples, the additional therapeutic is a cell cycle or checkpoint inhibitor. In some examples, the checkpoint inhibitor targets PD-1, PD-L1, CTLA-4, CDK4, and/or CDK6. Exemplary checkpoint inhibitors include ipilimumab, nivolumab, pembrolizumab, atezolizumab, avelumab, durvalumab, cemiplimab, palbociclib, ribociclib, and abemaciclib. In some examples, the subject is administered an ACT therapy, for example, a chimeric antigen receptor (CAR)-expressing T cell, engineered 25-TCR T cell, or a tumor-infiltrating lymphocyte (TIL).

The additional cancer treatment may be administered substantially simultaneously with the disclosed composition. In some examples, the additional therapeutic is administered prior to administering the composition, for example, at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 8 days, at least 9 days, at least 12 days, at least 14 days, at least three 30-weeks, at least four weeks, at least one month, or more prior. Multiple doses of the additional therapeutic can be administered to a subject, for example, administered twice daily, once daily, every other day, twice per week, weekly, every other week, every three weeks, monthly, or less frequently. A skilled clinician can select an administration schedule based on the subject, the condition being treated, the previous treatment history, tumor load and type, clinical stage and grade of the disease and overall health of the subject, and 35-other factors.

Clauses

[Clause 1] An antisense oligonucleotide (ASO) 14 to 20 nucleotides in length that specifically hybridizes with a gastrin mRNA molecule, wherein the ASO comprises at least one locked nucleic acid (LNA) nucleotide at each of the 5' and 3' termini.

5 [Clause 2] The ASO of clause 1, which is 15 to 17 nucleotides in length.

[Clause 3] The ASO of clause 1 or clause 2, which is 16 nucleotides in length.

[Clause 4] The ASO of any one of the prior clauses, comprising 1 to 4 LNA nucleotides at the 5' terminus and 1 to 4 LNA nucleotides at the 3' terminus.

10 [Clause 5] The ASO of any one of the prior clauses, comprising 1 LNA nucleotide at the 5' terminus and 2 LNA nucleotides at the 3' terminus.

[Clause 6] The ASO of any one of the prior clauses, comprising the nucleotide sequence of an ASO shown in FIG. 2.

[Clause 7] The ASO of any one of the prior clauses, comprising any one of SEQ ID NOs: 1-3, 8, 10, or 14.

[Clause 8] The ASO of any one of the prior clauses, comprising any one of SEQ ID NOs: 1-3.

15 [Clause 9] The ASO of any one of the prior clauses, comprising a phosphorothioate backbone.

[Clause 10] The ASO of any one of the prior clauses, further comprising a cholecystokinin-B receptor (CCK-BR) targeting moiety coupled to the 5' thiol of the ASO.

20 [Clause 11] The ASO of any one of the prior clauses, wherein the CCK-BR targeting moiety is a gastrin peptide or a DNA aptamer that specifically binds to CCK-BR; optionally wherein the gastrin peptide comprises SEQ ID NO: 5 and/or the DNA aptamer comprises SEQ ID NO: 16.

[Clause 12] The ASO of any one of the prior clauses, wherein the CCK-BR targeting moiety comprises a C-terminal maleimide.

[Clause 13] The ASO of any one of the prior clauses, wherein the C-terminal maleimide is a 3-maleimido-propionyl moiety.

25 [Clause 14] A pharmaceutical composition comprising the ASO of any one of the prior clauses and a pharmaceutically acceptable carrier.

[Clause 15] A method of treating a cholecystokinin-B receptor (CCK-BR) positive cancer in subject in need thereof, comprising administering to the subject a therapeutically effective amount of the ASO of any one of clause 1-13 or the pharmaceutical composition of clause 14.

30 [Clause 16] A method of inhibiting metastasis of a cholecystokinin-B receptor (CCK-BR) positive cancer in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the ASO of any one of clauses 1-13 or the pharmaceutical composition of clause 14.

[Clause 17] The method of clause 15 or clause 16, wherein the cancer expresses gastrin relative to a control.

35 [Clause 18] The method of any one of clauses 15-17, wherein administering the ASO decreases fibrosis and/or M2-polarized macrophages in a tumor.

[Clause 19] The method of any one of clauses 15-18, wherein administering the ASO decreases tumor size or weight relative to a suitable control.

[Clause 20] The method of any one of clauses 15-19, wherein administering the ASO decreases number of metastasis relative to a suitable control.

[Clause 21] The method of any one of clauses 15-20, wherein the control is a measurement obtained from the subject prior to treatment with the ASO, or an average or historical measure from a population of subjects having CCK-BR positive cancer and not receiving treatment with the ASO.

[Clause 22] The method of any one of clauses 15-21, wherein the CCK-BR positive cancer is pancreatic cancer, colon cancer, hepatocellular cancer, gastric cancer, esophageal cancer, small cell lung cancer, or medullary thyroid cancer.

[Clause 23] The method of any one of clauses 15-22, wherein the CCK-BR positive cancer is pancreatic cancer.

[Clause 24] The method of any one of clauses 15-23, wherein the CCK-BR positive cancer is a human cancer.

[Clause 25] The method of any one of clauses 15-24, wherein the subject is human.

EXAMPLES

Example 1

Materials and Methods

Pancreatic Cancer Cell Lines

Human pancreatic cancer cell lines AsPC-1, BxPC-3, and PANC-1 were obtained from the Georgetown Lombardi Tissue Culture Shared Resource (TCSR) facility and been purchased and authenticated by ATCC®. These cell lines were selected since that range in their surface membrane expression of the CCK-BR with PANC-1 having the highest number of receptors (283 ± 68 fmol/mg protein) and BxPC-3 having the fewer (125 ± 44 fmol/mg protein) based upon radioactive receptor ligand assays (Smith et al., Am J Physiol 266:R277-R283, 1994) and AsPC-1 has the lowest CCK-BR expression by qRT-PCR (Matters et al, Int J Oncol 38:593-601, 2011). AsPC-3 has the highest gastrin expression, followed by BxPC-3 and PANC-1 was low gastrin mRNA expression (Matters et al., Pancreas 38:e151-e161, 2009). Cells were grown in a humidified incubator with 5% CO₂ in the appropriate media (RPMI-1640; for AsPC-1 and BxPC-3), and (DMEM for PANC-1) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin.

Designing Anti-Gastrin LNA Gapmers

Using an online software tool developed by Integrated DNA Technologies®, Inc., Coralville, IA, and the mRNA protein coding sequence for human gastrin, 96 antisense oligonucleotides (16nt in length) were designed. The top 11, based upon GC content ~ 40-60, T_m~50-60°C, avoiding long repeats > 4, and avoiding secondary strand hairpins in mRNA structure, were selected for further modification and screening. To synthesize the Gapmers, the first 5' base and the last two 3' bases of each ASO were modified to be

locked nucleic acids (LNAs). In addition, a phosphorothioate alteration was made to all phosphate groups connecting the bases (the backbone).

CCK-B Receptor Targeting Moiety

Gapmer-90 was designed like the other Gapmers with the following structure: 5ThioMC6-D/+T*A*A*G*G*G*T*G*C*A*T*C*T*G*+G*+C. In order to render this molecule target-specific to bind to the CCK-B receptor in pancreatic cancer, instead of substituting the phosphate group into a phosphorothioate, the very last base is connected to a thiol modification on the 5' LNA T base. This was conjugated to maleimide functionalized Gastrin-10 peptide (Glu-Glu-Glu-Ala-Tyr-Gly-Trip-Met-Asp-Phe-NH₂, MW 1426.48 g/mol) (GenScript® USA Inc., Piscataway, NJ, USA) via a Michael addition reaction.

In Vitro Transfection of Cancer Cells with LNA Gapmers

Several different preliminary transfections were performed to determine the optimal concentration to apply to the pancreatic cancer cells to effectively knockdown the mRNA. Initially a commercial GAPDH Gapmer to calculate the estimated dose for the experiments was used (*see*, FIG. 1B). Human pancreatic cancer cells (AsPC-1, BxPC-3, PANC-1) were plated at a density of 200,000 cells/well in individual 6-well plates and allowed to adhere. After 24 hours, cells were transfected with Gapmer & Lipofectamine™-3000 (ThermoFisher, Cat# L3000001) in serum free Opti-MEM media for an additional 24 hours. Fresh media was placed and the cells allowed to grow. After 72 hours RNA was extracted and subjected to qRT-PCR analysis for gastrin expression. With dose-response experiments, it was determined that 30nM was the optimal *in vitro* dose.

RNA Extraction Confirmation of Gastrin mRNA Knockdown

After 72 hours from transfection in cell culture or after tumor excision from *in vivo* studies, RNA was extracted from pancreatic cancer cells/tumors using miRNeasy® kit (Qiagen®). Synthesis of cDNA was performed using an RT first strand kit (Qiagen®). Quantitative reverse transcription PCR (qRT-PCR) was performed using RT SYBR Green ROX® qPCR Mastermix (Qiagen®) in a 7300 Real-Time PCR System (Applied Biosystems®, Waltham, MA, USA) programmed at 1 cycle at 95 °C for 10 min followed by 40 cycles at 95 °C for 15s and 60 °C for 1 min). Samples were subjected in triplicate to qRT-PCR with selective primers. GAPDH was used as the normalizer for human samples. Single amplicons were confirmed by PCR dissociation curves. The relative differences between two groups were calculated using $\Delta\Delta CT$ method. Significance between data from two groups will be determined with Prism GraphPad® Version 5.03 by using unpaired Student t-test ($p < 0.05$).

Treatment of Pancreatic Tumors In Vivo

Orthotopic AsPC-1 tumors were established in nude mice by injecting 10⁶ cells into the mouse pancreas (n=3). The cells were luciferase tagged to assure tumor-take in all the mice prior to starting

therapy. Ten days after inoculation of cancer cells, tumors were confirmed by IVIS fluorescent imaging and ultrasound. Mice were treated with indicated treatments for 4 weeks and MRIs were performed on the mice prior to euthanasia and necropsy. Blood was collected by cardiac stick for biochemical analysis for toxicity. The primary tumors were excised, weighed and part formalin fixed and paraffin embedded for histology, and the other part was flash frozen in liquid N₂ for RNA analysis.

In the second experiment, luciferase labeled human PANC-1 cells (720,000/ mouse) were injected into the pancreas of (N=30) male mice. After 10 days and IVIS imaging as above, the mice were divided into three groups of equal tumor flux: PBS (control), untargeted Gapmer-90 (120nM), and targeted Gapmer-90 (120nM). Tumor growth was evaluated by luciferase bioluminescence after 3 and 6 weeks. The effect of the treatment on survival was evaluated.

Tumor Histology and Immunohistochemistry

Tumor sections (5µm) from paraffin-embedded pancreatic tumors and other organs were prepared. Hematoxylin and eosin staining was performed to confirm histology of the primary tumor and to confirm metastases. Intratumoral fibrosis was evaluated by staining with Masson's trichrome stain. Immunohistochemical staining was performed using a horseradish peroxidase-labelled polymer (Dako K4003) according to the manufacturer's instructions. Briefly, slides were treated with 3% hydrogen peroxide and 10% normal goat serum for 10 minutes each, and exposed to primary antibodies for Arginase (1/1800, Thermofisher, PA5-29645) and Ki67 (1/80, BIOCARE, CRM325) for 1 hour at room temperature.

Quantitative Measurement of Gapmer Uptake into Tumors

A nucleotide probe complementary to Gapmer-90 (modified at the 5' end with digoxigenin and at the 3' end with biotin, and with the first and last three nucleotides phosphorothioated) was designed. With increasing concentrations of the probe, a standard curve was generated. Protein homogenates were prepared from tumor tissues and incubated with the complementary probe then placed into a NeutrAvidin™-coated 96-well plates, black (Thermo Scientific®). After reacting samples with micrococcal nuclease reaction buffer (0.1 U/µl), anti-digoxigenin antibody (1:5000) blocking buffer was added to each well. After 30 min incubation, AttoPhos™ AP fluorescent substrate system (Promega®) was added to each well and the samples and the fluorescence of the samples read in a plate reader at the following parameters: 444 nm excitation, 555 nm excitation, and with a cutoff at 530 nm.

Statistical Analysis

For PCR analysis, the relative difference between the normalizer and target gene was calculated using $\Delta\Delta C_t$ method. A dissociation curve analysis of PCR products was done to confirm the specificity of amplification. Image J was used to analyze the area of fibrosis, Ki-67 positive cells and the number of arginase-stained tumor-associated macrophages from the slides. Mean Values for each Gapmer treatment was compared with controls using PRISM GraphPad version 9.0. T-tests were used to evaluate statistical

significance when two groups were compared and a one-way ANOVA when multiple groups were compared to control with a $P < 0.05$ considered to be statistically significant.

Example 2

LNA Gapmer Design and Knock-Down of Gastrin mRNA

The mRNA sequence of gastrin was used to design sequences that were 16mer nucleotides in size. The software program generated 246 sequences. Of these 96 were selected to further investigate due to their lack of secondary structure. Of these ninety-six, the top 11 were synthesized based upon optimal T_m (melting temperature), GC content, lack of secondary structures, and a ΔG no more than -10kcal/mol. The sequences and characteristics of the synthesized gapmers are shown in FIG. 2.

A control gapmer known to down-regulate the expression of GAPDH was used to confirm the transfection procedure and quantity of gapmer to apply to pancreatic cancer cells in culture. Using two sets of primers, a reduction in expression of GAPDH in human AsPC-1 pancreatic cancer cells was achieved (FIG. 3A).

Using conditions determined with the control GAPDH gapmer, gastrin-targeted Gapmers (30nM) were tested for their ability to down-regulate gastrin in AsPC-1 human pancreatic cancer cells. The relative gastrin mRNA expression was measured by RT-PCR in AsPC-1 cells after individual Gapmer *in vitro* transfection (N=3 replicates each) with the panel of eleven LNA Gapmers from FIG. 2. FIG. 3A demonstrates that six of the Gapmers effectively down-regulated the expression of the target gene including Gapmers 29, 31, 33, 82, 92, and 90, with Gapmer 90 exhibiting the greatest efficacy ($P < 0.0001$). The other five Gapmers from the panel, including Gapmer 31, 32, 93, 95, and 96 did not significantly decrease gastrin mRNA expression by PCR (FIG. 3B). These data show how high specific the Gapmers are in targeting the mRNA since a shift by just one nucleotide in the antisense oligonucleotide (ASO) sequence can change the effectiveness.

Growth of AsPC-1 cells was evaluated *in vitro* after exposure to the LNA Gapmers. AsPC-1 cellular proliferation by MTT assay was significantly decreased only with the Gapmers that were effective in downregulating mRNA expression of gastrin (FIG. 3C). In Contrast, there was no effect on cancer cell growth of AsPC-1 cells treated with the Gapmers that did not decrease the gene expression by PCR (FIG. 1D). From the panel of anti-sense gastrin Gapmers evaluated *in vitro*, Gapmer 90 exhibited the greatest downregulation of gastrin expression (decreased by 75%) compared to control cells. Of the panel of Gapmers tested, Gapmer-90 was the most effective ASO to inhibit growth of AsPC-1 cells with proliferation decreased by 66% compared to control cells.

Since Gapmer-90 was found to be the most effective Gapmer in decreasing growth and gene expression in AsPC-1 cells, it was also tested in two additional pancreatic cancer cell lines; human BxPC-3 and PANC-1. Gastrin mRNA expression was decreased by 53% in BxPC-3 cells and growth by 62% (FIG. 3E). Although PANC-1 cells produce less gastrin mRNA than AsPC-1 and BxPC-3 cells, they express a

high level of CCK-B receptors by radiolabeled binding assays, and Gapmer-90 significantly decreased PANC-1 cell proliferation by 66% compared to control cells (FIG. 3F).

Example 3

In Vitro Cell Proliferation Assays

Next, MTT cell proliferation assays were performed to determine if the Gapmers decreased growth of pancreatic cancer cells. The MTT assay measures cellular metabolic activity as an indicator of cell viability and proliferation. This colorimetric assay is based on the reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) to purple formazan crystals by metabolically active cells. A spectrophotometer is used to measure absorbance at 500-600 nanometers. The measurements are used to quantify cell growth and viability.

Gapmer-90 treatment was found to decrease AsPC-1 (FIG. 4A) and BxPC-3 cell growth (FIG. 4B). Gapmer-29 and Gapmer-30 decreased growth of BxPC-3 cells as compared to respective controls (FIG. 4C). However, treatment with Gapmer-30, Gapmer-32, or Gapmer-33 did not reduce growth of ASPC-1 (FIG. 4D).

Example 4

Target-Specific Modification of Gapmer-90

CCK-BR is a cell surface receptor that is not detected in normal human pancreatic cells, but becomes expressed in precancerous PanIN lesions of the pancreas and is over-expressed in pancreatic cancer cells. Moreover, CCK-BR is a G protein-coupled receptor for gastrin, a peptide that reportedly drives growth of pancreatic cancer. Hence CCK-BR represents a unique and specific target for pancreatic cancer.

Gapmer-90 was rendered target specific to the CCK-B receptor by conjugating via click chemistry a 10 amino acid targeting moiety to the antisense oligonucleotide. In particular, a maleimide-containing gastrin-10 peptide was conjugated to the 5' thiol end (see, FIGS. 5A and 5B). One goal of this modification was to permit use of the Gapmer *in vivo* without a transfection reagent. Without being bound by any particular theory, the mechanism by which the receptor-targeted Gapmer is expected to cross the plasma membrane is through internalization of the receptor after binding to the targeting moiety. In this example, the maleimide-containing gastrin-10 peptide has a structure of: 3-maleimido-propionyl-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH₂ (molecular formula: C₆₅H₇₉N₁₃O₂₂S; molecular weight: 1426.48 Da; *see*, SEQ ID NO: 5). The maleimide-containing gastrin-10 peptide can be conjugated to any thiol (-SH) group through Michael addition to form a stable thioether bond.

The CCK-B receptor targeted Gapmer-90 was found to decrease gastrin mRNA in cell culture similar to the untargeted Gapmer-90 transfected with Lipofectamine (FIG. 6A). Targeted Gapmer-90 is also shown to inhibit growth of AsPC-1 (via MTT assay) without the need of a transfection reagent (*e.g.*, lipofectamine; *see*, FIG. 6B). Without being bound by any particular theory, these results suggest that targeted Gapmer-90 binds to the CCK-B receptor and after internalization of the receptor, the Gapmer is

released to down-regulate gastrin mRNA by RNase H in the nucleus. Making the Gapmer target specific is expected to improve uptake, reduce toxicity to non-cancerous cells, and will allow for lower dosing regimens *in vivo*.

5

Example 5

Selective CCK-B Receptor Targeted LNA Gapmer Inhibits Growth and Metastases of Pancreatic Tumors In Vivo

To test the effects of the CCK-BR targeted Gapmer-90 *in vivo*, orthotopic AsPC-1 tumors were established in nude mice by injecting 10^6 cells into the mouse pancreas. The cells were luciferase tagged to assure tumor-take in all the mice prior to starting therapy. Ten days after inoculation of cancer cells, tumors were confirmed in all 9 of the mice by IVIS fluorescent imaging and ultrasound. Mice were divided into three groups: Group -1 Control- PBS treated; Group-2 LNA Gapmer (60nM BIW ip); and Proglumide (a CCK-BR antagonist, oral) (3 mice per treatment group). Mice were treated for 4 weeks and MRIs were performed on the mice prior to euthanasia and necropsy. The tumors were excised, weighted, metastases counted. RNA was extracted from the tumors for analysis. All metastases were confirmed by histology. FIG. 7A shows the mean tumor masses at necropsy, and FIG. 7B shows MRI images before necropsy.

The mean number of metastases per mouse and representative histologic figures of the confirmed metastases is shown in FIG. 8A. These results not only show that the targeted Gapmer-90 is effective *in vivo*, but also suggest that Gapmer is blocking the Gastrin-CCK-BR signaling pathway, similar to a receptor antagonist proglumide, but by a different mechanism. In addition, the reduction in metastases shown in FIG. 8A is significant as metastatic cancer is the cause of death in a majority of patients with pancreatic cancer.

RNA was extracted from the tumors of the control, CCK-BR targeted Gapmer 90 treated mice, and proglumide treated mice. RT-PCR was performed to measure gastrin mRNA in the tumors as further evidence that Gapmer 90 exerts its effect by knocking down gastrin mRNA. Indeed, gastrin mRNA was significantly downregulated only in tumors of mice treated with the LNA Gapmer 90 and not the controls or proglumide-treated mice (FIG. 9). To further understand the mechanism for decreased metastases seen in proglumide and CCK-BR targeted Gapmer-90 treated mice, tumors were analyzed for 2 transcription factors *ZEB1* and *ZEB2*, which regulate epithelial to mesenchymal transition (EMT). Both transcription factors were lower in the proglumide and Gapmer-90 treated mice, however due to variability in the *ZEB2* control, *ZEB2* data did not reach statistical significance with the number of samples available for analysis.

A follow-up experiment was performed to compare two different doses of targeted Gapmer-90, as well as untargeted Gapmer-90 (*see*, FIG. 12 for experimental set-up) and included a larger sample size for greater statistical power (10 mice per treatment group). Luciferase labeled AsPC-1 tumors were imaged about one week after orthotopic injection into the mouse pancreas. The radiance bioluminesces flux measurements were equal at baseline before initiation of therapy (FIG. 10A). A representative image of a tumor in the pancreas of a mouse imaged in the *in vivo* imaging system is shown in FIG. 10B. After treating the mice twice weekly for 4 weeks with PBS (control), untargeted Gapmer-90 (60nM), receptor targeted

Gapmer-90 (60nM) or receptor targeted Gapmer-90 (120nM), the mice were euthanized, blood collected for analysis, tumors resected and metastases counted. The pancreatic tumors of the mice treated with the receptor targeted Gapmers exhibited a significant decrease in mass (FIG. 10C); and the response was in a dose-related fashion with smaller tumors in the mice receiving the 120nM dose. In contrast, the mice treated with the same Gapmer but without the receptor targeting moiety were not different in weight from the control tumors. The number of metastases per mouse was significantly decreased in the mice treated with the receptor targeted Gapmers (FIG. 10D) with the fewest number of metastases seen in mice receiving the higher Gapmer dose (120nM). Compared to PBS treated control mice, metastases decreased in the mice treated with untargeted (60nM), targeted (60nM), and targeted (120nM) by 38%, 44% 71.3%, respectively. All of the metastases were confirmed by histology and representative images of tumor metastases in various tissues are shown in FIG. 10E.

To confirm that the Gapmer was successful in downregulating the intended gene, qRT-PCR was performed for gastrin (GAST) in each of the tumors. Similar to the tumor sizes, the mRNA gene expression was partially decreased in tumors of the mice treated with untargeted Gapmer (60nM) and the gene expression was dramatically decreased in mice treated with the receptor targeted Gapmers in a dose-dependent fashion (FIG. 10F). To understand the mechanism by which the Gapmers decreased tumor metastases, tumor RNA was also analyzed for the expression of *ZEB1* (FIG. 10G) and *ZEB2* (FIG. 10H), mediators of metastases through epithelial to mesenchymal transition (EMT). Indeed, the expression of both *ZEB1* and *ZEB2* were decreased in the tumors of mice treated with the targeted Gapmers 120nM > 60nM and mild decrease in the tumors of the untargeted Gapmer 60nM. To evaluate the off-target toxicity, mouse serum was analyzed for chemistries reflective of hepatic toxicity (ALT, AST, Alkaline phosphatase, and total bilirubin), renal injury (creatinine) and muscle or cardiac injury (CPK). Compared to PBS-control mice, no abnormalities in serum chemistries were identified (FIG. 10I).

Example 6

Effects of LNA Gapmers on Pancreatic Tumor Fibrosis and Tumor-Associated Macrophages

One reason pancreatic cancer is resistant to immune checkpoint antibodies and chemotherapeutic agents is likely the dense fibrosis observed in the tumor microenvironment (TME) (Waghray *et al.*, Curr Opin Gastroenterol 29:537-43, 2013) and lack of intratumoral CD8 T-cells. Since these tumors were grown in athymic nude mice that lack T-cells, tumor infiltrating T-cells could not be evaluated. Fibrosis was abundant in the PBS-treated control mouse tumors as demonstrated by the representative histologic section stained with Masson's trichrome (FIG. 11A; top left). Fibrosis was visibly less in the tumors treated with LNA Gapmers compared to control mouse tumors with modest decrease in tumors of mice treated with the untargeted Gapmer, less fibrosis in the targeted Gapmer treated tumors with the greatest decrease observed in the tumors of mice treated with the highest dose (120nM) of the receptor target- specific Gapmer (FIG.

11A, bottom right). The amount of fibrosis was analyzed by densitometry and mean values per treatment group of fibrosis as shown in FIG. 11B.

The pancreatic microenvironment has an abundance of cancer-promoting M2 polarized tumor-associated macrophages (TAMs) (Qian et al., Cell, 141:39-51, 2010; Zheng et al., Gastroenterology 144:1230-1240, 2013). Representative images from pancreatic cancer sections from each treatment groups showing arginase positive M2-polarizing tumor-associated macrophages is shown in FIG. 11C. The mean number of arginase positive TAMs per high powered field were counted for each group and evaluated (FIG. 11D). Although the targeted Gapmer-90 (60nM) decreased the number of immunoreactive TAMs by 29%, this did not reach statistical significance. In contrast, the high dose (120nM) targeted Gapmer decreased TAMs by 72% and this was statistically significant ($P < 0.001$; FIG 11D).

The Ki67 protein is widely used in Oncology as indicator of rapid tumor proliferation. Immunohistochemical staining for the proliferative marker Ki67 was performed on tissue sections of the pancreatic tumors and was abundant in tumors of control and untargeted Gapmer-treated mice and representative photos from each group are shown (FIG. 11A). Immunoreactive cells were counted and it was determined that all of the mice treated with the Gapmer-90 exhibited decreased Ki67 index scores compared to control PBS -treated mice (FIG. 11B). The untargeted Gapmer (60nM) decreased proliferation the least with scores of 29.8% less than controls. The targeted Gapmer (60nM) decreased proliferation by 34% and the targeted Gapmer (120nM) markedly decreased the Ki67 proliferative index by 76%.

Example 7

LNA Gapmers Decrease Ki67 Proliferative Index of Human Pancreatic Cancer Tumors

The Ki67 protein is widely used in Oncology as indicator of rapid tumor proliferation. Immunohistochemical staining for the proliferative marker Ki67 was performed on tissue sections of the pancreatic tumors and was abundant in tumors of control and untargeted Gapmer-treated mice and representative photos from each group are shown (FIGS. 11C-11F). Immunoreactive cells were counted and it was determined that all of the mice treated with the Gapmer-90 exhibited decreased Ki67 index scores compared to control PBS -treated mice (FIG. 11E-11F). The untargeted Gapmer (60nM) decreased proliferation the least with scores of 29.8% less than controls. The targeted Gapmer (60nM) decreased proliferation by 34% and the targeted Gapmer (120nM) markedly decreased the Ki67 proliferative index by 76%.

Example 8

Analysis of Gapmer Uptake into Tumors

Next, uptake of Gapmer-90 by AsPC-1 tumors was evaluated as described in Lim *et al.* (Lim *et al.* Detection of Locked Nucleic Acid Gapmers from Mouse Muscle Samples Using ELISA. Methods Mol Biol 2020;2176:233-9). Quantitative measurement of Gapmer uptake into the tumor using the complementary

bioluminescent probe showed some uptake of the untargeted Gapmer into the pancreatic tumors. This uptake may account for some of the modest effects that were observed in tumor weight compared to PBS-treated control mice (FIG. 10K). A dramatic improvement in Gapmer tumor uptake is seen in the tumors of mice treated with the receptor targeted Gapmers compared to the untargeted Gapmer (FIG. 10K; $P < 0.0001$).

5 These results support the rationale for receptor targeted therapy for improved precision medicine.

It will be apparent that the precise details of the methods or compositions described may be varied or modified without departing from the spirit of this disclosure. We claim all such modifications and variations that fall within the scope and spirit of the claims below.

10

What is claimed is:

1. An antisense oligonucleotide (ASO) 14 to 20 nucleotides in length that specifically hybridizes with a gastrin mRNA molecule, wherein the ASO comprises at least one locked nucleic acid (LNA) nucleotide at each of the 5' and 3' termini.
2. The ASO of claim 1, which is 15 to 17 nucleotides in length.
3. The ASO of claim 1, which is 16 nucleotides in length.
4. The ASO of claim 1, comprising 1 to 4 LNA nucleotides at the 5' terminus and 1 to 4 LNA nucleotides at the 3' terminus.
5. The ASO of claim 1, comprising 1 LNA nucleotide at the 5' terminus and 2 LNA nucleotides at the 3' terminus.
6. The ASO of claim 1, comprising a nucleotide sequence of an ASO shown in FIG. 2.
7. The ASO of claim 4, comprising any one of SEQ ID NOs: 1-3, 8, 10, or 14.
8. The ASO of claim 7, comprising a phosphorothioate backbone.
9. The ASO of claim 8, further comprising a cholecystokinin-B receptor (CCK-BR) targeting moiety coupled to the 5' thiol of the ASO.
10. The ASO of claim 9, wherein the CCK-BR targeting moiety is a gastrin peptide or DNA aptamer that specifically binds to CCK-BR.
11. The ASO of claim 10, wherein the CCK-BR targeting moiety comprises a C-terminal maleimide.
12. The ASO of claim 11, wherein the C-terminal maleimide is a 3-maleimido-propionyl moiety.
13. A pharmaceutical composition comprising the ASO of any one of claims 1-12 and a pharmaceutically acceptable carrier.

14. A method of treating a cholecystokinin-B receptor (CCK-BR) positive cancer in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the ASO of any one of claims 1-12 or the pharmaceutical composition of claim 13.

15. A method of inhibiting metastasis of a cholecystokinin-B receptor (CCK-BR) positive cancer in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the ASO of any one of claims 1-12 or the pharmaceutical composition of claim 13.

16. The method of claim 14 or claim 15, wherein the cancer expresses gastrin.

17. The method of any one of claims 14-16, wherein administering the ASO decreases tumor size or weight relative to a suitable control.

18. The method of any one of claims 14-17, wherein administering the ASO inhibits or decreases metastasis relative to a suitable control.

19. The method of any one of claims 14-18, wherein administering the ASO decreases tumor fibrosis and/or M2-polarized macrophages.

20. The method of any one of claims 14-19, wherein the control is a measurement obtained from the subject prior to treatment with the ASO, or an average or historical measure from a population of subjects having CCK-BR positive cancer and not receiving treatment with the ASO.

21. The method of any one of claims 14-20, wherein the CCK-BR positive cancer is pancreatic cancer, colon cancer, hepatocellular cancer, gastric cancer, esophageal cancer, small cell lung cancer, or medullary thyroid cancer.

22. The method of any one of claims 14-21, wherein the CCK-BR positive cancer is pancreatic cancer.

23. The method of any one of claims 14-22, wherein the CCK-BR positive cancer is a human cancer.

FIG. 1A

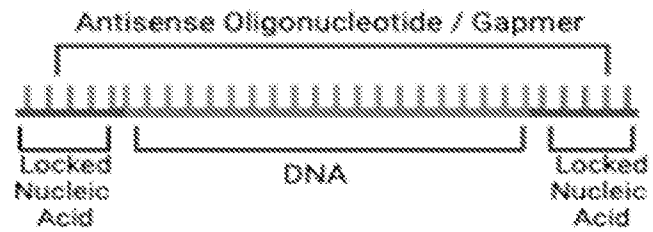


FIG. 1B

AsPC-1 GAPDH Gapmer Transfection

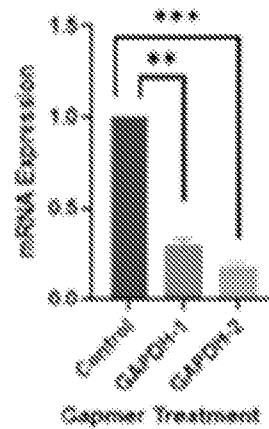


FIG. 2

Name	SEQ ID NO	ASO Sequence	Length nt (mer)	%GC Content	Melt Temp °C	Molecular Weight	Extinction Coefficient	nmole/OD260	µg/OD260	ΔG	Tm
Gapmer 29	2	AGC CAG TGC AAA GAT C	16	50	50	4863.2	142200	7.03	34.2	-1.33	42.2
Gapmer 30	7	GAG CCA GTG CAA AGA T	16	50	50.3	4823.2	139900	7.15	34.48	-1.33	42.2
Gapmer 31	8	AGA GCC AGT GCA AAG A	16	50	52.6	4814.2	133600	7.49	36.03	-1.33	42.2
Gapmer 32	9	CAG AGC CAG TGC AAA G	16	56.2	51.1	4839.2	135100	7.4	35.82	-1.33	42.2
Gapmer 33	3	CCA GAG CCA GTG CAA A	16	56.2	51.8	4879.2	138700	7.21	35.18	-1.33	42.2
Gapmer 92	10	ACC TAA GGG TGC ATC T	16	50	50.9	4881.2	155300	6.44	31.43	-1.44	48.4
Gapmer 93	11	TAC CTA AGG GTG CAT C	16	50	50	4881.2	156500	6.39	31.19	-2.25	50.7
Gapmer 95	12	TGT ACC TAA GGG TGC A	16	50	50.5	4841.2	152200	6.57	31.81	-2.9	48.2
Gapmer 96	13	CTG TAC CTA AGG GTG C	16	56.2	51.3	4866.2	154300	6.48	31.54	-2.25	50.7
Gapmer 82	14	GCA TCT GGC TGC TGG G	16	68.8	59.5	4836.2	148900	6.72	32.48	-2.8	50.9
GapmerR-90	1	TAA GGG TGC ATC TGG C	16	56.2	54.6	4826.2	150300	6.65	32.11	-1.08	39.7

FIG. 3A

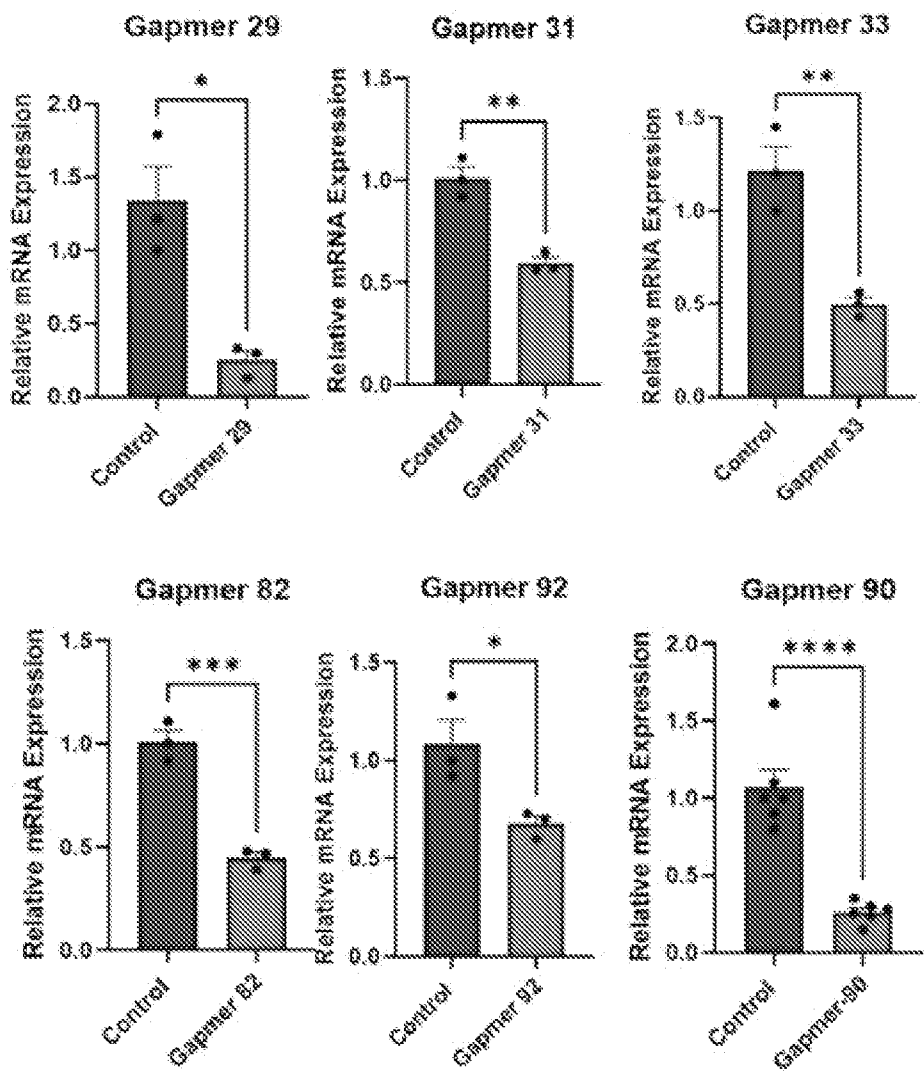
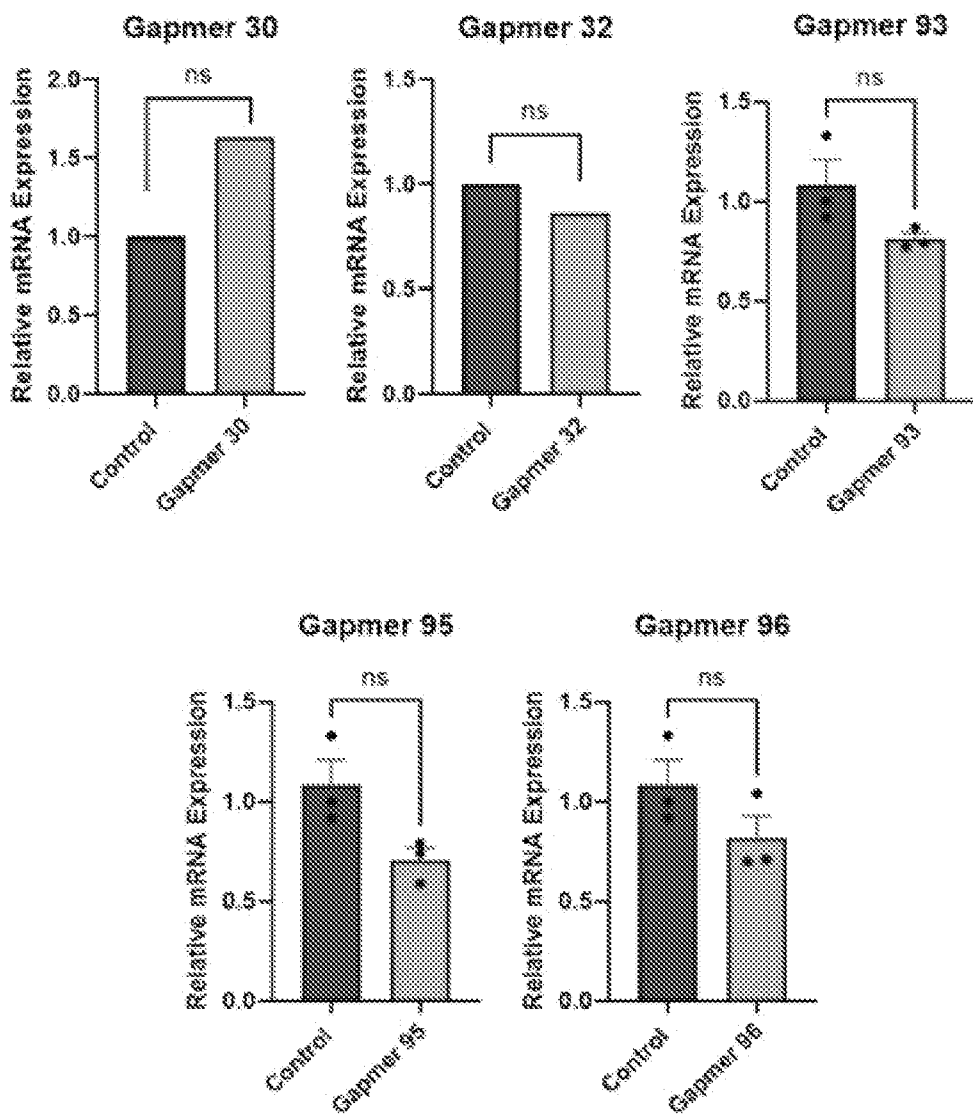


FIG. 3B



5/24

FIG. 3C

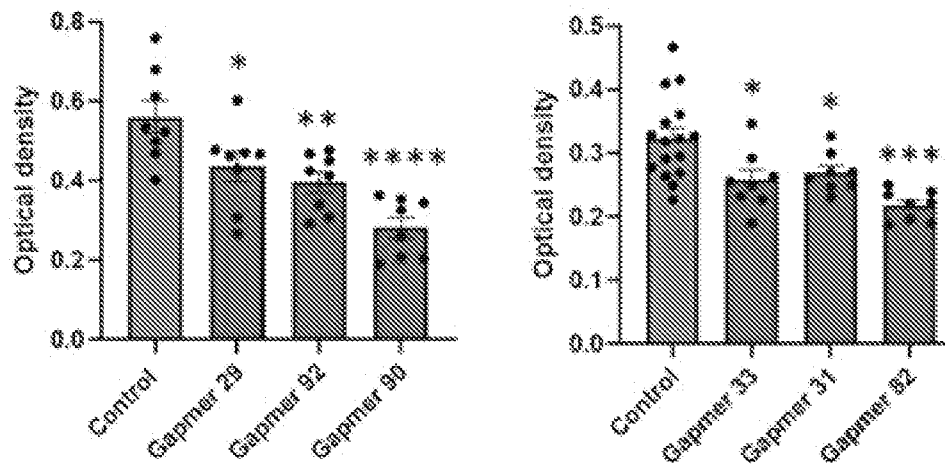
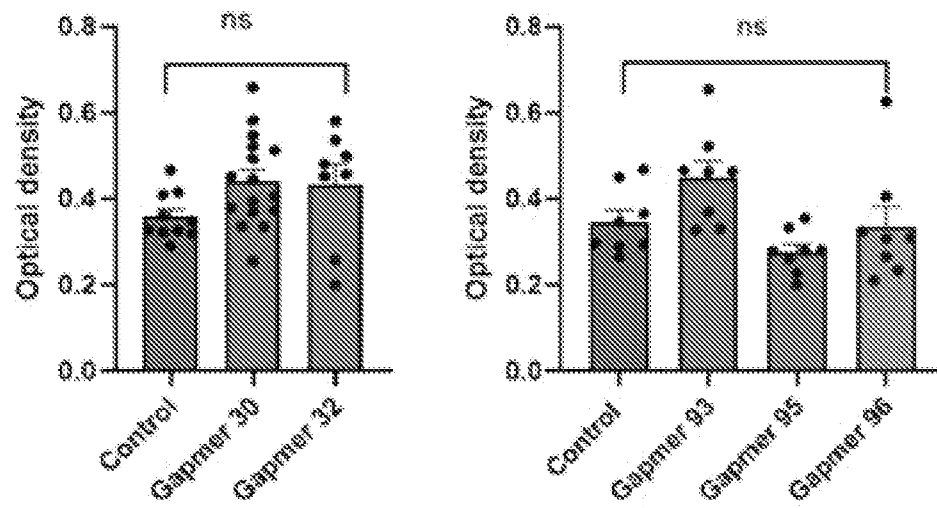


FIG. 3D



6/24

FIG. 3E

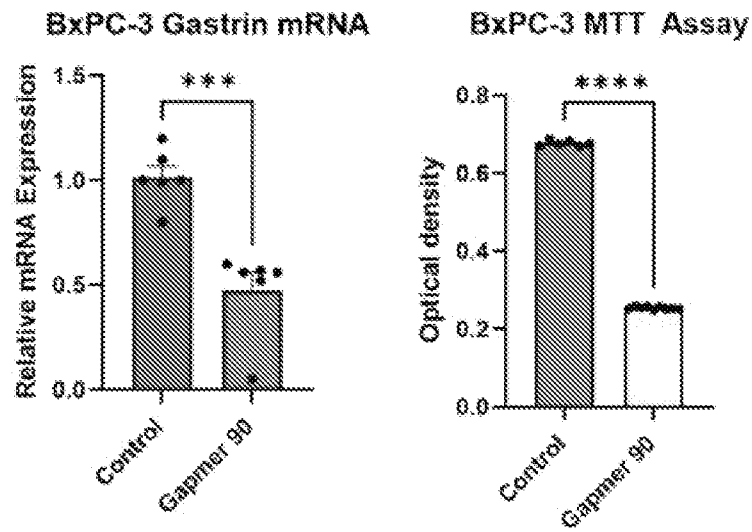
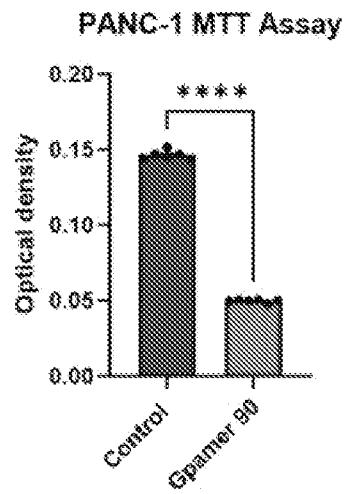


FIG. 3F



7/24

FIG. 4A

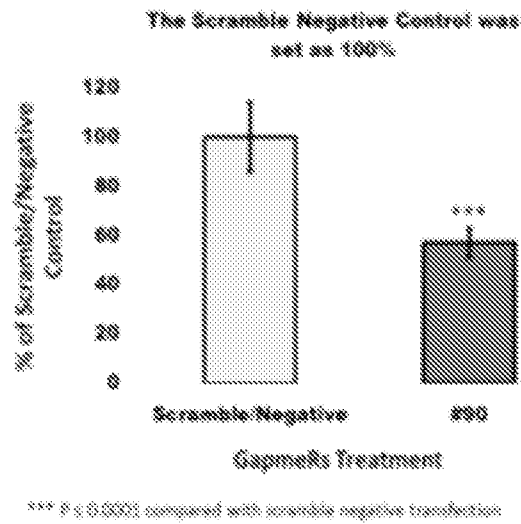


FIG. 4B

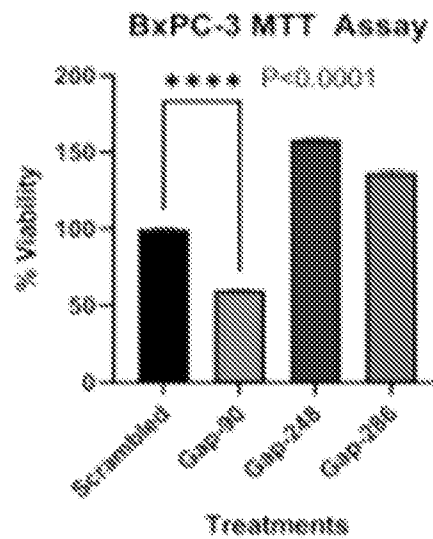


FIG. 4C

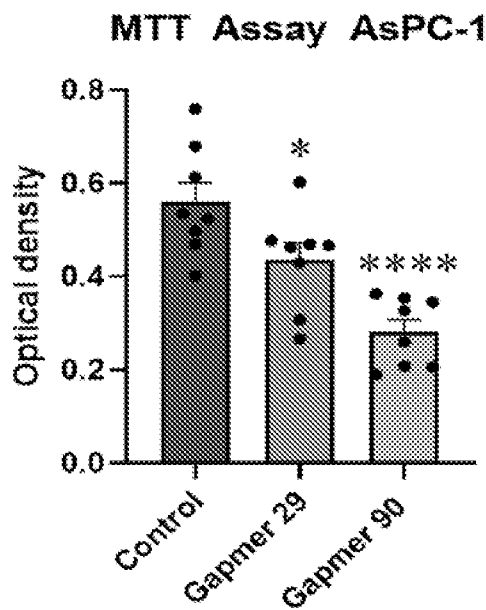


FIG. 4D

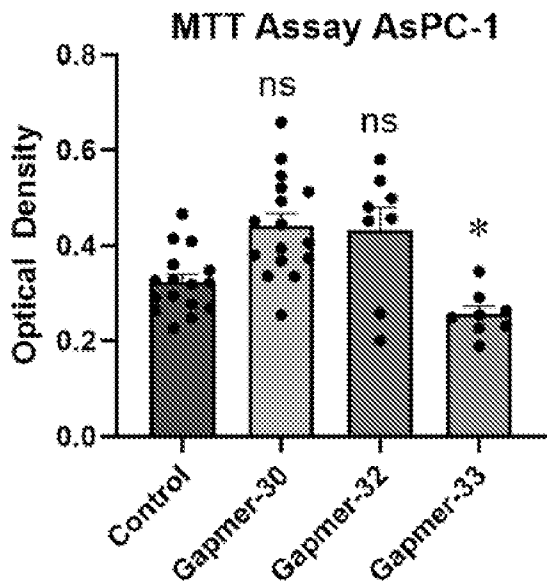


FIG. 5A

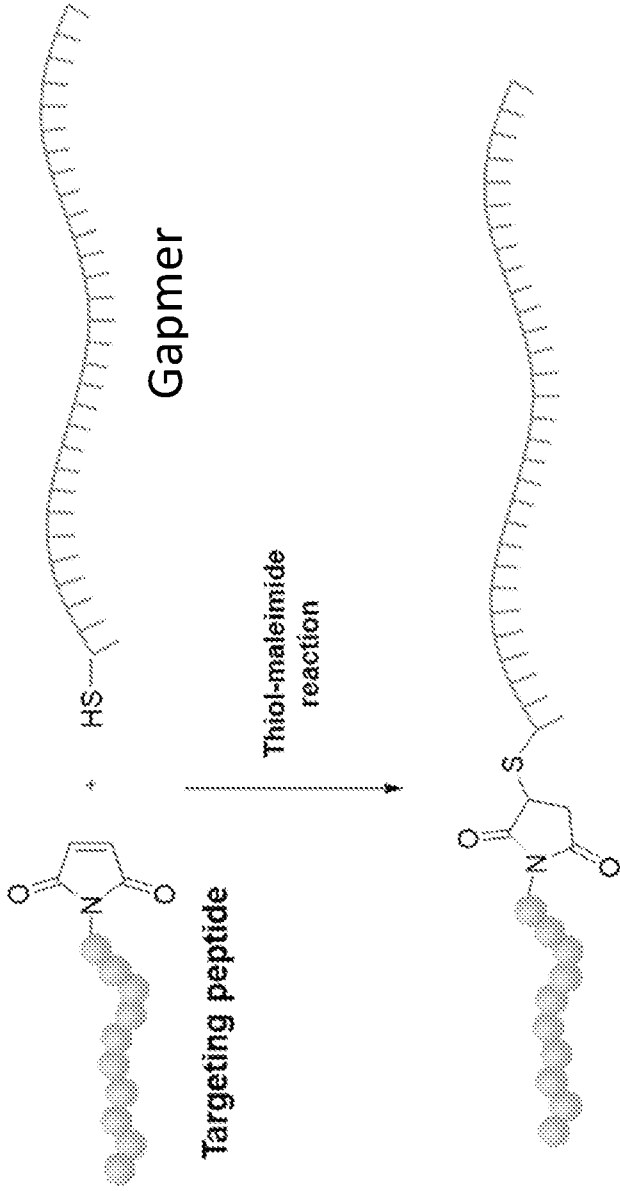
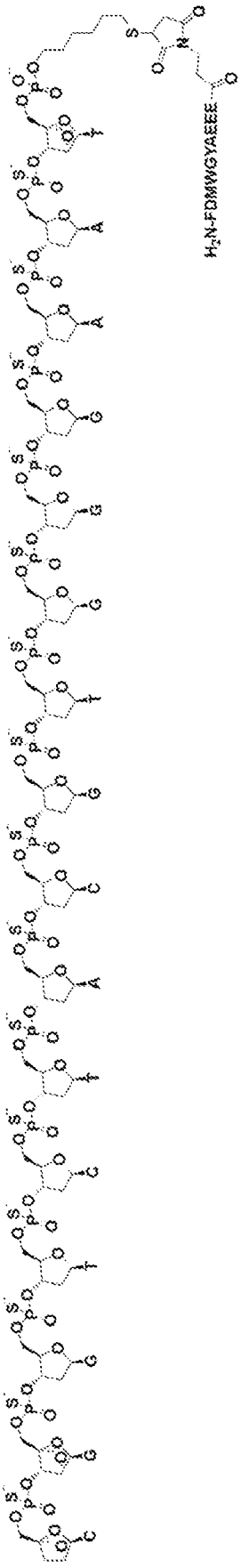


FIG. 5B



10/24

FIG. 6B

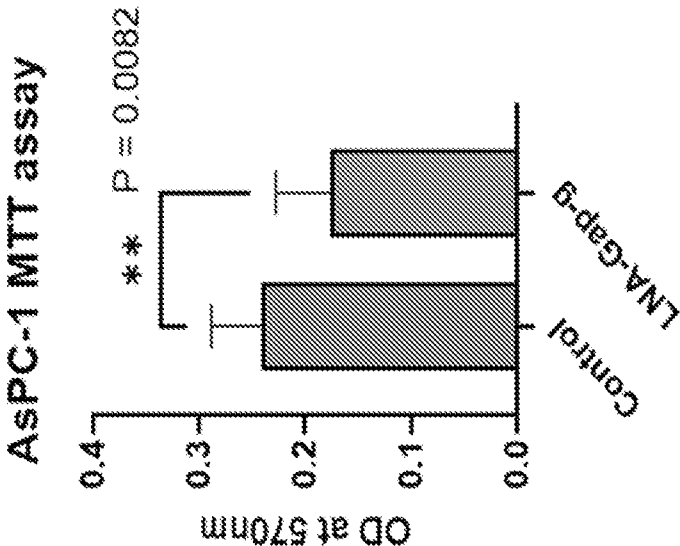


FIG. 6A

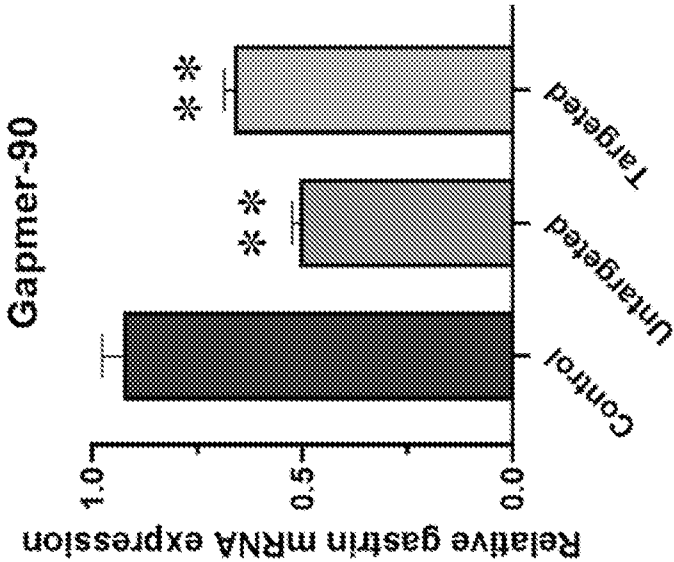


FIG. 7B

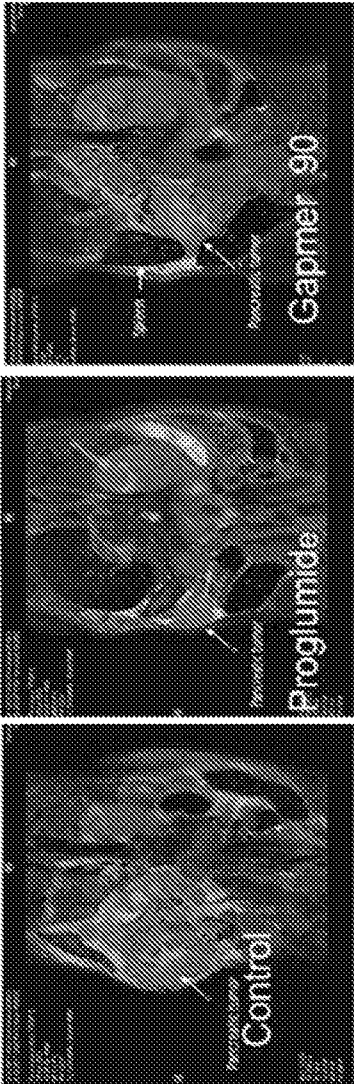


FIG. 7A

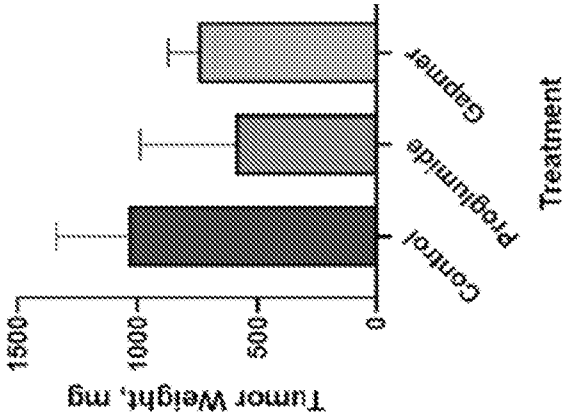


FIG. 8A

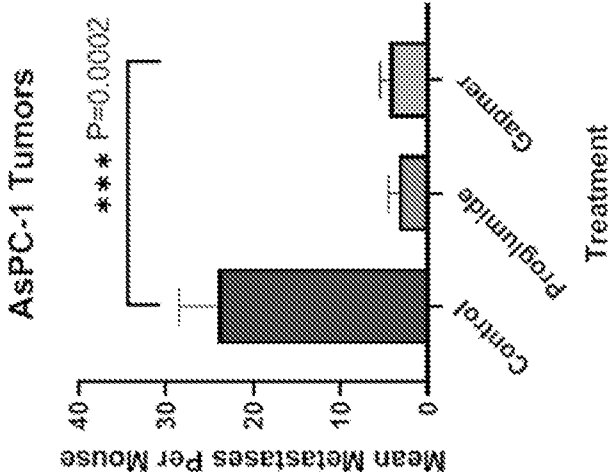


FIG. 8B

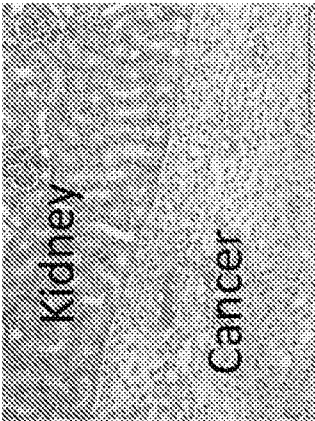


FIG. 8C

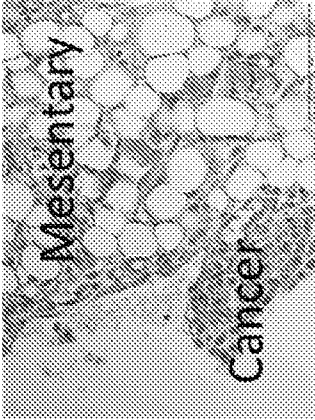
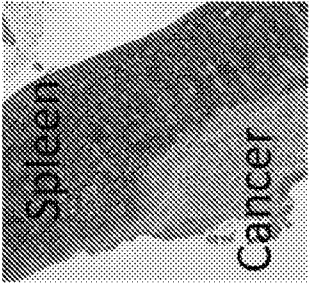


FIG. 8D



Metastases

FIG. 9A

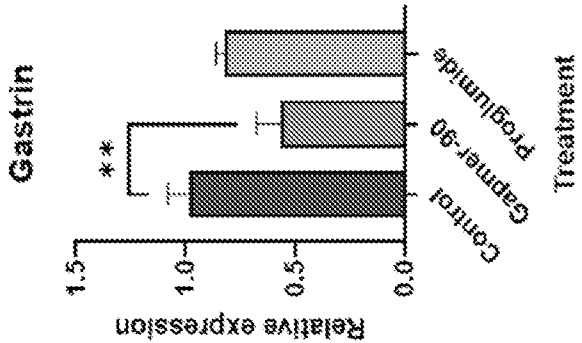


FIG. 9B

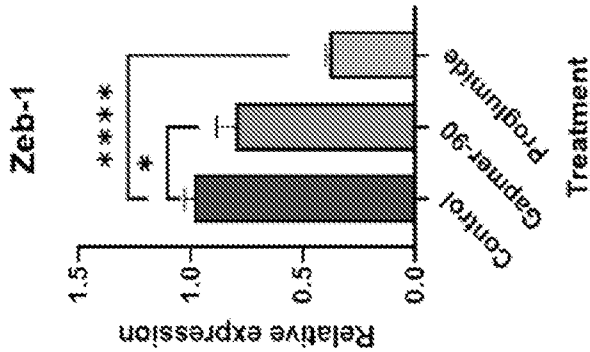


FIG. 9C

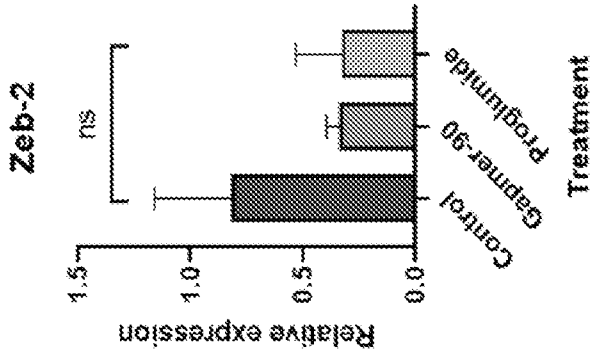


FIG. 10A

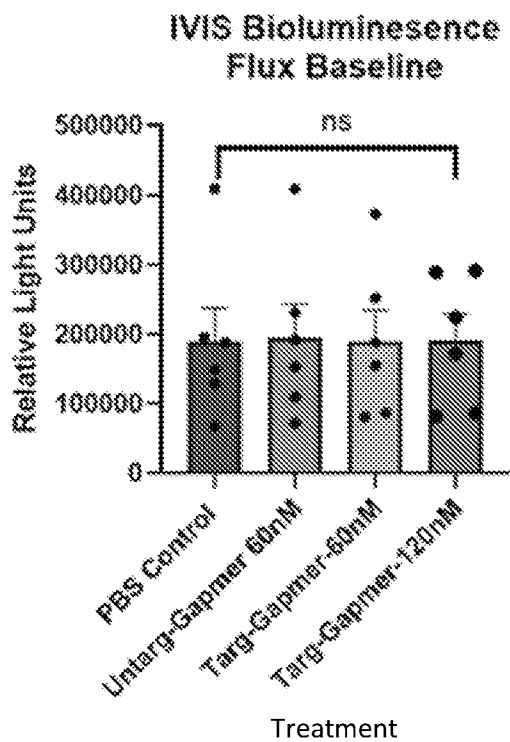


FIG. 10B

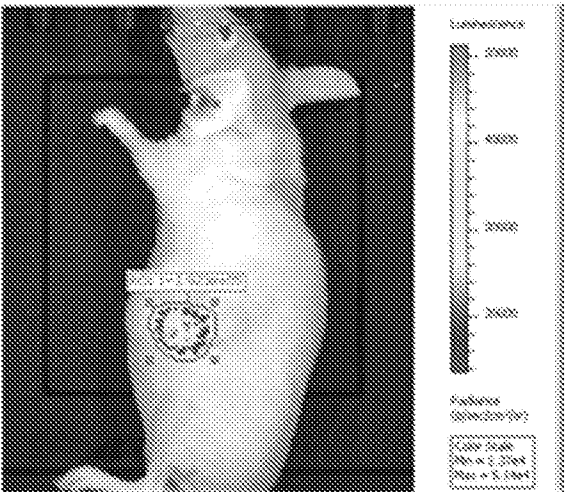


FIG. 10C

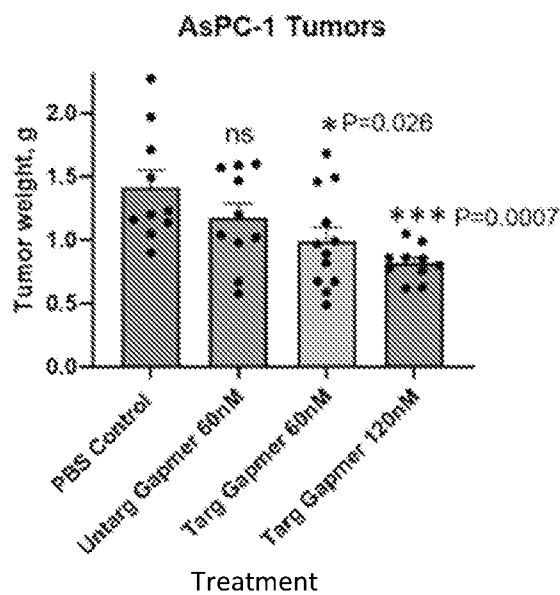


FIG. 10D

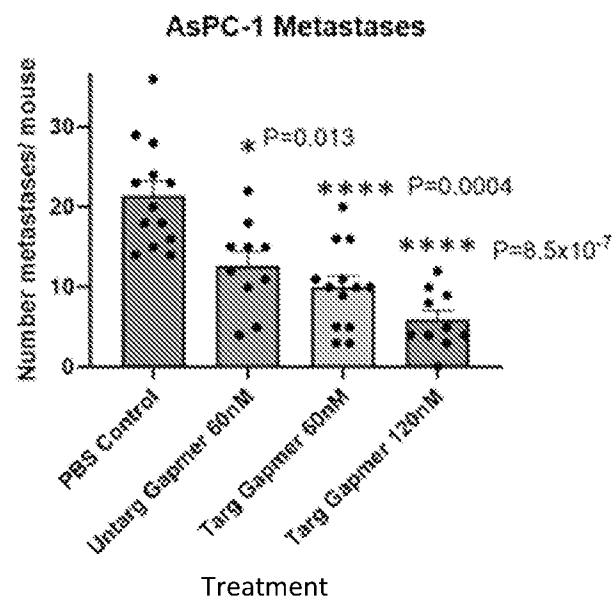


FIG. 10E

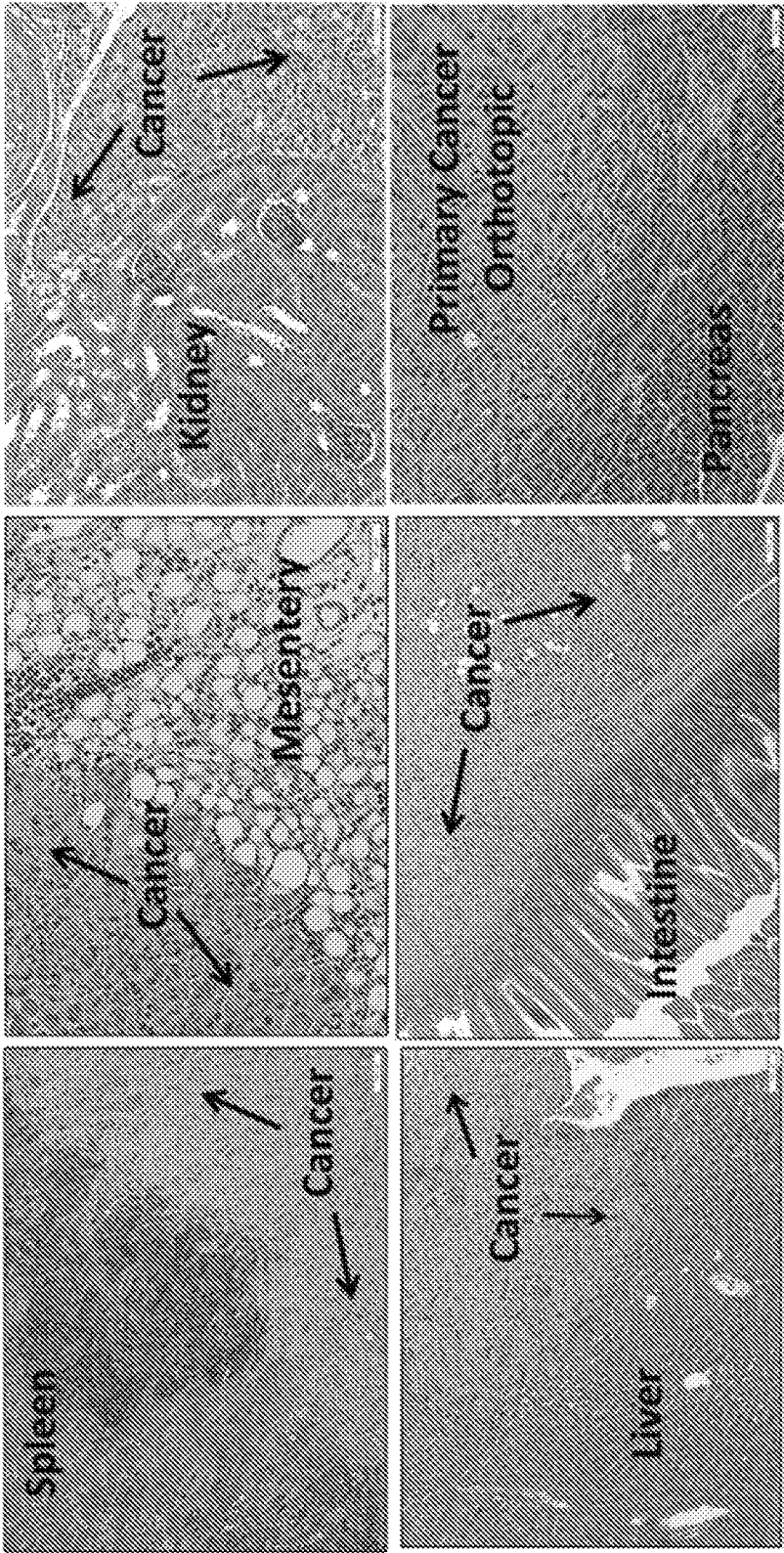


FIG. 10F

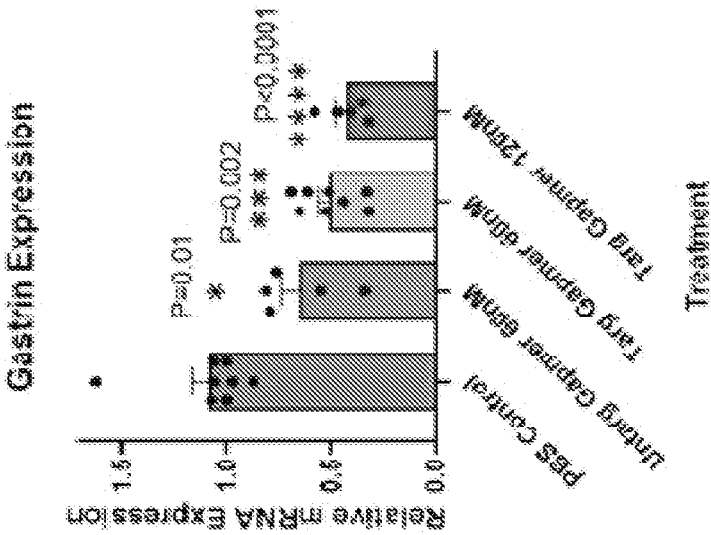


FIG. 10G

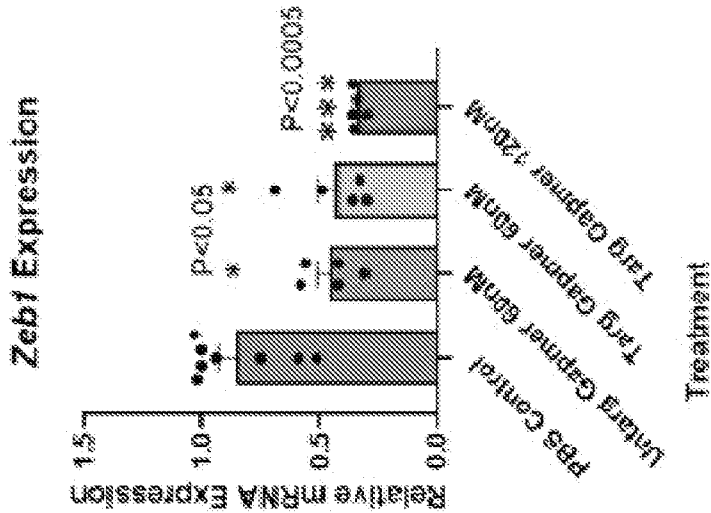


FIG. 10H

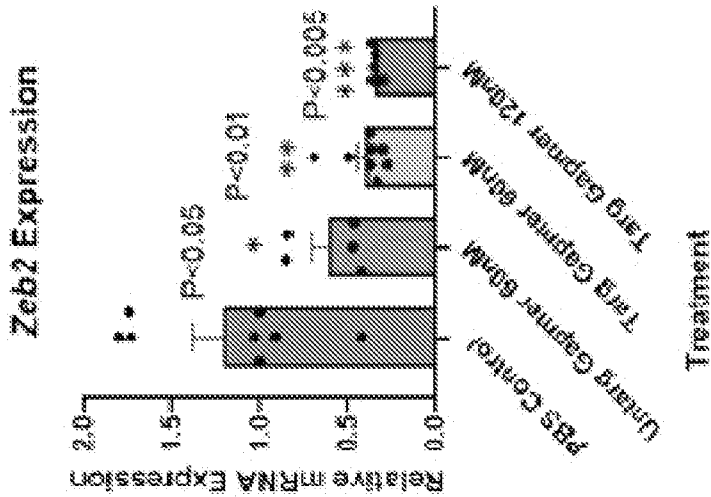


FIG. 10I

Serum Chemistry	PBS Control	Untarg-Gapmer-60nM	Targ-Gapmer-60nM	Targ-Gapmer-120nM
ALT, U/L	38.4±5.9	103±53	105±52	28±16
AST, U/L	125±20	172±53	226±74	89±14
Alk Phos, U/L	46±12	83±32	74±29	50±18
T Billi, mg/dL	1.5±0.7	4.0±2.1	3.0±1.7	1.1±0.7
Creatinine, mg/dL	0.38±0.06	0.31±0.03	0.29±0.03	0.31±0.02
CPK, U/L	305±44	183±17	386±105	83±31

FIG. 10J

Location	PBS Control	Untarg-Gapmer- 60nM	Targ-Gapmer- 60nM	Targ-Gapmer- 120nM
Spleen	58	38	29	23
Intestine	50	28	26	3
Liver	22	15	10	6
Mesentery	34	26	36	16
Kidney	6	4	4	2
Diaphragm	18	11	3	0
Abdominal wall	20	5	5	7

FIG. 10K

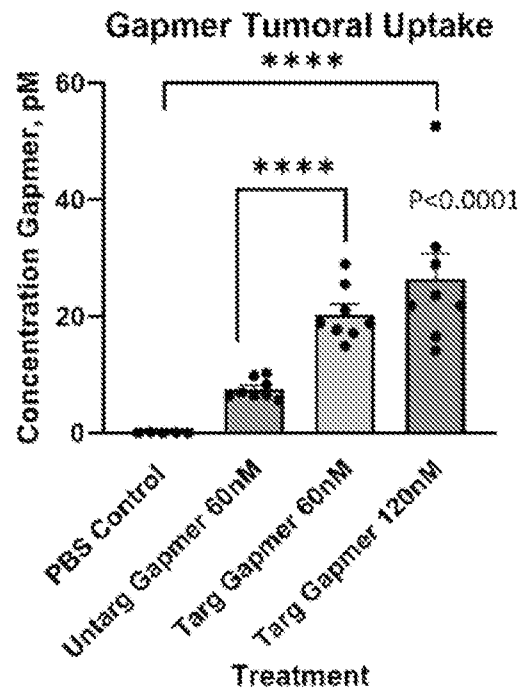


FIG. 11B

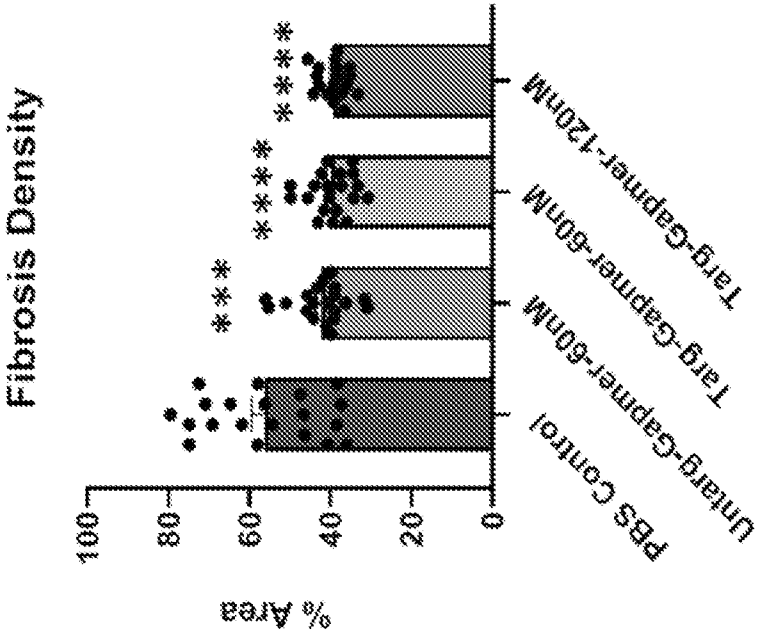


FIG. 11A

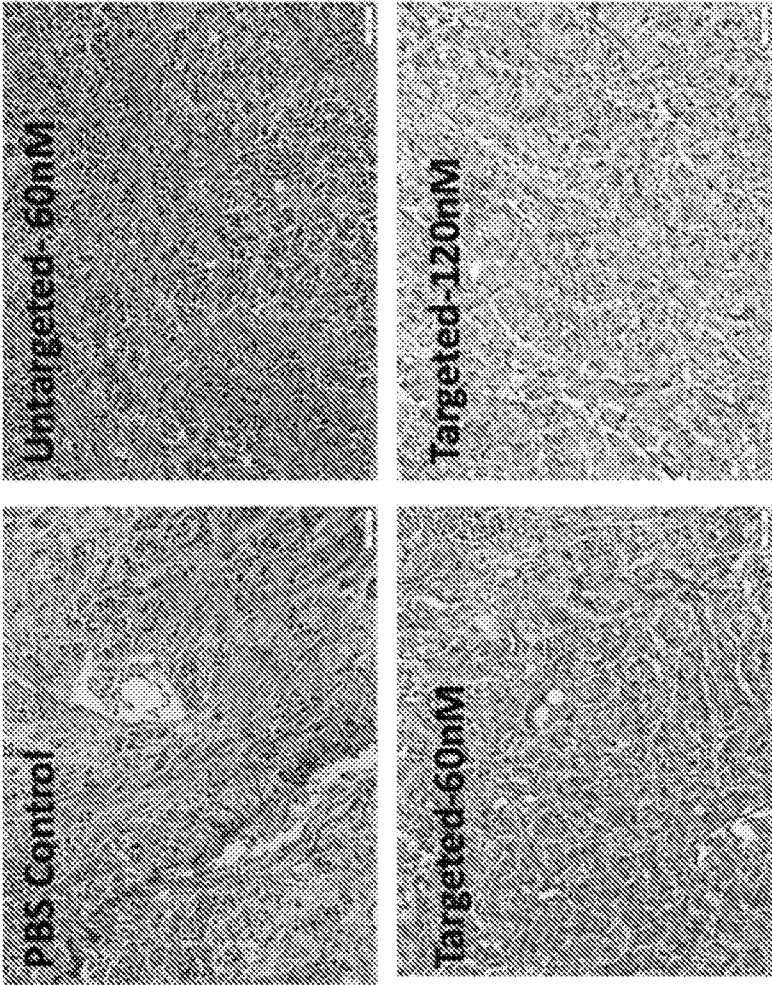


FIG. 11D

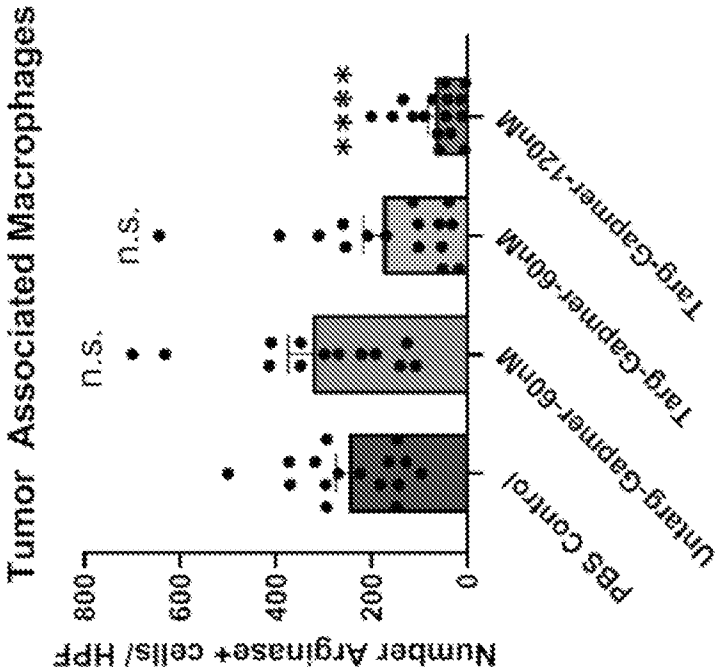


FIG. 11C

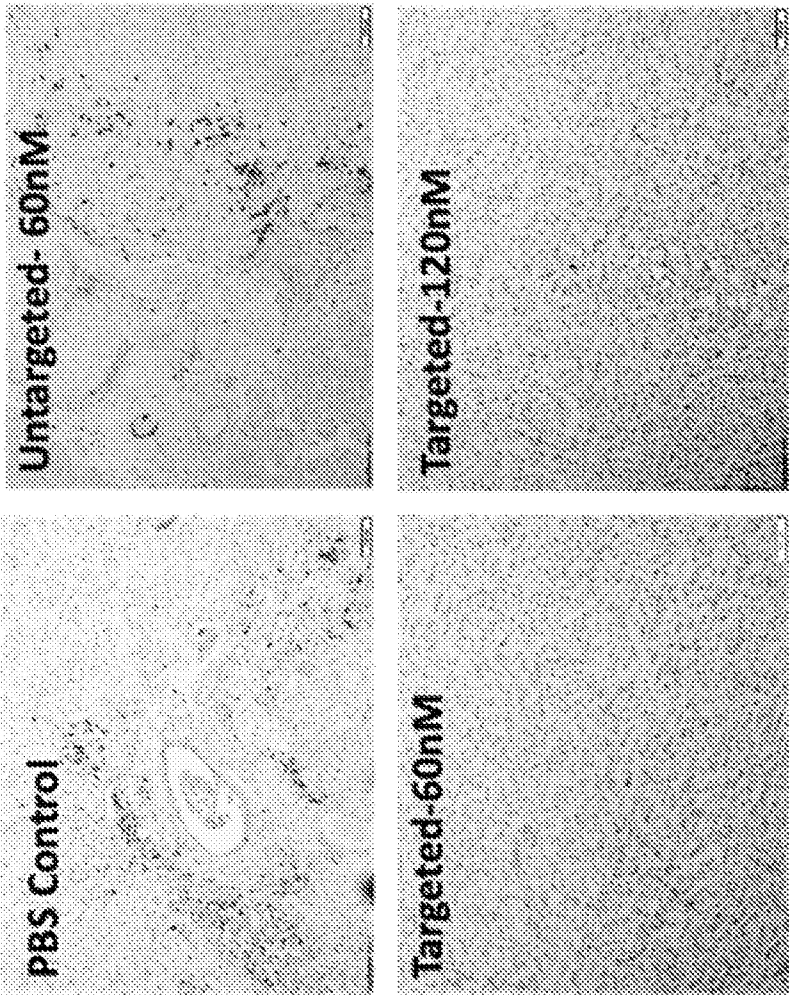


FIG. 11F

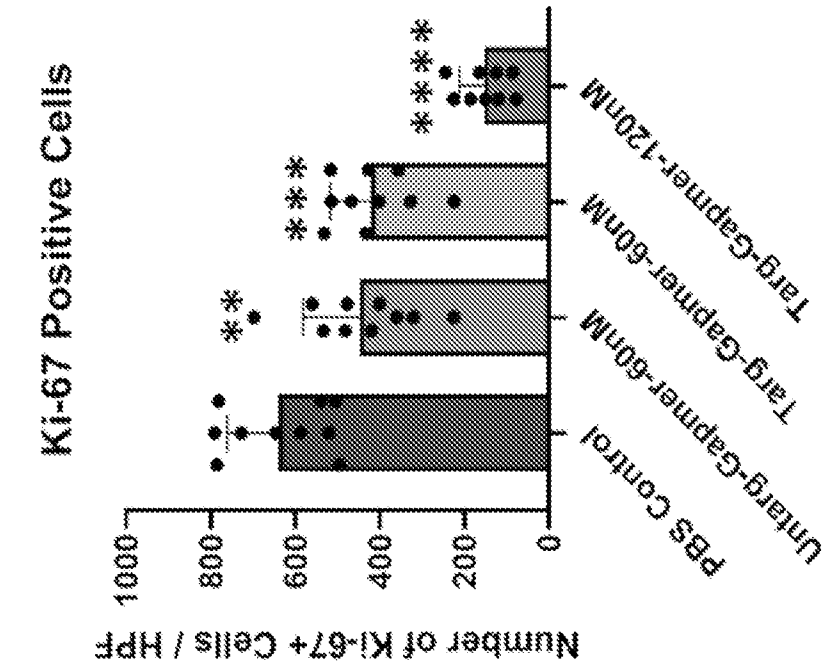


FIG. 11E

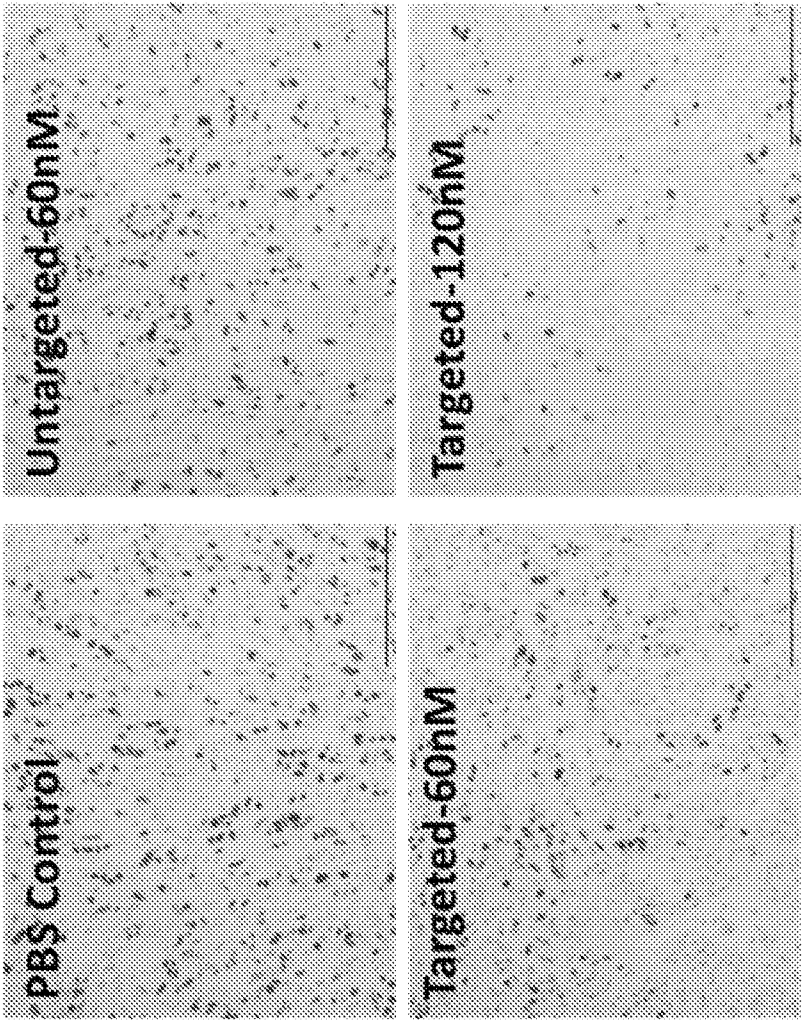


FIG. 12

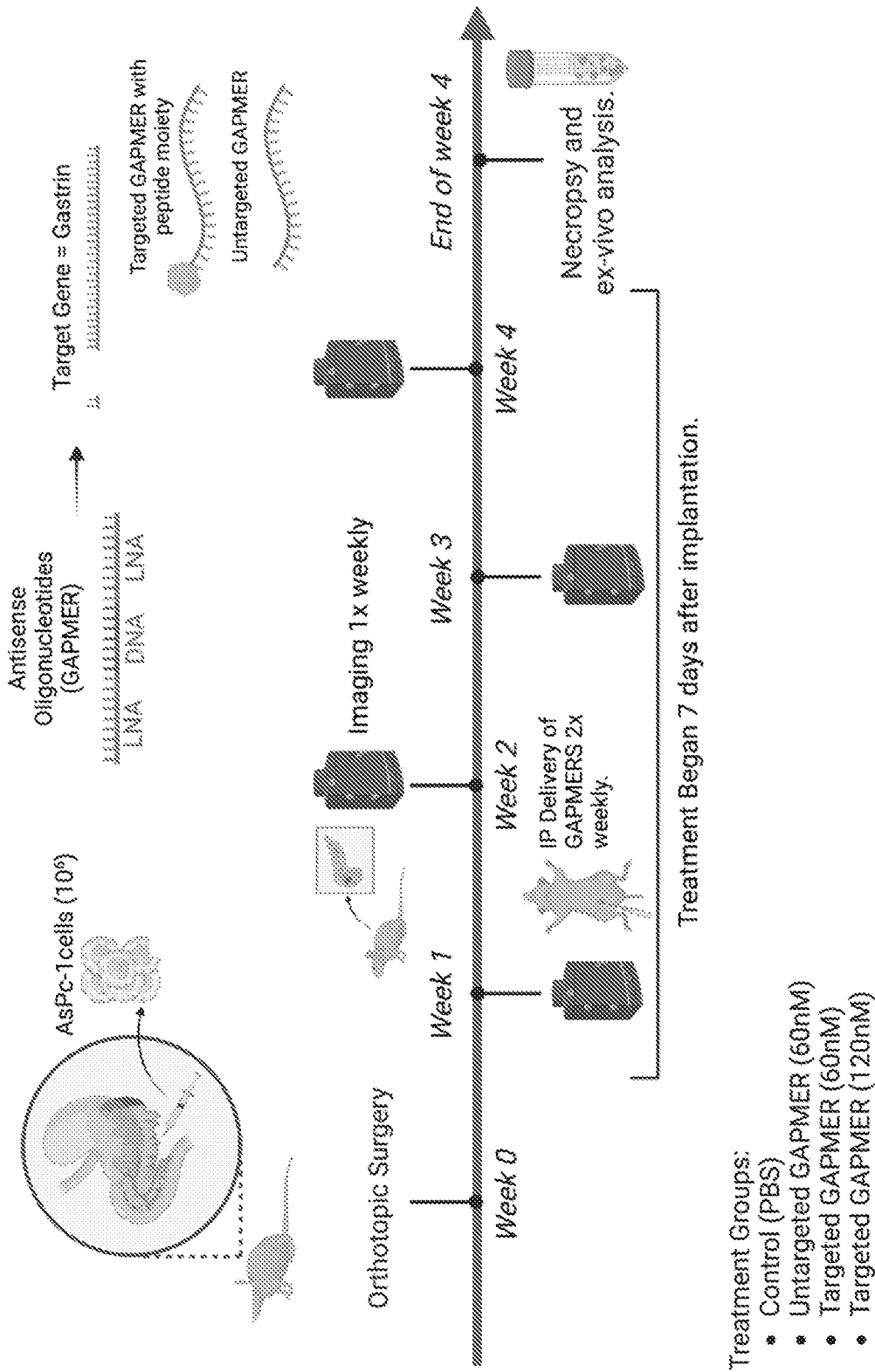
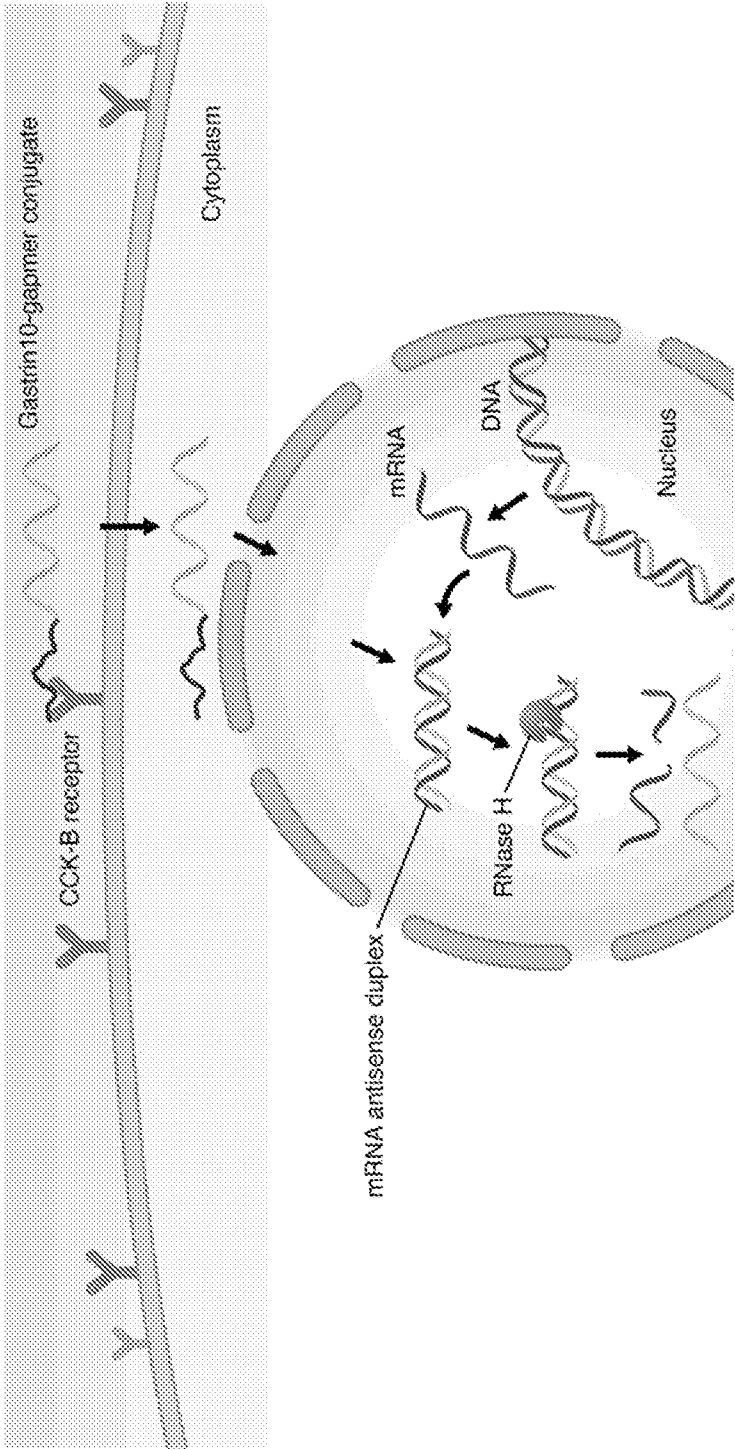


FIG. 13



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/041812

A. CLASSIFICATION OF SUBJECT MATTERIPC: **C12N 15/113** (2023.01); **A61K 48/00** (2023.01); **A61P 35/00** (2023.01)CPC: **C12N15/113; A61K48/005; A61P35/00; C12N2310/11; C12N2310/315; C12N2310/341**

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.
A	WO 2015/183920 A2 (CHILDREN'S HOSPITAL MEDICAL CENTER) 03 December 2015 (03.12.2015) paragraph [00179]	6-12, 13/6-12
A	US 2007/0059711 A1 (SLOMINSKI, ANDRZEJ et al.) 15 March 2007 (15.03.2007) page 16 - SEQ ID NO: 179	6-12, 13/6-12
Y	KUESPERT ET AL. "Antisense Oligonucleotide in LNA-Gapmer Design Targeting TGFBR2 —A Key Single Gene Target for Safe and Effective Inhibition of TGF-beta Signaling" 1-25. International Journal of Molecular Science, Vol. 21, Article 1952. Web. 12 March 2020; [DOI: 10.3390/ijms21061952]. abstract; pages 2-3	1-5, 13/1-5
A		6-12, 13/6-12

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

07 October 2024 (07.10.2024)

Date of mailing of the international search report

21 January 2025 (21.01.2025)

Name and mailing address of the ISA/US

COMMISSIONER FOR PATENTS
MAIL STOP PCT, ATTN: ISA/US
P.O. Box 1450
Alexandria, VA 22313-1450
UNITED STATES OF AMERICA

Authorized officer

SHANE THOMAS

Facsimile No. 571-273-8300

Telephone No. PCT Helpdesk: (571) 272-4300

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y A	SMITH ET AL. "Antisense oligonucleotides to gastrin inhibit growth of human pancreatic cancer" 107-112. Cancer Letters, Vol. 135. Web. 22 August 1998; [DOI: 10.1016/s0304-3835(98)00279-1] page 108, first column, last paragraph; page 111, first column, second paragraph	1-5, 13/1-5 6-12, 13/6-12
P,X	CHEKURI ET AL. "Abstract 3250 LNA gapmer to gastrin inhibits growth and metastases of human pancreatic cancer in mice" Cancer Research. 22 March 2023; [DOI: 10.1158/1538-7445.AM2024-3250] Entire document	1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/041812

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13ter.1(a)),
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

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.: **14-23**
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:

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. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-13, SEQ ID NO: 2 (ASO nucleotide sequence) are directed towards antisense oligonucleotides that hybridize with a gastrin mRNA molecule.

The antisense oligonucleotides that hybridize with a gastrin mRNA molecule of Claims 1-5, 6-7 (each in part), 8-13 are believed to encompass the first named invention of Groups I+ and are the claims that will be searched without fee to the extent that they encompass SEQ ID NO: 2 (ASO nucleotide sequence). This first named invention of Group I+ has been selected to encompass the first species of each of the genera found in claims 6-7 based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines.

Applicant is invited to elect additional ASO nucleotide sequence(s) to be searched. Additional ASO nucleotide sequence(s) will be searched upon the payment of additional fees. Applicants must specify the searchable claims that encompass any additionally elected ASO nucleotide sequence(s). 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/examined. Exemplary elections would be SEQ ID NO: 8 (ASO nucleotide sequence).

Groups I+ share the technical features including: an antisense oligonucleotide (ASO) 14 to 20 nucleotides in length that specifically hybridizes with a gastrin mRNA molecule, wherein the ASO comprises at least one locked nucleic acid (LNA) nucleotide at each of the 5' and 3' termini. These shared technical features are previously disclosed by the publication entitled "Antisense oligonucleotides to gastrin inhibit growth of human pancreatic cancer" to Jill Palmer Smith, et al. (hereinafter "Smith") in view of the publication entitled "Antisense Oligonucleotide in LNA-Gapmer Design Targeting TGFBR2—A Key Single Gene Target for Safe and Effective Inhibition of TGF-beta Signaling" to Sabrina Kuespert, et al. (hereinafter "Kuespert").

Smith discloses an antisense oligonucleotide (ASO) 14 to 20 nucleotides in length that specifically hybridizes with a gastrin mRNA molecule (synthetic antisense oligonucleotide was designed to bind to the gastrin mRNA. Where the oligonucleotide is 20 nucleotides long; page 108, first column, last paragraph; page 111, first column, second paragraph).

Smith does not disclose wherein the ASO comprises at least one locked nucleic acid (LNA) nucleotide at each of the 5' and 3' termini.

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

Kuespert discloses ASO comprises at least one locked nucleic acid (LNA) nucleotide at each of the 5' and 3' termini (LNA-gapmer [ASO] targeting specific mRNA; abstract; Figure 1). It would have been obvious to one of ordinary skill in the art before the relevant date of the invention to modify the Smith invention to provide ASO comprises at least one locked nucleic acid (LNA) nucleotide at each of the 5' and 3' termini, as taught by Kuespert, in order to provide antisense oligonucleotides that are capable of targeting specific mRNA, and further where LNAs protect the ASO from degradation (Kuespert reference, abstract; Figure 1).

Since none of the special technical features of the Groups I+ inventions are found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Smith and Kuespert references, unity of invention is lacking.

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.: **1-5, 6-7 (each in part), 8-13; SEQ ID NO: 2 (ASO nucleotide sequence)**

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.